VACCINATION AND COMPARISON OF THE POTENCY OF NEWCASTLE DISEASE VACCINE STRAINS I_2 AND LA SOTA IN RURAL CHICKENS IN PLATEAU STATE, NIGERIA

BY

MUSA, USMAN

DEPARTMENT OF VETERINARY SURGERY AND MEDICINE
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY
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MUSA, USMAN DVM (1992) M.Sc (2002)
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A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY ZARIA, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN VETERINARY MEDICINE

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FACULTY OF VETERINARY MEDICINE
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FEBRUARY, 2009

DECLARATION

I hereby declare that the work reported in this dissertation entitled **''Vaccination and comparison of the potency of Newcastle disease vaccine strains I_2 and La Sota in rural chickens in Plateau State, Nigeria''** was carried out and written by me in the Immunology and Virology Laboratory of the Department of Veterinary Surgery and Medicine, Ahmadu Bello University Zaria and Viral Research Laboratory of the National Veterinary Research Institute, Vom and Jos South, Barkin Ladi, Kanam and Shendam Local Government Areas of Plateau State under the supervision of Professors, P. A. Abdu, J. U. Umoh and I. I. Dafwang.

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 submitted for another degree or diploma at any University.

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Musa Usman	**Signature**	**Date**

CERTIFICATION

This dissertation entitled **"VACCINATION AND COMPARISON OF THE POTENCY OF NEWCASTLE DISEASE VACCINE STRAINS I_2 AND LA SOTA IN RURAL CHICKENS IN PLATEAU STATE, NIGERIA''** by Musa Usman meets the regulations governing the award of Doctor of Philosophy (Ph.D) of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

____________________________ ____________________________

Professor P. A. Abdu **Date**
DVM, M.Sc., Ph.D (ABU)
(Chairman, Supervisory Committee)
Department of Veterinary Surgery and Medicine,
Ahmadu Bello University, Zaria.

____________________________ ____________________________

Professor J. U. Umoh **Date**
DVM (ABU), MSPH., Ph.D (Missouri)
(Member Supervisory Committee)
Department of Veterinary Public Health and Preventive Medicine,
Ahmadu Bello University, Zaria.

____________________________ ____________________________

Professor I. I. Dafwang **Date**
BSc, M.Sc (ABU)., Ph.D (U.W. Madison)
(Member Supervisory Committee)
National Agricultural Extension Research and Liaison Services,
Ahmadu Bello University, Zaria.

____________________________ ____________________________

Professor A. B. Ogunkoya **Date**
DVM (Moscow), M.Sc., Ph.D (ABU)
Head, Department of Veterinary Surgery and Medicine,
Ahmadu Bello University, Zaria.

____________________________ ____________________________

Professor S. A. Nkom
BSc, MSc., Ph.D
Dean, Postgraduate School, **Date**
Ahmadu Bello University, Zaria.

DEDICATION

This dissertation is dedicated to my parents Alhaji Musa Gulma (late) and Hajiya Jumma Musa Gulma, my children Mohammad Zaharadeen Usman (Deenee), Mohammad Mustapha Usman (Musty), Aisha Usman, Amina Usman and to the memory of my friends late Dr. Hassan Abdul (died 1999), Dr. E.S.Haruna (died 2004) and my uncle Mamuda Mohammed Gulma. They died in ghastly motor accidents on their way to Kaduna from Zaria and from Ibadan to Jos and Gombe to Bauchi respectively, may their soul rest in peace Amen.

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ABSTRACT

Newcastle Disease (ND) has been recognized for a long time as one of the major production constraints to rural poultry. Management systems, thermoliability, maladministration of vaccines and lack of extension services have been suggested as possible causes of outbreaks, spread and maintainance of ND. A study on management systems in rural household chickens in Plateau State was, therefore, conducted in 32 rural households of four Local Government Areas (LGAs) involving 1,240 households using a structured questionnaire. Also studied was ND antibodies and field vaccination with ND vaccine strain I_2 ($NDVI_2$) on 1,208 chickens raised under traditional management system. Thermostability of $NDVI_2$, vaccination trial and comparative immunogenicity tests using $NDVI_2$ and La Sota administered via different routes and vaccine carriers were investigated. The cost benefit of ND control using $NDVI_2$ via intraocular (i/o) route was estimated. The ND antibodies in rural and commercial chickens were evaluated using haemagglutination inhibition (HI) tests and the vaccines were titrated using embryonated chicken eggs. Diseases were the main (83.2%) causes of losses of rural chickens followed by preying (8.85%) and herbs were main remedies used for managing ND. Out of 1,208 sera tested, 627 (51.9%) had detectable antibodies to ND virus, but only 170 (14.1%) of the chickens had a protective HI antibody titre of ≥ 4 Log_2. About 1,041 (86.2%) of the chickens sampled were at risk of suffering from clinical ND. In the field, $NDVI_2$ administered by i/o route gave higher geometric mean titre (GMT) of 8.3 ± 1.6 Log_2 and 92% protection rate than when administered through drinking water (dw) with GMT of 3.4 ± 0.6 Log_2, yielding a 46% antibody protection rate. Reconstituted and lyophilized $NDVI_2$ vaccines maintained a minimum infective dose of 6.8 Log_{10} and 8.4 Log_{10} for 3 and 35 days, respectively from an initial titre of 10.2 Log_{10}. Reconstituted $NDVI_2$

lost 3.4 Log_{10} titre after exposure at room temperature (RT) for 3 days. Lyophilized $NDVI_2$ lost only 1.8 Log_{10} titre after exposure at RT for 35 days. $NDVI_2$ or ND La Sota vaccines administered through commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and untreated sorghum gave little protection (0 to 22%) to birds when challenged with velogenic viscerotropic ND virus. The financial losses due to ND outbreak in unvaccinated flocks in terms of the value of birds and eggs amounted to ₦1,250,505.9 and losses avoided following vaccination were ₦10,296,264. The benefit cost ratio arising from adopting $NDVI_2$ via i/o route for the control of ND in rural chickens is 7.2. It was concluded that the prevalence and risk of ND in rural chicken are very high. The $NDVI_2$ or NDV La Sota vaccines can protect commercial and rural chickens vaccinated through i/o, im or dw routes, while feeds were not suitable as vaccine carriers. In the field, ND vaccination with $NDVI_2$ via dw route may not be appropriate but the benefits derived in the use of $NDVI_2$ via i/o route were greater than the cost of the ND outbreak in unvaccinated flocks.

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LIST OF ABBREVIATIONS AND SYMBOLS

ACIAR	Australian Center for Internationl Agricultural Research
CEC	Council of European commission
CFT	Complement fixation test
C.P	Crude protein
CRBCs	Chicken Red Blood Cells
CTA	Center for tropical agriculture
EEC	Europen economic commission
EID	Embryo infective dose
ELISA	Enzyme linked immunosorbent assay
EU	European Union
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statiscal Data base
FDLPCS	Federal Department of Livestock and Pest Control Services
FV	Future Value
GMT	Geometric mean titre
hr	hour
HA	Haemagglutination
HI	Haemagglutination Inhibition
IAEA	International atomic energy agency
IFPRI	International Food Policy Research Institute
i/o	Introcular
im	Intramuscular
K/cal	Kilocalorie
LIFDC	Low Income Food Deficient Countries

LGA	Local government area
Log_2	Log to base 2
Min	minute
ND	Newcastle Disease
NDV	Newcastle Disease Virus
$NDVI_2$	Newcastle Disease Vaccine Strain I_2
NDV4	Newcastle Disease Vaccine Strain V4
NDV4HR	Newcastle Disease Virus Heat Resistant Strain
NGO	Non governmental organization
NVRI	National Veterinary Research Institute
OIE	Office international organization
Pv	Post vaccination
PV	Present value
RBCs	Red blood cells
RIM	Resource inventory management
RT	Room temperature
SD	Standard deviation
VN	Virus neutralization
VND	Velogenic Newcastle disease
$<$	Less than
$>$	Greater than
$\leq$	Equal to or less than
$\geq$	Equal to or grater than
$\pm$	Plus or minus
$\sum$	Summation

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CHAPTER ONE

1.0 INTRODUCTION

The International Food Policy Research Institute (IFPRI) forecasts that, poultry will contribute about 40% of the global total animal protein by 2015 (IFPRI, 2000). The estimated population of family produced chickens in Africa is 700 million, compared with 191 million cattle, 182 million goats, 158 million sheep and 15 millions for pigs (FAOSTAT, 2000). In Nigeria, rural poultry production is an important activity (Nwanta, 2003). In rural communities, almost every household including the Fulani pastoralists keep local chickens purposely for meat, egg production, income, barter, special festivals, social obligations such as traditional ceremonies as well as traditional treatment of illness (Dipeolu *et al.,* 1996; Nwanta *et al.,* 2006; Musa *et al.,* 2008).

Sonaiya (2007) defined rural poultry as involving any genetic stock; improved or unimproved that is raised extensively or semi-intensively in relatively small numbers (usually less than 100 at any time). There is financial investment on inputs with most of the inputs generated in the homestead; labour is not salaried, but drawn from the family, while production is geared essentially towards home consumption and other uses. Rural poultry easily integrate into the main occupation (i.e.agriculture) of rural areas. The rural poor are more likely to keep family poultry as compared with large livestock because poultry requires less land and labour.

Several workers have reported the unique adaptive features of the Nigerian local chicken that enable it to adapt to the local environment (Adebimbo *et al.,* 1999; Ikeobi *et al.,* 2004; Peters *et al.,* 2007). They are also known to have higher degree of adaptability to diseases and less nutritional requirements (Hassan *et al.,* 1989; Dipeolu *et al.,* 1998).

Rural poultry makes up a major part of total poultry production in LIFDC (Sonaiya, 2007). In a survey in north eastern Nigeria, Kushi *et al.* (1998) found that 70.2% of the respondents kept chickens. In Kaduna state Nwanta (2006) reported that of the four species of local poultry investigated, the chicken was raised by all respondents. A mean flock size of 18 local chickens, 16 guinea fowls and 7 ducks were recorded per household. Over 50% of the households kept, two or more species of poultry with chickens and guinea fowls being the most common (79%). In Kenya, 90% of the populace kept chickens (Mbugua, 1990) and in Malawi 95% (Kampeni, 2000). Sonaiya (2007) reviewed reports from 24 countries and concluded that about 80% of the rural populace in Africa keeps family poultry.

Report by Nwosu and Okeke (1989) has shown rural poultry sector contributes 68.5% of our National poultry meat supply as against 11.39% from commercial chickens and 19.70% from other poultry. Similarly, the internal egg supply by rural chickens was reported to be as high as 72.7% (David-West, 1979). Other contributions of rural chickens include pest control and provision of organic manure derived from their droppings for soil fertility (Alders and Spradbrow, 2001).

Inspite of the importance of rural chickens to the rural and peri urban poor, mortality is one of the most important factors hindering the development of the rural poultry industry in Nigeria (Nwanta, 2003). Studies conducted by Ibrahim and Abdu (1992) revealed that predators and diseases were the main causes of stock attrition in rural poultry.

The control of diseases in rural poultry is made difficult because of the multiple age structure and small size of the flocks, the labour required in catching individual birds for vaccination and lack of skill in the use of vaccines.Newcastle disease (ND) has been recognized for a long time as one of the major production constraints to rural poultry, which are therefore exposed to repeated and severe epidemic of the disease. Studies have shown that ND is an endemic disease in rural and commercial poultry with occasional severity being recorded in highly susceptible poultry flocks (Fatumbi and Adene, 1979; Adu *et al.,* 1986; Adene, 1989; Orajaka *et al.,* 1999; Adene, 2004). Clinical and serological study of ND in Nigeria has confirmed the susceptibility of rural chickens, pigeons, guinea fowls, ducks and turkeys (Hill *et al.,* 1953; Gomwalk *et al.,* 1985; Durojaiye and Adene, 1988; Nawathe, 1988; Ezeifeka *et al.,* 1992; Echeonwu *et al.,* 1993; Mai *et al.,* 2004; Nwanta *et al.,* 2006).

In endemic villages, circulating strains of ND virus cause 100% mortality in unprotected flocks and outbreaks are unpredictable, hence discouraging villagers from paying proper attention to the husbandry and welfare of flocks (Alders and Spradbrow, 2001). About 200-250 outbreaks of ND were reported by Okeke and Lamorde (1988) to occur annually among chicks and adult chickens in Nigeria. These outbreaks of ND were observed to occur at certain periods of the year which in some cases may be attributed to seasonal condition (Nawathe, 1988). However, studies have shown that the outbreaks in October to December periods coincided with the time of greatest movement of birds for sale during festivities of Eid-el-fitr, Eid-el-kabir, Christmas and Easter (Abdu *et al.,* 1985; Nwanta *et al.,* 2006).

Because of the importance attached to ND by rural farmers, various local names have been given to the disease by people in many countries (Alders and Spradbrow, 2001).

In Northern, Nigeria ND is known in Hausa language as "*Haukan Kaji*" (Ibrahim and Abdu 1992), "*Koli*" (Yoruba) in Western Nigeria (Dipeolu *et al.*, 1998) and *Ogbunnakpo* (Igbo) in Eastern Nigeria (Nwanta, 2003).

Knowledge and understanding of the prevailing strains of ND virus infecting rural poultry population and the distribution of the infection as well as the factors responsible for the maintenance of the virus in circulation in Nigeria and poultry are crucial to the steps required for the formulation of appropriate policies and strategies for its control. The control of ND in rural poultry in Nigeria will not only boost productivity of the rural poultry sector but will also guarantee supply of animal protein to the populace (Nwanta *et al.,* 2006).

Any of the conventional commercial ND vaccines will protect rural chickens against Newcastle disease, if the vaccines reach the chickens in a potent form. But many commercial vaccines are thermolabile. Cold chains are expensive to develop and maintain in rural settings. Apart from this, effective vaccination with conventional vaccines involves, catching and handling individual birds using skilled vaccinators and repeating the whole procedure sufficiently often to ensure that every bird receives at least two doses of vaccines at different times (Nasser *et al.,* 2000). Also, rural flocks are small, scattered and multi-aged. The minimal unit vial for commercial vaccines in Nigeria which is usually 200 doses is expensive and inapplicable for very small flocks. Some of the logistical problems may be resolved if a thermostable NDV strain that does not depend on a cold chain is used in vaccinating rural chickens. Hope for rural chicken vaccination came with the advent of heat stable vaccines which in turn heralded the introduction of an innovative mode of ND vaccination namely, oral delivery through chicken feeds (Aini, 1990). This presented a feasible method for vaccination of rural chicken flocks (Echeonwu *et al.,* 2007). The immunogenic potential of the thermostable V4 strain of ND virus was reported by several workers (Ibrahim *et al.,* 1981; Westbury, 1984; Spradbrow, 1992a).

Until 1998, thermostable ND vaccine, NDV_4 HR and $NDVI_2$ were not available in Nigeria. The I_2 strain of Newcastle disease seed virus was developed through the assistance of the Australian Centre for International Agricultural Research (ACIAR) and was made available to developing countries at no cost for the production of the vaccine (Bensink and Spradbrow, 1999).

1.1 STATEMENT OF RESEARCH PROBLEM

Rural chickens can be found in all developing countries and plays a vital role in many poor rural households. Newcastle disease is the most important viral disease of poultry in the world including developing countries (Adu *et al.,* 1986; Adene, 1990; Adene, 1997; Nwanta *et al.,* 2008). It is said to be a major factor affecting the development of poultry industry in Nigeria and Africa in general (Ambali *et al.,* 1985; Orajaka and Ezema, 2004). It is a serious constraint to poultry production and has a devastating effect on the productivity of commercial and rural poultry industry (Shamaki *et al.,* 1989; Nwanta *et al.,* 2006). Vaccination is the fundamental control method used to reduce mortalities due to ND in rural chickens (Meulemans, 1988). However, rural chickens are not vaccinated because the use of conventional vaccines for control of ND in rural poultry poses a difficult logistic problem.

1.2 JUSTIFICATION OF THE STUDY

Development of semi intensive enterprises, particularly rural chicken production, can be a useful way to meet the nutrition, income, employment and gender needs of rural population. The population of poultry in Nigeria is put at about 150 million with the rural poultry accounting for about 80% of the number (FAO, 1992). Extensive vaccination campaigns have not been launched by government as a result of financial constraints as well as a perception that rural chickens do not play any important role in the economy of the country (Barileng, 2006). Problems have been identified that hamper the introduction of ND vaccines to rural areas in Africa (Spradbrow, 1992b). One of the major problems is the heat liability of conventional ND vaccines and the consequent requirement for elaborate cold chains for vaccine storage in villages. The second was limited education and lack of extension information available to rural communities about the importance of poultry diseases in general. Thirdly, poverty of the rural population also contributes to the poor health management status of the poultry (Spradbrow, 1993/94). Some of the households have little or no income; that makes it difficult if they have to purchase conventional ND vaccines which comes in large dose package and at high price. A fourth problem is the catching of rural chickens for individual vaccination. Rural chickens are often not housed and have to be individually caught, for vaccination.

1.3 OBJECTIVES OF THE STUDY

The goal of the study was to improve the productivity of rural poultry and reduce rural household poverty through the control of ND.

Objectives

The objectives of the study were to:-

(i) Determine the production constraints and remedies adopted by farmers in controlling ND in Plateau State.

(ii) Conduct survey for ND antibodies and field vaccination trials in rural chickens in four LGAs of Plateau State.

(iii) Compare the serological response of rural chickens to $NDVI_2$ and La Sota vaccine strains.

(iv) Evaluate the thermostability and potency $NDVI_2$ stored at different temperatures.

(v) Compare the antibody titres and post challenge responses in rural and commercial chickens vaccinated with $NDVI_2$ and La Sota vaccine strains.

(vi) To assess financial implication of intraocular vaccination of rural chicken with $NDVI_2$.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DEFINITION AND AETIOLOGY

Newcastle disease is a highly infectious, contagious viral disease of domestic fowl and other species of bird characterized by variable morbidity and mortality in unvaccinated birds (Hanson, 1978; Alexander, 1997). Clinically, ND is characterized by reduction in egg production, laying of soft shelled and malformed eggs, partial suppression and at times complete caesation of immunological response (Ezeokoli *et al.,* 1984; Orajaka and Ezema, 2004). The disease is also characterized by respiratory and nervous signs (Alexander, 1997).

2.2 THE NEWCSATLE DISEASE VIRUS

Newcastle disease is caused by a paramyxovirus that affects domestic and wild birds and has become a major problem in many countries where poultry are reared (Allan *et al.,* 1978; Alders and Spradbrow, 2001).

Newcastle disease virus belong to *paramyxoviridae* family; subfamily *paramyxovirinae* and under genus *rubula* virus. The genus *rubula* is formed from mumps virus, human para influenza virus types 2 and 4, Newcastle disease virus (PMV-1) and the avian paramyxovirus PMV-2 to PMV-3 (Alexander, 1997).

Paramyxovirus 1 is the most important pathogen for poultry but PMV-2 and PMV-3 has been associated with a respiratory disease and egg reduction following natural infection in chickens (Lalita, 2002).

The rate at which infectivity is destroyed is found to be dependent on the strain of the virus, the length of time of exposure, the quantity of the virus and the nature of the suspending medium and interactions between treatments. No single treatment has been able to destroy the entire virus but that such treatment may result in a low probability of infective virus remaining (Alexander, 1997).

2.3 CLASSIFICATION OF NEWCASTLE DISEASE VIRUS

Newcastle disease virus is an RNA virus belonging to the genus *Paramyxovirus* (PMV) of the family *Paramyxoviridae* within the avian *Paramyxoviruses*, nine distinct serotypes designated PMV-1 to PMV-9 are recognized and NDV is a serotype 1 *Paramyxovirus* PMV-1 (McFerran and McNulty, 1993).

Members of the *Paramyxoviridae* are enveloped RNA viruses which posses non-segmented single-stranded genome of negative polarity. By negative contrast electron microscope, particles appear pleomorphic, usually 100-500 nm in diameter (Melnik, 1982). The family consists of three genera (1) *Morbilli virus* (2) *Pneumovirus* (3) *Paramyxovirus.*

2.4 BIOLOGICAL PROPERTIES OF NEWCASTLE DISEASE VIRUS

Newcastle disease viruses possess a number of biological and physical characteristics by which it may be distinguishable qualitatively or quantitatively from other paramyxoviruses and by which strains of NDV may be distinguished from each other. Among these properties are the abilities to agglutinate and to lyse erythrocytes, to induce toxic changes without multiplication, to infect and incite various signs and lesions in certain hosts and to cause the death of some hosts, to be specifically neutralised by or to react with antibodies and to be inactivated by selected chemical agents under confined conditions.

2.4.1 *Haemagglutination Activity*

The activity of NDV to agglutinate RBC was first described by Burnet (1942). The ability of NDV to agglutinate RBC is due to the binding of the haemagglunation-neuraminidase (HN) protein to receptors on the surface of the RBC (Alexander *et al.,* 1992). This property and the specific inhibition of agglutination by antisera have proven powerful tools in the diagnosis of the disease (Burnet, 1942). Chicken RBCs are usually used in haemagglutination (HA) tests, but NDV will cause agglutination of all amphibian reptiles and avian cells (Lancaster, 1966). Winslow *et al.* (1950) showed that human, mouse and guinea pig RBC were agglutinated by all NDV strains tested but the ability to agglutinate, cattle, goat, sheep, swine and horse cells varied with the strain of NDV. Other avian paramyxoviruses also appear to be able to agglutinate a wide range of RBC, but the exact range may vary with isolate as well as serotype (Alexander, 1997).

The process of agglutination occurs in two stages:-

(1) Attachment of the virus to the receptor substance on the cell surface (agglutination) and the destruction of the receptor substance by the enzyme neuraminidase (Alkaemono, 1962 cited by Alexander, 1997).

(2) The second stage is associated with the release of the virus from the surface of the cell (elution) (Sasik and Levine, 1967 cited by Alexander, 1997).

Approximately 100,000 virus infective units are equal to one, haemagglutinating unit (Hanson, 1978). The number of viral particles in a preparation may be approximated on the basis of agglutinating activity (Hanson, 1978). The rapidity with which NDV elutes from cells as measured by a standard test varies from less than 60 mins to over 24 h and is related to the strain of the virus (Hanson, 1975).

2.4.2 *Haemolysis*

Newcastle disease virus possesses a haemolysin. The virus is capable of lysing those erythrocytes that it can agglutinate (Burnet, 1950). The hemolytic activity of the virus is enhanced by freezing and thawing, dialysis, source vibration and osmotic shock (Burnet, 1950).

2.4.3 *Neuraminidase Activity*

The exact function of the neuraminidase in virus replication is unknown but it is likely that it removes virus receptors from the host cell and this prevents the reattachment of released viral particles (Alexander, 1997; Oladele, 2003). An obvious consequence is of the possession of this enzyme is the gradual elution of agglutinated RBC (Alexander *et al.*, 1999). In Nigeria, neuraminidase activity was detected in Newcastle disease virus (NDV) Kudu 113 strain in sufficient amount in both crude allantoic fluid and lyophilized stock (Oladele, 2003).

2.4.5 Resistance to Environmental and Chemical Agents

The ability of the NDV to infect and to induce immunogenic response can be destroyed by exposure to environmental and chemical agents.

(1) *Environmental Temperature*

Environmental conditions especially warm temperatures and solar radiation, facilitates the destruction of NDV by chemicals. NDV is destroyed by exposure to ultraviolet rays and all virus activity is destroyed within 1 min at 100^{o}C. At 56^{o}C the destruction of infectivity, HA activity and immunogenicity occur within periods of five min to six h. At 37^{o}C, hours to days may be required to induce these charges. At

20 ^{o}C and 8 ^{o}C, months to years may pass before all reactivity in the virus is lost and freezing temperatures inactivate NDV (Lalita, 2002). In Nigeria, the stress of cool dry harmattan is known to worsen the outcome of NDV infection (Abdu *et al.*, 1992).

(2) *Ultraviolet Rays and pH*

Newcastle disease virus is destroyed by exposure to ultraviolet light rays in a similar way to that of other paramyxoviruses (Lancaster, 1966). It has a rather broad stability in the presence of varying hydrogen ion concentrations. The infectivity is retained for many hours at a pH as low as two and as high as ten (Lancaster, 1966).

(3) *Environment*

The stability of NDV in nature is dependent on the medium in which the virus is present, for example decaying carcasses, feaces, drying or fermenting matter (Alexander, 1997). Proteinaceous matter may not only be protective, but may nullify the action of disinfectants. When disinfection is required it should follow physical cleaning.

(4) *Chemical Agents*

Formalin, beta propiolactone and phenol have been used to destroy the infectivity without severely, damaging the immunogenicity of the preparation. At low temperatures, diluted formalin will destroy the infectivity without markedly affecting the haemagglutinin ability and without effect on the immunogenicity. All known viricidal chemicals will destroy NDV (Cunningham, 1948 cited by Alexander, 1997).

2.5 STRAIN CLASSIFICATION OF NEWCASTLE DISEASE VIRUS

The term strain is generally used to mean a well characterized isolate of the NDV. The important objective in characterizing viruses is to group similar viruses. For NDV isolates this has inevitably meant the distinction between viruses of high and low pathogenicity for chickens or perhaps more pertinently between enzootic and epizootic viruses (Alexander, 1990).

A strain of NDV is a culture that has been recovered from chicken or other animals by inoculation of a suitable host system like embryonated chicken eggs or cell cultures (Hanson, 1978). Moderately, mesogenic strains such as Roakin, MK 107, Mukteswar H and Haifa either have evolved in nature from the original highly virulent strains or have been developed in the laboratory. These viruses have been referred to as mesogenic strains, in contrast to the more virulent velogenic field strains (Hanson and Brandly, 1955).

Mesogenic strains of NDV occurring in nature, as exemplified by the Roakin strain, have been recognised by Brandly *et al.* (1946). Mesogenic strains have also been developed by repeated passages in other hosts; for example, strains MK 107 and Haifa were modified in duck embryos by Clancy *et al.* (1949). The Mukteswar strain was modified by repeated passage in chicken embryo. Strains of NDV have evolved in nature which is less pathogenic than the mesogenic strains. These have been named the lentogenic strains by Hanson and Brandly (1955) because of their slower growing capacity in chicken embryos and their lower intracerebral pathogenicity index in chicks. The three lentogenic strains B1, La Sota and F are used as live virus vaccines and have largely replaced the mesogenic strains in NDV vaccine. The advantage they have is that they can be used in very young chicks without severely affecting the host and they do not possess the degree of virulence of the mesogenic strains. Hitchner and Johnson (1948) first reported on a virus of low virulence suitable for vaccinating chickens by the intranasal route. Immunity following vaccination of day-old chicks was reported to be effective for at least 4 months. Later observation by Hitchner (1950) revealed that immunity lasted for at least 19 weeks when chicks, hatched from

B1 vaccinated dams were vaccinated intramuscular. However, Doll *et al.* (1950) found chicks hatched from susceptible hens and vaccinated at 3 weeks of age intranasally with B1, were susceptible to re-infection with Texas GB strain 51 days after vaccination. Re-infection occurred by direct contact, intranasal exposure or air borne exposure, but resulted only in subclinical infection.

2.5.1 Australian Newcastle disease virus Strains

Australia has been free of clinical ND, although avirulent strains of the virus are endemic in some poultry growing areas (Turner and Kovesdy, 1974). Since 1930 and 1932 clinical outbreaks of ND in Australia were controlled with a slaughter and eradication programme and application of quarantine measures (Westbury, 1984). Plans developed to control any clinical ND that might develop in the country did not allow for the use of vaccines. The immunogenicity of Australian strains of NDV has been studied and strain V4 was considered a candidate for use in ND vaccine production (Simmons, 1967; French *et al.,* 1969; Turner *et al.,* 1977; Spradbrow *et al.,* 1978).

2.5.2 Some Properties of Australian Newcastle disease virus Strains

Westbury (1979) tested seventeen Australian strains of NDV for their biological properties: mean death time, heat stability of the haemagglutinin and infectivity of the virus at 46^{o}C; the elution time of the virus from chicken erythrocytes and the ability to haemagglutinate equine red blood cells. The results obtained indicated that, the strains varied considerably in reaction, they ranged from fast eluting strains with heatlabile haemagglutinin and infectivity (e.g. ST) to slow-eluting strains having heat stable haemagglutinin and infectivity (e.g. V4). The result with V4 differed from some published results, suggesting that this strain does not haemagglutinate equine erythrocytes (Hanson, 1975). Fluctuation in the dominance of viral sub populations within NDV strains could explain the variability in the ability of different stocks of strain V4 to haemagglutinate equine erythrocytes (Westbury, 1979). No Australian strain was identified that eluted within ten h or in which haemagglutinin was inactivated at 56 ^{o}C in two min or less.

2.5.3 Websters V4 and V4 Heat Resistant Newcastle Disease Vaccine Strains

The V4 strain is a naturally occurring Australian ND virus isolated, identified and typed in 1966 by Simmons (Heath *et al.*, 1992). The strain is described as slow eluting with heat-stable haemagglutinin and infectivity (Westbury, 1979). It was developed by Webster as a vaccine strain because of its avirulence and high immunogenicity (Westbury, 1979). Soon after original isolation of V4 strain for ND vaccination in Australia, a small vaccination trial was conducted in Australian chickens (Heath *et al.*, 1992). Chickens infected with the V4 strain of NDV were protected against challenge with the virulent Albiston Gorrie strain of NDV in Victoria in 1930 and 1932. Strain V4 or similar avirulent Australia strains have since been shown to protect against experimental challenge with NDV strains such as Roakin, Texas G.B, Fontana 108 and Hert's 33 (Webster *et al.*, 1976; Turner *et al.*, 1977).

Webster's V4HR ND vaccine has been developed specifically for use in hot climates, so as to reduce dependence on cold chain for vaccine transport and to have improved virus stability when applied mixed with feed for rural poultry (Heath *et al.*, 1992). The efficacy and economic benefits of Webster's oral V4HR ND vaccine for rural poultry have been assessed in South-East Asia by the Australian Centre for

International Agricultural Research (ACIAR) (Johnston, 1990; Johnston and Cumming, 1991).

2.5.4 Safety of V4 Newcastle disease vaccine

The safety performance of V4 (avirulent) ND vaccine is superior to both the B1 (lentogenic) and La Sota (mesogenic) vaccine strains (Webster, 1984).

2.6 HISTORY OF NEWCASTLE DISEASE IN NIGERIA

Newcastle disease had existed since time immemorial and has been recognized from every continent and almost every country in the world with the possible exception of Antarctosa (Alexander, 1990).

The first documented and confirmed outbreak of ND in Nigeria occurred between December 1952 and February, 1953 in and around Ibadan (Hill *et al.,* 1953). Subsequently, the disease appeared to be widespread in domestic and exotic chickens (Fatumbi and Adene, 1979; Ezeokoli *et al.,* 1984, Gomwalk *et al.,* 1985; Abdu *et al.,* 1985, Nwosu and Okeke, 1989). Abdu *et al.* (1985) confirmed that ND was currently Nigeria's single most dreaded disease of the poultry industry.

2.7 HOST RANGE FOR NEWCASTLE DISEASE

It is probable that most avian hosts, domestic and feral, can be infected with strains of NDV. Kaleta and Baldauf (1988) listed more than 250 species of free-living and caged birds that have been infected with NDV. This is in addition to the common species of domestic birds that become infected. The consequences of these infections will vary with strain of virus and the species of host. Chickens are the most important host for NDV. However, rural flocks are not limited to domestic chickens alone but the possible role of other avian species must be taken into consideration.

Newcastle disease virus has been isolated from a number of mammalian species and its replication in these hosts are probably not of epidemiological significance to avian disease (for example, laboratory workers, vaccination crews and abattoir staff) and there are very few occasional reports of an association with more serious ailments (Alders and Spradbrow, 2001). Carrier chickens and partially immune chickens can develop infection with velogenic NDV and display mild or no clinical signs and can shed the virus for up to 5 weeks (Lancaster, 1966; Okeke and Lamorde, 1988).

Wild birds have been found to habour velogenic, mesogenic and lentogenic strains of NDV and hence shed the virus (Hanson, 1976; Alexander, 1988). Like influenza virus, NDV was also isolated from migrating feral water fowl and other aquatic birds. Most of the isolates were of low virulence for chickens (Alexander *et al.*, 1992). A large number of wild birds were considered to be naturally infected with NDV (Lancaster and Alexander, 1975). An epizootic due to a virulent NDV in feral birds was reported in North America (Alexander, 1995).

Virulent NDV isolates were obtained from captive caged birds (Senne *et al*., 1983). Kaleta and Baldauf (1988) suggested that such infections might have originated from holding stations or as a result of the spread of NDV to the holding stations from near by poultry such as a backyard flock. Outbreaks of ND in pet birds were reported in six states in the United States of America (USA) in 1991. Illegal importations were suspected to be responsible for the introduction of the NDV to USA (Panigraphy *et al.*, 1993).

Physical environment plays a role as reservoir of NDV in villages (Lancaster, 1966). Infected birds can shed the virus in faeces where it can survive for three months at temperature of 20-30^{o}C and longer at cooler temperature (Lancaster, 1966).

Ducks are reported to be readily infected with NDV and to be capable of spreading the virus. There are few reports of clinical NDV in ducks (Alexander, 1995). However, there are several accounts indicating a high rate of carriage of Newcastle disease virus by ducks. For example, in a rural situation in Indonesia, Kingston and Dharsana (1979) found that the virus persisted for one year in a flock of only 300 ducks. In Vietnam, duck plague virus (a herpes virus) has been contaminated with NDV (Alexander, 1995). It would be useful to determine whether the two viruses act synergistically in dually infected ducks. These findings were with Pekin ducks. Even less is known of Newcastle disease in the Muscovy ducks that seem to be more common in Africa. At IAEA/FAO meeting in Morocco, it was observed that in Tanzania ND is a greater problem in chickens in villages where ducks are also kept (Alexander, 1995).

Turkeys can also be infected with NDV, but are apparently not very susceptible to the disease. Certain strains of NDV have become adapted to pigeons in some countries and cause disease in both pigeons and chickens. Veterinary authorities in Africa gave very diverse views on ND in guinea fowl (Alexander, 1997). Opinions vary, from these birds being entirely refractory to guinea fowl being highly susceptible and a danger to commercial chickens. From China there has been a report of a strain of NDV adapted to, and caused disease in geese (Luchuan and Hong, 1998).

NDV can infect mammals. Human infection occurs and, at least with virulent strains of the virus, causes severe conjunctivitis. There has been an isolation of NDV from a pig in Indonesia (Alexander, 1997).

2.8 TRANSMISSION OF NEWCASTLE DISEASE VIRUS

Apart from predator birds or the feeding of untreated swill containing poultry meat to poultry, the spread of NDV from bird to bird appears to occur as a result of either inhalation of excreted droplets or the ingestion of infective materials such as faeces (Alexander, 1995). There is always difficulty in explaining the first event in an outbreak, the infection of the first bird in an isolated flock. With most commercial flocks, the entrance of an infected chicken should not occur. Aerosol transmission is a possibility if there is a large concentration of infected chickens in the area. Estimates of airborne spread have been reported to extend up to 40 kilometers (Alders and Spradbrow, 2001). Other factors involved in the spread of ND include contaminated human shoes and clothing, egg crates, cartons, eggs and contaminated food and food containers, drinking water, vaccines, other animals and poultry products (Majiyagbe and Nawathe, 1981; Alders and Spradbrow, 2001). Although, it is clear from administration of live vaccines by aerosol that infection may be established via the respiratory route, there is little experimental evidence that infected birds will pass on the virus to susceptible birds in this way. The success of this route of transmission will depend on many environmental factors such as temperature, humidity and stocking density. However, there is no doubt that when climatic conditions are suitable and poultry farms are sufficiently concentrated as in Northern Ireland in 1973, air-borne spread may play a significant role in the epidemiology of ND (McFerran and McCraken, 1988). It is easily demonstrated that NDV infection can be passed from one bird to another via contaminated faeces. It is most likely that the "asymptomatic enteric" NDV and the pigeon strain of NDV were transmitted primarily in this way (Alexander *et al.*, 1984).

2.8.1 Movement of Live Birds

Migratory feral birds may be responsible for the primary introduction of infection, but nearly all NDV isolates obtained from feral birds have been of low virulence. An exception to the presence of virus of low virulence in migratory birds has been the report of highly virulent NDV in cormorants in North America (Alexander, 1995).

World trade in captive caged birds is enormous and in many countries virulent NDV has been isolated frequently from such birds. For example, 147 virulent NDV isolates were obtained from 2,274 captive birds held in quarantine in the USA between 1974 and 1981 (Senne *et al.*, 1983). Some infected psittacines have been shown to excrete virulent NDV intermittently for extremely long periods, in some cases for more than one year which further emphasizes the potential role these birds may have in the introduction of NDV to a country or area (Erickson *et al.*, 1977).

The potential for racing pigeons to carry and introduce NDV into a country or an area has been highlighted by the panzootic in such birds over the last 15 years (Alexander, 1995). Trade in backyard flocks and other birds kept for recreational purposes, hobby birds, has been implicated in the introduction and spread of NDV in the outbreaks in the European Union (EU) in the period 1991 to 1994 (Alexander, 1995).

2.8.2 Movement of Poultry Products and By-Products

Modern methods of commercial poultry slaughter, marketing of poultry meat and veterinary inspection, have induced the movement of live commercial poultry; poultry products and by-products in many developed countries of the world.

In the past, poultry meat has been incriminated as the main vehicle for the introduction and spread of NDV. For example, in 1974 it was considered that one third of the first 542 outbreaks of ND in England and Wales could be directly attributed to feeding poultry waste to chickens and sampling of a lot of frozen poultry imported into Britain in the same year produced an isolation rate of up to 66 per cent (Reid, 1961). Modern method of poultry carcass preparation and legislation on the feeding of untreated waste to poultry have greatly reduced the risk of spread of NDV from poultry products and by-products but nevertheless the possibility of spread in this way remain (Alexander, 1995).

2.8.3 Contaminated Poultry Feed and Water

In the British Isles outbreaks of ND in commercial poultry have been associated with feed contaminated with infective faeces from feral pigeons infected with NDV; similarly, water contaminated with infective faeces may introduce NDV to a flock (Alexander *et al.*, 1985; O' Relly *et al.*, 1994).

2.8.4 Movement of People and Equipment

Secondary spread during most epizootics of ND in recent times has been a result of movement of personnel or equipment. Man plays an important role in the transfer of infective faeces from one site to another on hair, clothing, foot wear, crates, feed sacks, egg trays or vehicles (Alexander, 1995).

2.8.5 Other Methods

Other methods of spreading the NDV include air-bone spread, spread by incorrectly attenuated or contaminated ND vaccines and spread by non-avian species. The last method is likely to be mechanical transfer of infective faeces, for example by insects, rodents or scavenging animals. In hot countries, reptiles which may enter poultry houses should not be ignored as potential spreaders of NDV as their susceptibility to infection has been reported (Alexander, 1995). There is no evidence of transovarian transmission of NDV from the hen to chicks. Nevertheless, in eggs laid by diseased hens infection and death of embryos may occur particularly during the first 4-5 days of incubation (Bivins *et al.,* 1950 cited by Alexander, 1995).

2.9 INCUBATION PERIOD OF NEWCASTLE DISEASE

The incubation period (IP) of ND after natural exposure has been reported to vary from 2 to 5 days or longer with an average of 5-6 days. The IP, as well as the severity of the disease decreases gradually from hatching to maturity (Hanson, 1978). Outbreak of ND may be as acute and severe as to kill all or nearly all the birds in a flock within 3 or 4 days. At the other extreme the disease may be so mild that symptoms are scarcely noticeable (Hanson, 1978).

After experimental infection the IP of ND period varies from 1 to 25 days with an average of 4 to 5 days (Doyle, 1927). The IP is shorter following aerosol infection (Sinha *et al.,* 1957). This incubation period was also reported to be shorter at warmer temperature and longer at colder environment temperature (Sinha *et al.,* 1957).

2.10 FACTORS CONTRIBUTING TO NEWCASTLE DISEASE OUTBREAKS IN RURAL CHICKENS

2.10.1 The Newcastle disease virus

The NDV strains vary in pathogenicity for rural chickens, from velogenic to apathogenic. It is likely that NDV strains found in rural poultry vary in their ability to establish infections by different routes with the less virulent strains relying more on the enteric route (Alexander, 1988). In addition, a strain of NDV comprises several genetically and phenotypically distinct virus 'clones'. The ‘clones’ that make up a field strain of NDV have varying infectivity, thermostability, shed-ability, replicability and pathogenicity (Hanson, 1988). Their combined presence allows the strain to survive under a variety of conditions and to adapt rapidly to hostile environmental circumstances. There is considerable variation in thermostability of "clones" and strains of NDV (Martin, 1992).

These properties of NDV make it hard to generalize concerning the conditions favouring outbreaks of ND among rural poultry, especially when the situation in rural environments may be complicated by the presence of several strains of NDV. In some villages this is further complicated by the presence of one or more vaccine strains (Martin, 1992).

2.10.2 Bird and Host Factors

(1) *Age*

It was reported that chickens become increasingly resistant to ND with age (Beard and Hanson, 1984). All ages are susceptible to the velogenic form of the ND (Sinha *et al.*, 1957). However, susceptibility to mesogenic strains of NDV decreases with age (Bradly *et al.*, 1946). Velogenic NDV strains will cause disease in healthy non-immune adults, but some birds will survive. Ezeokoli *et al.* (1984) concluded that in backyard management systems, birds around 16-24 weeks of age had the highest risk of NDV infection. Furthermore, studies have suggested that the sex of the birds may influence the morbidity and mortality of NDV (Lalita, 2002).

(2) *Acquired immunity*

Immunity induced by previous exposure to NDV can be protective. Circulating antibody, secretory antibody, cell-mediated immunity and non-specific surface immunity all play a role. Protective immunity is generally accepted as corresponding to a $\log_2$ HI antibody titre of 3 or more although some birds with low or undetectable HI antibody titres following vaccination or infection are nevertheless protected against NDV challenge (Allan and Gough, 1974; Beard and Hanson, 1984; Saglid and Henresnapes, 1987). The duration of active protective immunity varies with the immune status of the bird at the time of exposure to the immune stimulus and the nature of the immune stimulus, which depends on the doses of NDV, the route of infection and the strain of NDV (Ibrahim *et al.*, 1981; Westbury, 1984). The avirulent V4 HR NDV strain acquired by contact with orally vaccinated birds give only transient protective HI antibody levels and at the other end of the spectrum, immune birds surviving artificial challenge with VNDV have maintained a mean log_2 HI antibody titre of 7 for 15 months (Samuel and Spradbrow, 1991). In general, HI antibodies from natural infection declined after 3-4 months and were absent by 8-12 months, while vaccine induced protection lasts 2-12 months, depending on vaccine strain and route of administration (Beard and Hanson, 1984). Antibodies induced by one strain of NDV are protective against all strains of the virus (Martin, 1992).

Rural chickens are a mixed population in terms of immunity. Maternally derived antibody in newly hatched chickens may be present for up to 5 weeks. Chicks become fully capable of mounting an immune response at about the same age (Spradbrow, 1987). An impaired immune response may occur in rural poultry, and possible causes include infectious bursal disease (Okoye and Shoyinka, 1983), chronic aflatoxicosis and vitamin A deficiency (Spradbrow, 1987). The presence of lentogenic NDV in some rural chicken populations may result in constant cycles of infection which periodically boost the immunity of all exposed birds (Samuel and Spradbrow, 1991). This immunity may or may not be sufficient to protect against VND challenge, the degree of protection depending on the stage of the infection cycle. It is possible that mesogenic strains could behave similarly and also possible that both lentogenic and mesogenic infection cycles could be superimposed in a rural population. Evidence for the presence of such non-velogenic infections in rural chickens comes from isolations of virus from healthy rural birds and from HI titres in high proportions of non-vaccinated rural birds, which would normally not be expected to survive VND (Saglid and Henresnapes, 1987; Bell and Mouloudi, 1988; Bell *et al.*, 1990).

Some rural chicken populations, on the other hand, have uniformly low HI titres, implying that the birds sampled have not encountered NDV. This could be due to the rural being free of ND or ND occurring in classical epizootic pattern, with close to 100% case fatality rate. In endemic regions, presumably the latter is the case and only highly virulent strains are present in the region (Martin, 1992).

Periodic vaccination complicates the situation further; especially when live vaccine is used, since vaccine strains, particularly V4HR, spread among the population. Vaccine administered to immune or partially immune birds may not induce protection.There are considerable practical difficulties in ensuring that vaccine is given only to birds that will respond in a rural flock of mixed age and immunity. With periodic vaccinations of all birds or all young birds, it is inevitable that a proportion will not respond and was susceptible to VND infection sometime thereafter (Martin, 1992).

(3) Concurrent Disease

Rural chickens are affected by a wide range of bacterial, viral and parasitic diseases (Abdu *et al.,* 2000), many of which, particularly parasites are constantly present. Nutrition of rural chickens is often poor in birds dependent on scavenging for a limited quantity of available food. Postmortem examinations may conclude that the cause of death was ND, but there will usually be other concurrent infections. These concurrent diseases render rural birds more susceptible to ND (Adene, 1990; Abdu *et al.,* 2000).

(4) Breed Susceptibility

Opinions differ on the relative susceptibility of indigenous breeds and commercial breeds. Cherdchai (1988) stated that in Thailand indigenous rural birds are more resistant to ND than commercial breeds. While Higgins and Shortridge (1988) reported that there was no evidence for differences in susceptibility between local breeds in Hong Kong small holdings and imported breeds. In Nigeria, Ezeokoli *et al.* (1984) and Sa'idu (2006) reported that local chickens are less likely to develop ND than the improved breeds (intensively reared chicken) because the local breed of chickens may be infected by the mild strain of the ND virus when they are scavenging for food and gradually develop immunity thus, reducing their susceptability to severe outbreaks.

2.10.3 Environment

Newcastle disease occurs year round in most rural poultry populations, but is most common and severe at times of climatic stress. Outbreaks are often associated with change of season, particularly the start of the wet season (Ronohardjo *et al.,* 1988; Thitisak *et al.,* 1988). Cold and hot weather has been cited as a contributory factor in ND outbreaks (Dao and Pham, 1985; Bell *et al.*, 1990).

A high rural poultry density results in greater opportunities for a contaminated environment and for spread of NDV relative to a more dispersed population (Martin, 1992).

2.11 PATHOGENICITY OF NEWCASTLE DISEASE VIRUS

Pathogenicity of ND varies greatly with the host, specie, immune status of the bird, type of ND virus and age of the bird . For example, ducks and geese may be infected but show few or no clinical signs even when the strain of the NDV is lethal for chickens (Higgins, 1988).

In chickens the pathogenicity of NDV is affected by the strain of the virus, age of the chickens, route of administration and environmental conditions. Chicks may experience sudden death without major clinical signs while in older birds the disease may be more protracted and with characteristic clinical signs (Alexander, 1995). The pathogenicity of NDV infection in chickens is not affected by genetic make up (Cole and Hutt, 1961).

The routes of infections (nasal, ocular and oral) appear to emphasize the respiratory nature of the disease. The intramuscular, intravenous and intracerebral routes appear to enhance the neurologic signs (Beard and Hanson, 1984).

2.12 CLINICAL SIGNS

Clinical signs and the speed at which they appear vary widely and depend upon infectivity and dose of the virus, the species, age and immune status of the host, infections with other organisms, environmental conditions and the route of exposure (Alexander, 1997). Infection with NDV displays a complete continuum from very rapid fatal disease to inapparent infection. Newcastle disease virus was grouped into five "pathotypes" by Beard and Hanson (1984) on the basis of predominant signs in affected chickens.

2.13 LABORATORY DIAGNOSIS OF NEWCASTLE DISEASE

Diagnosis can be made by direct or indirect methods. Indirect methods include among others haemagglutination inhibition test (HI), enzyme linked immunosorbent assays (ELISA), virus (plaque) neutralization test (VN), complement fixation test (CFT) (OIE, 1996).

Direct methods include virus isolation, electron microscopy and virus characterization. Samples from dead birds should consist of oro-nasal swabs, as well as sample collected from lungs, air sacs, intestine (including contents), spleen, brain, liver and heart tissues collected separately or as a pool and placed in phosphate buffer Fisotonic saline (pH 7.0-7.4 containing antibiotics). The importance and impact of NDV is directly related to the virulence of that isolate. It is however necessary to carry out laboratory assessment in order to determine the pathogenicity (Alexander, 2000).

Routine diagnosis of ND usually requires two distinct steps. First it is necessary to establish that the virus isolated is NDV and not any other avian paramyxovirus or influenza virus. The second is whether or not the virus isolated is vaccinal or enzootic, since a variety of live vaccines are used in the face of an outbreak, which is likely to interfere in the virus typing, experiments (Alexander, 1990).

2.13.1 Serology

The presence of specific antibodies to NDV in the serum of bird gives little information on the infecting strain of NDV and therefore has limited diagnostic value. Nevertheless, in certain circumstances the demonstration that infection has taken place is sufficient for the needs of a diagnostician. Post vaccinal serology can be used to confirm successful application of vaccine and an adequate immune response by the bird (Alexander, 1995).

A wide range of tests may be used to detect antibodies to NDV in poultry sera and tests based on neutralization or enzyme linked immunosorbent assay (ELISA) reactions have been used in support of the diagnosis of Newcastle disease (Jordan, 1990). However, the standard test for poultry is the haemagglutination inhibition (HI) test, which has been described in detail in the Office Internationale des Epiozootics manual (OIE, 1996) and for European Union countries in Directive 92/66/EEC (CEC, 1992 cited by Alexander, 2000). In these documents, it was pointed out that sera from different species may cause agglutination of chicken red blood cells. To eliminate this, it is necessary to either use red blood cells from the same species as the serum sample. The value of any serological method in the diagnosis of ND is clearly dependent on the expected immune status of the birds involved and is therefore complicated in the case of ND by the world wide use of vaccines. For most avian sera a positive HI antibody titre may be regarded as 1/8 (titre is more usually expressed as the reciprocal of the end point dilution in several forms and notations) for example, 2^3 or 3.0 Log_2. In unvaccinated birds, positive serology and clinical signs may be considered as strong diagnostic evidence of the disease (Jordan, 1990).

In some cases, the serology of vaccinated flocks may strongly indicate an amnestic response resulting from challenge but such interpretation of elevated titre needs great care and should not be regarded as unequivocal evidence of infection by NDV (Jordan, 1990). Haemagglutination inhibition and other tests may be used to measure the immune status of vaccinated birds. In vaccinated birds the mean HI titre levels range from log_2 4.0 to Log_2 6.0 after a single live vaccine to log_2 9.0 to Log_2 11.0 with multidose programmes including oil emulsion inactivated vaccines (Jordan, 1990).

Some authors have attempted to correlate HI antibody titre to NDV with protection against a fall in the egg production or disease when stocks were challenged with a virulent NDV (Lancaster, 1966; Alexander, 1990). These estimates have been based on laboratory experiments and at best are an over simplification as it is impossible to allow for the multifarious exacerbating conditions that may be encountered in the field (Jordan, 1990). Other tests that may be used in detecting antibodies to NDV in poultry sera includes ELISA, single radial immunodiffusion, single radial haemolysis, agar gel precipitation and virus neutralization (VN) in chick embryos (Beard, 1980; (Chu *et al.,*1982; Hari, 1986; Gelb and Cianci, 1987; Cadman *et al.,* 1997).

2.14 RURAL POULTRY PRODUCTION

2.14.1 Management Systems

Poultry is an ideal livestock for small farms because of the little individual requirement for feed, water and other production inputs. There are three distinguishable systems for managing rural poultry. They are the two extensive systems, free-range and backyard and the small-scale intensive system. In the free-range system there is little intervention in the life cycle of the birds. The major intervention is in the areas of feed and water supplementation, overnight housing, and to a much lesser degree, health management. Family flocks are usually integrated with crops, fishes and other livestock species such as chicken/cattle, chicken/guinea fowl, chicken/duck, chicken/turkey, duck/rice/fish and duck/pig (Adene, 1990; Sonaiya, 1990).

Under the extensive systems, production cycles are continuous with poultry, unsorted by sex; at different stages present in the flock at any given time. Foundation stock is usually obtained from the market or as gifts. Flock composition is heavily skewed towards chickens in Africa and towards ducks in Asia and Latin America. Household flock size ranges from 3 to 97 in Africa, 10-31 in South America and from 50 to 2000 in Asia (Sonaiya, 1990a).

The system of management appears to have an influence on the breeds used. The free range systems uses almost exclusively local breeds as it has been found, from the fate of exotic cocks in the numerous cock exchange programmes that exotic birds do not survive under this system (Sonaiya, 1990; Gueye, 1998b).

2.14.2 Diseases and Predators

Newcastle disease (ND) is the most important disease of rural poultry recognized in virtually every country (Spradbrow, 1990). Mortality due to ND is however, variable and in most countries, the disease is seasonal (Chabeuf, 1990; Abdu *et al.*, 1992; Alders and Spradbrow, 2001). In Nigeria, the disease is more common in the dry harmmatan period and the cold harmmatan wind is known to worsen the outcome of the disease (Abdu *et al.*, 1992).

While the importance of ND is recognized, conventional vaccination techniques are expensive to use and do not provide adequate cover and protection for rural birds which have been identified as reservoir source of infection for the commercial flocks (Bell and Mouloudi, 1988). Pilot and field studies on the use of orally fed ND vaccines have been carried out in the Gambia, Ethiopia, Mozambique, Tanzania and Zimbabwe, results in Africa are not really encouraging (Alders and Spradbrow, 2001). Aerial (hawks, kites) and terrestrial (rats, snakes, dogs, cats, foxes and racoons) predators account for far more mortality than is usually recognized. Prevention by means of proper housing, shelter and bush clearing is the best policy. Hunting, poisoning, trapping and the use of natural enemies of the predators are control methods available. Moreover, traditional remedies are widely used to control poultry diseases (Gueye, 1998b).

2.14.3 Feeds and Feeding

On the range and in the backyard, a bird can certainly not find all the nutrients it needs for optimal production all the year round. During the dry season, poultry can quickly develop vitamin deficiency because of the scarcity of succulent vegetables on the range (Sonaiya *et al.*, 1999). There is thus a need to supplement their scavenging with sources of minerals and vitamins. Most of the materials available for scavenging are not concentrated enough in terms of energy because they contain a lot of crude fibre. That is why grains are given to poultry in the traditional rural system. It has been estimated that 35 g of grain supplement per hen per day is given to local chickens in the free range system in south-western Nigeria (Sonaiya *et al.*, 1999). Insects and their larvae are identified as protein sources for scavenging poultry. Atteh and Ologbenla (1993) cited by Sonaiya *et al.* (1999), reported that maggots could make up to 3% of the diets of chickens without compromising performance.

In the semi-intensive system, producing a well balanced feed is uncommon if not impossible for the smallholder. The feed situation for birds in this system is therefore usually poorer than for birds in the extensive or fully intensive systems (Bessei, 1990a).

Energy supplements such as maize, sorghum and millet are offered early in the morning and late in the evening. Birds scavenge during the day mostly for protein (insects, worms, larvae, etc.), minerals (stones, grits, shells), and vitamins (leafy greens, pepper, oil palm nuts) in-between these meals. There is evidence to show that such a cafeteria system is not inferior to the offer of complete feeds (Bessei, 1990a).

2.14.4 Utilization and Sale

Poultry products have social and spiritual benefits and play an important role in rural economy. In many customs of indigenous people, poultry is used for ceremonies, sacrifices, gifts and savings. In the Sahelian region of West Africa, guinea fowls are used as gifts to visitors more than chickens. In cases when no poultry is available (e.g. after a ND outbreak), in order to meet customary family obligations, the family will buy or borrow a bird. In the rural, chickens are given or received to show or to accept good relationship, or to say thank you for a favor or help. Poultry, particularly in the free range, provide meat, eggs, feathers, manure (convertible to fertilizer and natural gas), pest control, weed clearance, seed cleaning of grasses for mulch, scratching and foraging (Sonaiya *et al.*, 1999).

Under normal conditions, birds are sold when the household is in need of money. The income from the sale of chickens is additional revenue to earnings from cash crops from the field. The sale of birds and eggs takes place in the rural market. Prices fluctuate during the year, being low during the dry season when the granaries are empty and the crops are still growing and everybody needs ready cash. At such times, traders come to buy to resell in big cities. Sometimes, middlemen are involved. They buy the birds in the villages and sell them at the market or to traders. Poultry products which are sold contribute about 15% to the annual financial income of the household (Alders and Spradbrow, 2001).

Generally, indigenous birds are preferred over exotic varieties because they are readily available, easier to look after, tastier and more nutritious and better brooding hens. Other features include: low egg production but birds are hardier in nature, more in demand and birds fetch higher prices, have better adjustability to local environmental conditions and the rural people are more familiar with rearing Indigenous birds (Sonaiya *et al.*, 1999).

2.14.5 Breeds

There are many types, breeds and strains of indigenous poultry which are well adapted to their environment. There is need for their genetic improvement in order to;

improve their productivity within their local environment; make use of the improved indigenous birds in crossing with imported exotic birds; and conserve the desirable genes (e.g. for disease resistance) of the indigenous breed for future breeding (Sonaiya *et al.*,1999).

2.14.6 Productivity of rural poultry

The great differences in productivity between extensive and intensive poultry production are due largely to differences in how the birds are managed. In industrial poultry, housing and management and even the breeds and strains used are fairly stereotyped whereas under extensive systems these vary enormously.

Productivity increases in direct proportion to the level of confinement and hence management. Under the free-range and backyard systems, egg production by chickens is 20-100 per year. Under these systems, ducks produce 30-80 eggs, turkeys 60 - 80 eggs, and guinea fowls 100-120 eggs per year. In Mali, hatchability was reported to be uniformly high in guinea fowl, chickens are used for hatching guinea fowl eggs and brooding keets. Body weight of about 1.2 kg (normal size) and 800 g (dwarf) is achieved in chickens in about 32 weeks (Gueye, 1998b).

In general, the limitations on production in rural flocks include:

1. Newcastle disease periodically decimates flocks, emphasizing to the owner that all his birds may die at any time. The result is that the farmer does not invest any of his scarce resources in his chicken flock. In addition, the owner allows each hen to incubate her eggs to replace the birds that have died.
2. The feed supply is variable, depending on the season and rainfall. In most situations, energy is probably the first limiting nutrient, but protein may be more critical in others. Over population can play a crucial role as well (Huchzermeyer, 1993).

3. The environment for the newly hatched chickens is very hostile as evidenced by the huge mortalities over the first six weeks of life. Usually, the hen hatches her chickens close to or in the owner's house. The ground is usually bare and checked out for anything edible several times a day by older birds, plus dogs and cats. Very young chickens cannot walk to more fertile areas, e.g. vegetable gardens, paddy fields, where suitable food is more readily available. Thus, young chickens up to six weeks of age-literally starve to death, as well as being affected by predators and common disease organisms (Gueye, 1998b).

2.14.7 Disease control

Rural poultry suffer losses from predators and from disease caused by viruses, bacteria and parasites. The losses attributable to morbidity are not known but it has been estimated that more than 750 million chicks, guinea keets and ducklings in Africa die each year as a result of various infections. In addition, predators particularly hawks, snakes, dogs, cats and rats kill or wound an approximate 75 million poultry every year. Surviving birds show various signs of sickness depending on the type of infection. Rural poultry has been identified as reservoir host for pathogenic organisms causing ND in industrial chickens which is more financially disastrous because of the high capital investment required in that sector (Sonaiya, 1990).

Any of the usual commercial ND vaccines will protect rural chickens against ND, if the vaccines reach the chickens in a potent form. There are however, special problems. Many commercial vaccines are thermolabile. Cold chains are impossibly expensive to develop and maintain. Rural flocks are small, scattered and multi-aged. The minimal unit vial for a commercial vaccine ranges from 800 to 1000 doses, which is expensive and inconvenient for very small flocks (Alders and Spradbrow, 2001).

The Australian Centre for International Agricultural Research (ACIAR) has supported projects on the vaccination of rural chickens since 1984. The original concept was the production of a thermostable strain of ND virus, developed by artificial selection. Hofstad and Yoder (1963) had suggested that robust ND vaccines might be produced by seeking strains of virus that possessed superior thermostability. Heat resistant strains of ND virus had actually been produced in the 1950s for use as phenotypic markets in genetic experiments (Alexander, 1995). The thermostable vaccine that has been most used is a variant of the avirulent Australian V4 strain of ND virus, described by Simmons (1967). This virus had an inherent degree of thermostability and it responded to selection for enhanced heat resistance (Kim and Spradbrow, 1978). A detailed data on the selection of heat resistant variants of strain NDV-V4 has been published and heat-resistant variants of NDV-V4 are now contained in commercial vaccines (Ideris, 1990).

The focus on ND control turned to local production of thermostable vaccine and the supply of seed material free of commercial ownership. Australian Centre for International Agricultural Research sponsored the development of a second thermostable vaccine; strain NDV-I_2, from an avirulent Australian isolate similar to NDV-V4. The selection was made after studying a collection of 42 Australian isolates. Newcastle disease vaccine strain I_2, already a thermostable virus, was selected for enhanced thermostability. Successful laboratory and field trials led to strain NVDI_2 being adopted as the vaccine strain for use in rural chickens in Vietnam (Tu *et al.*, 1998).

2.14.7.1 Liquid Vaccine

Newcastle disease virus vaccines are less heat stable in liquid form than in freeze-dried form. However, under rural conditions, ND vaccines must often be stored and transported in liquid form. Various additives are available to protect virus infectivity during freeze drying and subsequent storage (Spradbrow, 1992b).

The Malaysian thermostable variant of NDV4 survived exposure at 56°C for at least 9 h; the parent stock was entirely destroyed after exposure for 3 h (Ideris, 1990). It was also noted that for long term protection (3 weeks) at 20-25°C, polyvinyl-pyrollidone (PVP) was more effective than skim milk, gelatin, methy1cellulose or carboxymethy1cellulose. Claxton and Leonard (1987) reported that NDV4 virus lost less than Log_{10} unit after storage for 1 hour at 50°C and less than 3 Log_{10} units after 6 hours.

2.14.7.2 Freeze Dried Vaccine

The thermostability of heat resistant NDV-V4 vaccine, obtained by selection in a liquid medium, is also evident in the freeze-dried vaccine. The commercial vaccine was stable in freeze dried form for three months at 11-22°C, and lost only about 1 Log_{10} on storage for a further three months (Heath *et al.,* 1992).

2.14.7.3 Vaccine on Food

Not all foodstuffs are virus friendly (Spradbrow, 1992b). Problems are probably associated with substances that inactivate viruses and with binding to food lectins. The role of heat inactivation of ND virus on food can be estimated only with the most innocuous of food carriers. Cooked white rice is possibly the best for this purpose (Samuel and Spradbrow, 1991). The residue from the production of maize porridge has been used as a carrier for NDV-V4 vaccine in Nigeria. This vaccine remained viable for over 3 months on storage at 20°C (Olabode *et al.,* 1996).

2.14.8.0 Methods of vaccination

2.14.8.1 *Eye Drop*

The most effective way to administer thermostable ND vaccines is by eye drop- provided the chickens can be caught. Unless the chickens are provided with some form of night housing this task becomes difficult. There will usually be an antibody response to a single application of vaccine and the vaccinator can be relatively certain that each chicken has received vaccine. Vaccine can then be applied by trained rural vaccinators or by the owners of the chickens. However, training is essential (Spradbrow, 1992b).

2.14.8.2 *Nose Drop and Mouth Drop*

The chicken eye is a small and mobile target for a vaccinator. It is simpler to apply a drop of vaccine to a nostril and to see it inhaled. This technique may be less effective than eye drop application because the lymphoid tissue of the Harderian gland is not exposed to the vaccine. Application of thermostable vaccine by mouth drop has also proved effective in Indonesia and Malawi. It is suggested that vaccine could be diluted and placed in the mouth in larger volumes (1.0 ml) to reduce errors by unskilled vaccinator (Saglid and Spalatin, 1982; Darminto and Daniels, 1992).

2.14.8.3 *Drinking Water*

Drinking water is not an option for vaccination in all areas. In the wet tropics chickens find their own sources of water. Where surface water is unavailable or where chickens are confined, application of thermostable vaccines in drinking water is possible. Vaccination through the drinking water usually gives good results, sometimes similar to those obtained with eye drop route. Examples of drinking water approach can be found in reports of Tantaswasdi *et al. (*1992), Foster *et al. (*1996) and Tu *et al.* (1998),

2.14.8.4 *Food Carriers*

Most of the investigations of food carriers for thermostable ND vaccine have been undertaken in Asia and Australia. There have been fewer studies on the foodstuffs, and especially the grains, that are available in Africa. In Ethiopia, parboiled barley was shown to be a suitable carrier for NDV-I_2 vaccine. Untreated barley, and parboiled or untreated sorghum were not effective (Nasser *et al.,* 1998). In laboratory trials in Ghana, wheat bran was an effective carrier for NDV-V4 vaccine, while millet, maize meal, corn chaff and milk waste were not (Amakye-Anim *et al.,* 2000). Vaccine supplied on boiled sorghum to rural chickens in central Tanzania gave only low levels of protection, although this substrate had proved successful in earlier laboratory trials (Foster *et al.,* 1996). In further trials with NDV-V4 vaccine in southern Tanzania, dried cassava granules were found to be an adequate food carrier (Salum *et al.,* 1997).

In Asia, suitable food carriers have been paddy (unhusked) rice, cooked white rice and cooked parboiled rice. Uncooked white rice is not a suitable food carrier. Sufficient food vaccine must be supplied so that each chicken in a feeding group has a chance of receiving the vaccine. For most grains this is 7-10 g per chicken. For cooked white rice some 20 g is required for each chicken (Spradbrow, 2005).

2.14.8.5 *Sprays and Aerosols*

Conventional ND vaccines have sometimes been applied to confined rural chickens as sprays. This technique has not been tested with thermostable vaccines in villages. However, under experimental conditions NDV-V4 vaccine has been effective when administered via aerosol (Schalkoort and Spradbrow, 1980).

2.14.8.6 *Lateral Spread*

The thermostable variants of NDV strains NDV-V4 and $NDVI_2$ spread readily between groups of chickens confined on solid floors. Spread is uncertain or does not occur, when chickens are maintained on wire. This indicates the importance of oral/faecal spread with these viruses. Similar spread from vaccinated chickens to rural chickens has been demonstrated under rural conditions. Probably the conditions of night housing or of congregation around food and watering points will influence the efficacy of spread (Tu *et al.,* 1998).

2.15 EXTENSION SERVICES

The production of thermostable ND vaccines and their testing under laboratory conditions were relatively easy tasks. Pilot rural trials were more difficult and either farmers or rural vaccinators will have to be trained to administer the vaccine. Systems for cost recovery must be developed. However, the major task was with the extension workers who must explain the concept of vaccinating chickens and have the concept accepted (Alders and Spradbrow, 2001).

CHAPTER THREE

3.0 RURAL CHICKEN PRODUCTION SYSTEMS IN PLATEAU STATE

3.1 ABSTRACT

Studies on management systems in rural chickens in Plateau State were conducted in 32 villages of four LGAs, involving 1,240 households. The objectives of the study were to determine the rural chickens production constraints, Identify various causes of losses in rural chickens and to determine the remedies adopted by farmers for management of ND in their flocks. Demographic data of the respondents and information on source of stock, common causes of losses and management systems were collected using interviews and structured questionnaires. The biodata of the respondents showed that 82% were farmers and 8 % house wives who are also farmers. Domestic chicken was the most popular specie of poultry reared. While goats were the most common small ruminants raised. The study also revealed that the main source of foundation stock was open live bird market (62.5%) which has serious implication for spreading diseases, while gifts accounted for (17.2%). Women were found to be responsible for most of the daily routines such as cleaning the chicken house (44.1%) and letting the birds out in the mornings (43.2%). Majority (75.3%) of the farmers provided housing for their chickens and the most popular source of water was the well (68.3%). About (39.42%) of the farmers provided supplementary feed to chickens twice a day usually in the morning and evening. Diseases were the main (83.2%) causes of losses of rural chickens followed by preying (8.85%). The study further showed that the rural poultry farmers used traditional ethnoveterinay medicine for treating sick poultry (50.2%) and some (29.4%) seek conventional veterinary services. It was concluded that the productivity of rural chickens is likely to improve

through adequate measures to control diseases and improvement of management practices in form of better feeding, housing and extension services.

3.2 INTRODUCTION

Estimate of the population of poultry in Nigeria from various sources showed that rural poultry and commercial poultry make up 84% and 16% of the total poultry population, respectively (FDLPCS, 2003; Adene and Oguntade, 2006; Sonaiya, 2007). In Nigeria rural poultry production is an important activity (Nwanta, 2003). In rural communities almost every household including Fulani pastoralists keep chickens purposely for meat, egg production, income, barter, special festivals, social obligations such as traditional ceremonies as well as traditional treatment of illnesses (Dafwang, 1989; 1990; Dipeolu *et al.*, 1996; Nwanta *et al.*, 2006).

In Northern Nigeria, the rural chickens constitute the majority of the chicken population and are mainly kept under extensive management system where they roam freely and scavenge for food (Adu *et al.*, 1986; Olabode *et al.*, 1992; Musa, 2002). Their movement is uncontrolled and they rarely receive any prophylactic treatment or vaccination. Constraints to the production of rural chickens include a range of bacterial and viral diseases, internal and external parasites, poor nutrition and predation (Fatumbi and Adene, 1979; Mahaka, 1989; Otchere *et al.*, 1989; Permin and Hansen, 1998; Sonaiya *et al.*, 1999; Abdu *et al.*, 2000).

A clinical and serological study of ND in Nigeria has confirmed the susceptibility of rural chickens (Hill *et al.*, 1953; Abdu *et al.*, 1985; Nwanta *et al.*, 2006), pigeons (Ezeifeka *et al.*, 1992), guinea fowls, ducks and turkeys (Majiyagbe and Nawathe, 1981; Gomwalk *et al.*, 1985; Echeonwu *et al.*, 1993). In Nigeria, studies have shown that ND is the most endemic in both rural and commercial poultry with occasional epidemic outbreaks being recorded in highly susceptible flocks (Adu *et al.*, 1986; Orajaka *et al.*, 1999; Sa'idu *et al.*, 2006). About 200-250 outbreaks of ND occur annually among chicks and adult chickens (Okeke and Lamorde, 1988). Rural chicken farmers are not happy of these losses caused by ND virus (Nwanta, 2003). In villages, circulating strains of ND virus are commonly found causing 100% mortality in unprotected flocks and such outbreaks are unpredictable, hence discouraging ruralrs from paying proper attention to the husbandry and welfare of their flocks (Alders and Spradbrow, 2001).

It was suggested that disease control in rural poultry sector could be the best way to improve the economic status of the communities engaged in poultry farming and guarantee the supply of animal protein to the populace (Ikeme, 1990; Nwanta *et al*., 2008).

The objectives of the study were to:

- Determine the rural chickens production constraints.
- Identify various causes of losses in rural chickens in four LGAs of Plateau State.
- Determine the remedies adopted by farmers for management of ND in their flocks.

3.3 MATERIALS AND METHODS

3.3.1 Study Area

Plateau state is made up of 17 LGAs (Appendix 1) and is located in the Central zone of Nigeria on latitude 9 ^{o} 56 N and longitude 8^{o} 53 E. Covering an area of 26,899 square kilometers, the state derives its name from its unique geographical relief of undulating tabloid highland with an average height of about 1, 200 meters above sea level rising from the Benue valley to the peaks of Shere hills. The estimated population figure of the state stands at 2.56 million people. Plateau State was chosen because it has the enviable position of having two agro-ecological zones: a cool humid sub-temperate climate in its northern region and a hotter sub-humid region in the South that is part of the Guinea Savanna Ecological Zone that spans across much of central Nigeria, and central Africa. The northern part of Plateau State has been classified as the Humid Jos Plateau Ecological zone (Ojanuga, 2006). It has an average annual rainfall of about 1,400 mm and an average daily temperature of about 22 ^{o}C in contrast to the Southern part which has a mean rainfall of about 1100mm and average daily temperature of 30 ^{o}C. The ecological zonal classification is sub-humid Central Niger Benue trough. About 90% of the rain falls in six months between April and September and the rain usually comes with thunder storms of high intensity particularly at the beginning and towards the end of the season. As a result of the high elevation of the plateau, the average annual temperatures are lower than that in the surrounding plains with March and April as the hottest while December and January are the coolest. Mean maximum temperature fluctuates around 26.7 ^{o}C while mean minimum temperature is around 16.7 ^{o}C. The low relative humidity in Jos (Plateau State capital), which is less than 25% between November and March, is one of the most comfortable climatic in Nigeria (Anon, 2006).

3.3.2 Sampling

A multi stage sampling procedure was adopted to select four LGAs and eight villages per local government area for the study. The four LGAs were purposively selected to represent the Northern Plateau ecology and the Southern guinea savannah ecology of Plateau State. The LGAs were Jos south and Barkin Ladi in the North and Kanam and Shendam in the South. Random sampling procedure was used to select the eight villages from each LGA as listed in Table 3.1. The study was conducted from January 2006 to June 2007. Thirty two villages with 1,240 households were selected and visited.

Table 3.1: Thirty two villages in the four Local Government Areas of Plateau State sampled.

	Local Government Area			
	Jos South	Barkin Ladi	Kanam	Shendam
	Cooler climate		Warmer	
Villages	Kuru	Roop	Jarmai	Nyuun
	Rahol	Heipang	Bagyar kwalkal	Gidan Adamu
	Rabin	Wereh	Kyamsangi	Kawo TV
	Hwak	Rabwak	Sabongida	Kwasan
	Vwang	Rashalang	Gar	Kopgalwa
	Hwolshe	Bwom	Tatung	Longuel
	Gyel	Rakung	Gumshar	Kurum
	Vom	Larwing	Dengi	Laraba

3.3.3 Data Collection

A total of 1,240 structured questionnaires (Appendix II) were administered to rural poultry farmers. Based on the sample size per cluster, 1,240 farmers were randomly selected and given the questionnaires and where necessary interviewed.

The flock structure was defined as chicks (0- 10 weeks old), cockrells (11-20 weeks old), pullets (11-20 weeks old), adult cocks (≥ 21 weeks old) and adult hens (≥ 21 weeks old). Season of ND outbreaks was categorized as follows: November to March (Dry season), April to May (Pre rainy season), June to August (Rainy season) and September to October (Pre dry season).

Strains were identified by phenotypic features such as the most dominant plumage color and well known genes such as Naked neck, frizzled feathers, long leggedness and dwarfness which all have phenotypic expressions (Kitalyi, 1998).

Plateau state has about 20 different ethnic groups which include Ankwai, Berom, Jarawa, Pyem, Ron and others. In some of these LGAs there are about 2 to 4 ethnic groups. For this reason interpreters were sought where necessary and interviews were conducted in the mornings or evenings when the farmers are in their homes and at their convenience. Aspects captured in the questionnaire included household demography and flock dynamics, management systems, health and productivity and season of ND outbreak.

3.3.4 Data Analysis

Data obtained through questionnaire on chicken population, health, causes of losses, management system, productivity and ethnoveterinary practices adopted was coded and analyzed using the Statistical Package for Social Sciences (SPSS) Programme (version 13) to determine the frequency of response to each of the questions. The number recorded for each question was then converted into percentages and tabulated against the LGAs sampled. Chi-square was used to assess the impact of ND as a major cause of loss in rural chicken in comparison with other agents.

3.4 RESULTS

The results of the interview and questionnaire administered revealed that out of 1,240 questionnaires printed, only 980 were actually distributed to the households of which 865 (88.3%) were correctly filled and returned.

The respondents were mainly farmers (81.75%), civil servants (8.18%) and house wives who are also farmers (8.00%) and a few traders (2.25%). Males constitute the majority (65.30%), while 34.70% were women (Table 3.2).

The main source of foundation stock was open live bird markets (70 %), while gift as a source accounts for 17.70 % and 12.30% of the respondents inherited their stock (Table 3.3). The number of chickens by category of birds raised showed that 31.40% of the birds kept were hens, 29.50% were chicks, 20.50% were growers and 18.66% cocks (Table 3.4).

Each household was found to be rearing two or more strains as shown in Plate 1. The distribution of chickens by strain is shown in Table 3.5. Chickens with white, black or mottled plumage colour constituted 57% of the total population. The long legged and the barred plumage colours constituted about 10% each while the naked neck, dwarf and frizzled feathered constituted 8.3, 6.4 and 5.2% respectively. Although there was a higher percentage of naked necks in the hotter ecological zone (9.5 versus 6.6%), their overall presence which constitutes only 8.3% of the total population indicates that they are not a dominant strain in any of the zones. (Table 3.5).

Table 3.6 shows the mean number of livestock in households raised with rural poultry. The mean number per household was 5.6 goats, 1.2 sheep, 1.0, pigs 0.3 cattle and 0.1 rabbits. The mean number per households of other poultry was ducks 6.0, turkeys 0.3, 1.0 pigeon and 1.2 guinea fowls (Table 3.7).

Table 3.8 shows estimates of some reproductive traits for various species of poultry. The ducks had the highest number of eggs per clutch and hatched the highest number of ducklings per hatch and number of chicks weaned. Turkeys had lowest number of poults weaned.

Table 3.2: Gender and occupation of rural poultry farmers in four local government areas of Plateau State.

Factor	Frequency	Percentage (%)
Gender		
Male	565	65.30
Female	300	34.70
Occupation		
Crop farming	687	81.75
Civil servant	69	8.00
House wives	68	8.00
Business	19	2.25

Table 3.3: Main sources of foundation stock for rural chicken farmers in the four LGAs of Plateau State.

Source of birds		LGA				Total
		Jos South	Barkin Ladi	Kanam	Shendam	
Live bird market	Frequency	126	177	162	136	601
	% of total	14.7	20.6	18.85	15.8	70.00
Gift	Frequency	53	41	30	28	152
	% of total	6.17	4.77	3.49	3.26	17.70
Inheritance	Frequency	6	19	39	42	106
	% of total	0.70	2.21	4.54	4.89	12.30
Total	Frequency	185	237	231	206	859
	% of total	21.54	27.59	26.89	23.98	100

X^2 = 89.2, df = 6, significance level = $P < 0.05$

Table 3.4: Number of rural chickens by sex and age raised in four Local Government Areas of Plateau State.

Local government area	Number of chickens				Total
	Cocks	Hens	Growers	Chicks	
Jos South	605	2116	1178	1780	5,679
Barkin Ladi	886	1594	1075	1531	5,086
Kanam	2130	2899	1624	2838	9,490
Shendam	1432	1890	1662	1838	6,823
Total	5053	8498	5539	7987	27,078
Mean	1263.3	2124.5	1384.8	1996.8	6769.5
(%)	(18.66)	(31.40)	(20.50)	(29.50)	(100)

Table 3.5: Strains of Chicken Reared by Rural Poultry Farmers in Plateau State.

	Agro - Ecological zone					
	Humid Jos Plateau (Jos South and Barkin Ladi)		Sub-Humid Central Niger Benue trough (Kanam and Shendam)			
Strain	F	%	F	%	Total	%
Dwarf	83	8.3	65	5.0	148	6.4
Naked Neck	66	6.6	124	9.5	190	8.3
Frizzled	58	5.8	61	4.7	119	5.2
White	176	17.6	266	20.4	442	19.2
Black	196	19.6	280	21.5	476	20.7
Mottled	241	24.1	146	11.2	387	16.8
Barred	104	10.4	125	9.6	229	9.9
Long legged	51	5.1	224	17.2	275	11.9
Others	26	2.6	10	0.8	36	1.6
Total	1001	100.0	1301	100.0	2302	100.0

Table 3.6: Number of livestock in a rural households living with chickens in four Local Government Areas of Plateau State.

Poultry specie	Mean per Local Government Area	Mean per household
Goats	1341	5.6
Sheep	294	1.2
Pigs	242	1.0
Cattle	69	0.3
Rabbits	35	0.1

Table 3.7: Number of livestock and poultry raised in the 32 villages of four Local Government Areas of Plateau State.

Animal Specie	Mean per local government	Mean per household
Chickens	1593.4 ±728	20.3 ± 7.8
Ducks	1445 ± 231	6.0 ± 3.1
Turkey	61.8 ± 38.7	0.3 ± 0.0
Pigeon	208.5 ± 76.4	1.0 ± 0.2
G/fowls	277 ± 14.2	1.2 ± 1.0

Table 3.8: Estimates of reproductive traits of rural poultry as reported by respondents in Plateau State Nigeria.

Reproductive traits	Chickens	Guinea fowls	Ducks	Turkeys
No eggs/clutch	11.80 ±4.10	10.20±3.20	12.10 ±6.40	0.53 ±5.70
No of eggs /hatch	9.0 ± 2.40	7.0 ±2.06	10.50 ± 2.50	12.0 ± 3.30
No chicks/ hatched	8.0 ± 4.12	6.10± 2.70	8.30 ± 2.20	5.32± 1.90
No of chicks surviving	7.26± 2.13	4.29 ±1.06	7.91± 3.35	3.27± 1.29

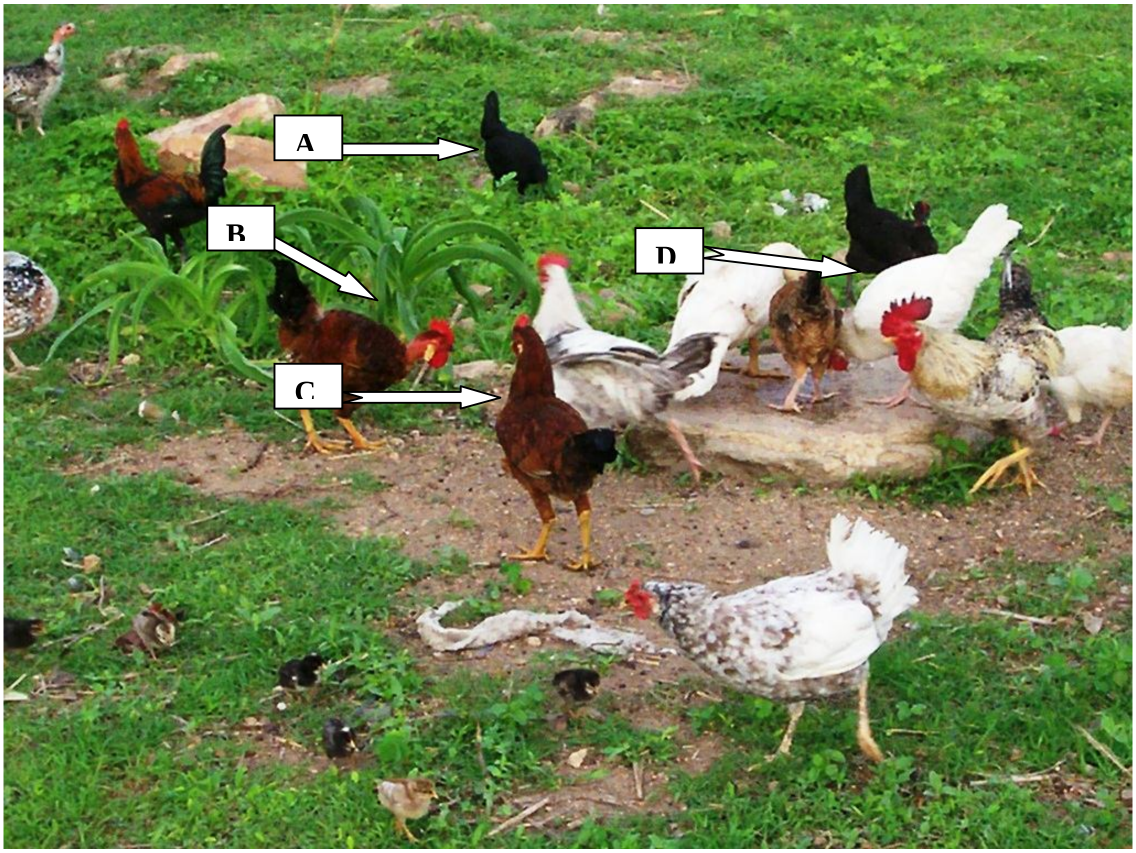

Plate I: Some major strains of rural chickens in Kanam Local Government Area.

A = Black, B = Naked neck, C = Barred, D = white

Plate II: Naked neck (arrowed) chicken in one of the households sampled.

Majority of the respondents (75.3%) provided housing for birds (Table 3.9). About 70.10% of the chickens roost in a mud house, and 14.3% roost under buildings. For farmers that do not provide housing for birds 13% of birds roost on the top of trees while 2.50% sleep anywhere around the homestead (Table 3.10).

There is significance P $P < 0.05$ difference in the source of drinking water for rural poultry with well being the most popular (68.3%) followed by river/stream (26.1%). These two main sources are actually the main sources of drinking water for rural poultry farmers in the survey area. Borehole accounted for 3.8%, tap (1.43%), pond (0.12%) and rain water (0.24%) (Table 3.11).

About 85.7% of the households always provided supplementary feed to their chickens. The responsibility of giving this supplementary feed was mostly with the wife 43.5% and husband 31.4% (Table 3.12).

The responsibility of providing water to the rural chickens lies with the wife (40.7%), husband (34.6%) and combination of husband, wife, son and daughter accounted for the remaining percentage (Table 3.12). The wife/mother is mostly responsible (44.1%) for cleaning the rural chicken house (Table 3.12). The wife/mother is also responsible (43.2%) for letting the birds out of the house and for shutting the birds in the house in the night (43.1%) and if they are sick (42.5%) and other non specified activities the husband/father is mainly (56.8%) responsible for sourcing of treatment (Table 3.12).

Table 3.9: Number of farmers that provides housing to rural chickens in the four Local Government Areas of Plateau State.

			LGA				Total
			Jos South	**Barkin Ladi**	**Kanam**	**Shendam**	
HOUSING	Yes	Frequency	137	192	142	134	605
		% of total	17.06	23.91	17.68	16.69	75.3
	No	Frequency	36	21	86	55	198
		% of total	4.48	2.62	10.71	6.85	24.7
Total		Frequency	173	213	228	189	803
		% of total	21.54	26.53	28.39	23.54	100

Table 3.10: Rural household chickens roosting place.

Roosting place		Local Government Area				
		Jos South	Barkin Ladi	Kanam	Shendam	Total
On tree	Frequency	11	8	66	26	111
	% of total	1.29	0.94	7.75	3.05	13.3
In mud house	Frequency	139	201	123	134	597
	% of total	16.31	23.59	14.44	15.73	70.10
Under a building	Frequency	32	23	29	38	122
	% of total	3.76	2.70	3.40	4.46	14.3
Any where	Frequency	3	6	7	6	22
	% of total	0.35	0.70	0.82	0.70	2.57
Total	Frequency	185	238	225	204	852
	% of total	21.71	27.93	26.41	23.94	100

X2 = 94.7, df = 9, Significance level = 0.000

Table 3.11: Source of drinking water for rural household chickens in Plateau State.

Source of water		Local Government Areas				
		Jos South	Barkin Ladi	Kanam	Shendam	Total
Well	F	166	214	110	85	575
	%	20.00	25.80	13.30	10.20	69.30
River stream	F	4	9	103	104	220
	%	0.48	1.08	12.41	12.50	26.50
Tap	F	0	1	0	0	1
	%	0	0.12	0	0	0.12
Rain water	F	0	1	1	0	1
	%	0	0.12	0.12	0	0.24
Bore hole	F	3	9	4	16	32
	%	0.36	1.08	0.48	1.93	3.85
Total	F	173	234	218	205	830
	%	20.8	28.20	26.37	24.70	100

X2 = 94.7, df = 9, significance level = 0.000

Table 3.12: Role of husband or wife in daily management routines for rural poultry.

Activity	Gender	Frequency	%
Watering	- husband	263	34.60
	- wife	310	40.70
Feeding	- husband	233	31.40
	- wife	322	43.50
Cleaning the chicken house	- husband	157	23.60
	- wife	294	44.10
Letting the birds out	- husband	165	25.30
	- wife	281	43.20
Shutting the birds house	- husband	179	27.50
	- wife	280	43.10
Checking if birds are sick	- husband	267	38.30
	- wife	296	42.50
Unspecified Routine	- wife	75	56.80
	- wife	41	31.10

Kitchen waste was the major (631) feed supplement provided to chickens (Table 3.13). Other feed supplements provided include, Maize offal (451) millet (411), maize (393), (63.02%) (Table 3.13). Only about 20 of the respondents provide nothing to their birds as a supplement food. While 121 of the respondents fed anything available to this flocks.

Tables 3.14 and 3.15 show the number of times in a day and time of the day rural farmers provide supplementary feed to their flock. Most of the respondents (39.42%) provided supplementary food twice in a day. Those farmers that feed their rural chickens anytime of the day (2.1%) were the least in number. These feeds were given in the early morning (39%) of the day. About 62.4% of the respondents are aware of ND, while 37.6% are not aware of the existence of the disease (Table 3.16).

Table 3.17 show the main causes of losses in rural chickens. The results indicated that diseases and preying were the major causes of losses in rural chickens with 83.2% and 8.85% respectively. The least cause of loss in the rural chickens was starvation (0.61%).

Newcastle disease outbreaks occurred year round in the villages sampled (Table 3.18). However, highest incidence (86.6%) of ND was observed from November to March (Dry season) and September to October 8.31% (Pre-dry season). During outbreaks of ND, infected birds exhibit the following major clinical signs; nervous signs (32.4%), weakness (16.6%), watery-whitish/greenish stool (16.2%), coughing/sneezing (13.6%), not eating (9.39%) and other signs (11.8%) (Table 3.19).

Table 3.13: Types of supplementary feed given to rural chickens by farmers in Plateau State.

Type of feed	Response		Jos South	Barkin Ladi	Kanam	Shendam	Total
			Local Government Area				
Millet	Yes	Frequency	65	435	177	134	411
		% total	15.82	8.52	43.07	32.60	100
Maize	Yes	Frequency	90	182	169	152	393
		% total	15.18	30.69	28.50	25.63	100
Maize offal	Yes	Frequency	147	178	83	43	451
		% total	32.59	39.47	18.40	9.53	100
Millet offal	Yes	Frequency	78	51	53	33	215
		% total	36.28	23.72	24.65	15.35	100
Kitchen waste	Yes	Frequency	192	145	168	126	631
		% total	30.4	23	26.6	20	100
Anything	Yes	Frequency	17	24	10	70	121
		% total	14.05	19.83	8.26	57.85	100
Nothing	Yes	Frequency	8	1	6	5	20
		% total	40	5	30	25	100

Table 3.14: Number of times that rural farmers gave supplementary feed to their birds in a day.

How often feed birds		Local Government Area: Jos South	Barkin Ladi	Kanam	Shendam	Total
Once a day	Frequency	72	117	67	50	306
	% total	8.71	14.15	8.71	6.05	37.00
Twice a day	Frequency	89	80	56	101	326
	% total	10.76	9.67	6.77	12.21	39.42
Three times a day	Frequency	13	24	93	48	178
	% total	1.57	2.90	11.25	5.80	21.52
Any time	Frequency	8	1	4	4	17
	% total	0.97	0.12	0.48	0.48	2.06
Total	Frequency	182	222	220	220	827
	% total	22.01	26.84	26.60	26.60	100.00

Table 3.15: Time of day for supplementary feeding of rural chickens.

Time of the day		Local Government Area				Total
		Jos South	Barkin Ladi	Kanam	Shendam	
Morning	Frequency	57	101	74	75	307
	% total	7.26	12.87	9.43	9.55	39.11
Afternoon	Frequency	3	0	1	1	5
	% of total	0.38	0	0.13	0.13	0.64
Evening	Frequency	1	1	0	1	3
	% of total	0.13	0.13	0	0.13	0.40
Morning/ afternoon	Frequency	8	6	8	11	33
	% of total	1.02	0.76	1.02	1.40	4.20
Morning/ evening	Frequency	91	80	74.0	45	290
	% of total	11.59	10.19	9.43	5.73	37.00
Three times/ any time	Frequency	37	21	6.00	83	147
	% of total	4.71	2.68	0.76	10.57	18.70
Total	Frequency	197	209	20.7	216	785
	% of total	25.10	26.62	6	27.52	100.00

Table 3.16: Awareness of Newcastle disease by rural poultry farmers in Plateau State.

Aware of Newcastle disease		Local Government Area Jos South	Barkin Ladi	Kanam	Shendam	Total
Yes	Frequency	58	151	113	218	540
	% of total	6.70	17.50	13	25	62.4
No	Frequency	90	91	61	83	325
	% of total	10.40	10.52	7.05	9.59	37.6
Total	Frequency	148	242	174	301	865
	% of total	17.12	27.98	20.2	34.79	100

Table 3.17: Main causes of losses in rural chickens as responded by rural farmers in Plateau State.

Cause of losses		Local Government Area				
		Jos South	Barkin Ladi	Kanam	Shendam	Total
Disease	Frequency	142	155	188	193	678
	% of total	17.21	18.8	22.8	23.3	83.0
Theft	Frequency	14	14	9	6	43
	% of total	1.70	1.70	1.1	0.73	5.20
Preying	Frequency	5	45	21	2	73
	% of total	0.60	5.45	2.55	0.24	8.85
Accident	Frequency	2	4	9	0	15
	% of total	0.24	0.48	1.09	0	1.82
Dogs/cats	Frequency	4	1	4	2	11
	% of total	0.48	0.12	0.48	0.24	1.33
Lack of feed	Frequency	4	0	1	0	5
	% of total	0.48	0	0.12	0	0.60
Total	Frequency	171	219	232	203	825
	% of total	20.73	26.55	28.12	24.61	100.0

X2 = 181.4, df = 21, significance level = 0.000

Table 3.18: Season of Newcastle disease outbreaks in rural household chickens of Plateau State.

Season		Local Government Area				
		Jos South	Barkin Ladi	Kanam	Shendam	Total
November to March (dry season)	Frequency	103	192	199	198	692
	%	13	24	25	24.6	86.0
April to May (pre rainy season)	Frequency	17	14	3	0	34
	%	2	1.74	0.4	0	4.0
June to August (rainy season)	Frequency	1	5	6	1	13
	% of total	0.12	0.62	0.74	0.12	1.60
September to October (pre dry Season)	Frequency	46	2	16	3	67
	%	5.71	0.25	1.99	0.37	8.00
Total	Frequency	167	213	224	202	806
	%	20.72	26.43	27.79	25.06	100.0

Table 3.19: Major clinical signs observed by farmers in sick rural chickens suspected of suffering from Newcastle disease.

Clinical signs		Local Government Area				Total
		Jos South	Barkin Ladi	Kanam	Shendam	
Abnormal nervous signs	Frequency	15	45	32	146	238
	%	2	6	4.4	20	32.40
Not active	Frequency	78	73	33	6	190
	%	10.6	10	4.5	0.8	25.9
Watery whitish/ greenish stool	Frequency	14	60	27	18	119
	%	2	8.2	3.7	2.5	16.2
Coughing/ sneezing	Frequency	3	2	79	16	100
	%	0.41	0.27	10.76	2.18	13.62
Others	Frequency	44	5	29	9	87
	%	5.99	0.68	3.95	1.22	11.85
Total	Frequency	154	185	200	195	734
	%	20.95	25.17	27.35	26.53	100.00

Table 3.20: Awareness of preventive value Newcastle disease vaccines by rural chicken farmers in Plateau State.

Knowledge that vaccines can prevent Newcastle disease		Local Government Area				Total
		Jos South	Barkin Ladi	Kanam	Shendam	
Yes	Frequency	83	93	51	141	368
	% of total	10.26	11.50	6.30	17.43	45.5
No	Frequency	92	128	164	57	441
	% of total	11.37	15.82	20.27	7.05	54.51
Total	Frequency	175	221	215	198	809
	% of total	21.63	27.32	26.58	24.47	100.0

Majority 54.51% of the respondents were not aware that there are vaccines that prevent ND (Table 3.20) while 45.49% are aware of vaccines that prevent ND. However, because of this lack of awareness, 50.16% of the respondents sourced their prescription and medicaments for treating rural chickens suffering from ND from traditional source, 29.40% veterinary services, 9.74% pharmacy, and 10.71% sourced from shops/markets (Table 3.21).

Some major medicaments used by rural chicken farmers for the treatment of rural chickens are listed in Table 3.22. Some (17.7%) of the farmers from Jos South LGA did not indicate the types of remedies given to their chickens when affected with ND. Those respondents that revealed the type of medicaments used mentioned mahogany (*Khaya senegalensis*) bark/roots (25.1%), wild garden egg (*Solanum nodiflorum*) (20.3%) and bitter leaf (*Vernonia amygdalina*) (7.8%) as the main remedies.

Various local names was given to ND in the areas sampled were itemized in Table 3.23. About 48% of the households do not know the local name of ND in their dialect. Majority of common names given to ND by rural farmers are *Mufkyl* (63%), *Borin Kaza* and *Mutuwan Kaji* (49%) (Table 3.23). Various methods have been devised by farmers to dispose off their chickens when suspected of being infected by NDV. These methods include offer for sale 80.63%, slaughter for consumption 16.07% and others (allow to die or disposed off as gift) 3.29% (Table 3.24).

Table 3.21: Major sources of prescription and procurement of medicaments for treating rural chickens suffering from Newcastle disease in Plateau State.

	Local Government Area					
Source of treatment		Jos South	Barkin Ladi	Kanam	Shendam	Total
Veterinary	Frequency	46	28	53	54	181
services	% of total	7.47	4.55	8.60	8.77	29.40
Ethnoveterinary	Frequency	88	63	65	93	309
	% of total	14.29	10.23	10.55	15.10	50.16
Pharmacy	Frequency	5	5	48	2	60
	% of total	0.81	0.81	7.8	0.32	9.74
Shops/market	Frequency	2	39	25	0	66
	% of total	0.32	6.33	4.06	0	10.71
Total	Frequency	141	135	191	149	616
	% of total	23	22	31	24	100

Table 3.22: Medicaments used by farmers to treat Newcastle disease in four Local government Areas of Plateau State.

Local Govt. Area	Medicament	Botanical name	Local name	Preparation	Frequency	%
Jos South						
	(i) Wild garden egg.	*Solanum nodiflorum*	Gautan kaji (H)	Cut into two halves and soaked in water	45	12.2
	(ii) Mahogany bark	*Khaya senegalensis*	Madaci (H)	Soak in water	38	10.3
	(iii) Bitter leaf	*Vernonia amygdalina*	Shiwaka (H)	Soak the bark in water	20	5.4
	(iv) Hairy thorn apple	*Datura metel*	Zakami/Haukata yaro (H)	Soak in water	5	1.4
	Not indicated	Not applicable	Not applicable	Not applicable	65	17.7
Barkin Ladi	(i) wild garden egg	*Solanum nodiflorum*	Gautan kaji (H)	Soak in water	16	4.3
	(ii) Ground Pepper	*Capsicum spp.*	Yaji(H)	Soak in water	6	1.6
	(iii) Not indicated	Not applicable	Not applicable	Not applicable	48	13.04
Kanam	(i) Mahogany bark/roots	*Khaya senegalensis*	Madaci (H)	Soaked in water	30	8.2
	(ii) Herbs un specified	Not applicable	Not applicable	not applicable	27	7.3
Shendam	(i) Mahogany bark	*Khaya senegalensis*	Madaci (H)	Soak in water	28	7.6
	(ii) Wild garden egg	*Solanum nodiflorum*	Gautan kaji (H)	Soak in water	14	3.8
	(iii) Bitter leaf	*Vernonia amygdalina*	Shiwaka (H)	Soak in water	9	2.4
	(iv) Zakami	*Datura metel*	Zakami/Haukata yaro (H)	Soak in water	10	2.7
	(v) White magi	*Monosodium glutamate*	Magi (H)	Soak in water	7	2.0
Total					**368**	**100**

H = Hausa

Table 3.23: Local names of Newcastle disease as given by the respondents in Plateau State.

			Local Government Areas				
Local Names of ND	**English translation**		**Jos South**	**Barkin Ladi**	**Kanam**	**Shendam**	**Total**
Farfadiyan kaza (H)	Epilepsy	Frequency	1	9	2	11	23
		% of total	0.34	3.09	0.69	3.78	7.90
Mukyl (A)	To die	Frequency	7	2	0	53	63
		% of total	2.41	0.69	0.00	18.56	21.65
No response (E)	Don't know	Frequency	1	0	0	47	48
		% of total	0.34	0.00	0.00	16.15	16.49
Bitkye (A)	Day of chicken	Frequency	0	0	0	36	34
		% of total	0.00	0.00	0.00	12.33	11.64
Muran kaji (H)	Chicken cough	Frequency	0	0	0	3	3
		% of total	0.00	0.00	0.00	1.03	1.03
Mutuwan Kaji (H)	Chicken death	Frequency	1	26	0	3	30
		% of total	0.34	8.93	0.00	1.03	10.31
Kabwak (M)	Chicken sickness	Frequency	0	1	0	1	2
		% of total	0.00	0.34	0.00	0.34	0.68
Gokye (A)	Chicken sickness	Frequency	0	3	0	3	6
		% of total	0.00	1.30	0.00	1.30	2.60
Farin kashi (H)	White stool	Frequency	0	1	2	0	3
		% of total	0.00	0.34	0.69	0.00	1.03
Borin kaza (H)	Chicken madness	Frequency	5	42	0	2	49
		% of total	1.72	14.34	0.00	0.69	16.83
Lolonk chongo (B)	Chicken madness	Frequency	24	2	0	0	27
		% of total	8.25	0.68	0.00	0.00	9.27
Total		Frequency	40	89	4	158	291
		% of total	13.75	30.58	1.37	54.30	100

Language key: A = Ankwai, B = Berom, E= English, H = Hausa, M = Mikan

Table 3.24: Methods of disposal of sick rural chickens.

What you do with sick birds		Local Government Area				Total
		Jos South	Barkin Ladi	Kanam	Shendam	
Dispose off/sell	Frequency	144	168	161	139	612
	% of total	18.97	30.82	21.21	18.32	80.63
Slaughter	Frequency	11	7	43	61	122
	% of total	1.45	1	5.67	8	16.07
Others (gift, allow to die etc)	Frequency	6	14	5	0	25
	% of total	0.79	1.84	0.66	0	3.29
Total	Frequency	161	189	209		759
	% of total	21.21	24.90	27.54		100

Table 3.25: Rural poultry vaccination by farmers in Plateau State.

		Local Government Area				Total
Those that vaccinated their birds against Newcastle disease		Jos South	Barkin Ladi	Kanam	Shendam	
Yes	Frequency	16	39	0	86	141
	% of total	3.72	9.06	0	20	32.8
No	Frequency	66	106	6	111	289
	% of total	15.34	24.65	1.39	25.81	67.2
Total	Frequency	82	145	6	197	430
	% of total	19.06	33.72	1.39	45.81	100.0

Table 3.26: Farmers that sell their birds when affected with Newcastle disease.

		Local Government Area				Total
Do you sell sick bird		Jos South	Barkin Ladi	Kanam	Shendam	
Yes	Frequency	16	15	2	67	100
	% of total	3	2.85	0.4	12.71	19.0
No	Frequency	55	91	2	100	248
	% of total	10.44	17.3	0.4	19	47.0
Sometimes	Frequency	61	49	34	35	179
	% of total	11.57	9.30	6.45	6.64	34.0
Total	Frequency	132	155	38.	202	527
	% of total	25.05	29.41	7.21	38.33	
100.0						

Table 3.27: Number of respondents that are predisposed to consumption of meat from sick rural chickens.

		Local Government Area				Total
Do you eat sick birds?		Jos South	Barkin Ladi	Kanam	Shendam	
Yes	Frequency	91	77	58	104	330
	% of total	12	10	7.7	14	43.7
No	Frequency	63	101	86	68	318
	% of total	8.3	13.4	11.4	9	42.0
Sometimes	Frequency	27	47	3.0	31	108
	% of total	3.6	6.2	0.4	4.1	14.3
Total	Frequency	181	225	147	203	756
	% of total	23.9	29.8	19.4	26.9	100.0

3.5 DISCUSSION

Although 1240 households were targeted for the study only 980 questionnaires were recovered. This is because some households in the rural households sampled were not up to the 30 households that were targeted.

The occupation of the respondents showed that 81.75% of them were crop farmers, 8.18% of them were civil servants, 8.06% house wives and 2.25% were business men. Majority (65.3%) of these respondents were males that were heads of the households visited. These men answered questions on behalf of their wives who could not be interviewed for cultural and religious reasons. Farming as main occupation of the respondents as observed in this study is to be expected since in the rural areas the main activity is farming. The involvement of civil servants in keeping rural poultry is also to be expected as most of these civil servants are children/family of men that are still living in the rural households despite the collapse of the mining industry.

The flock size of 20.3 chickens found in this study is similar with the findings of Dafwang (1989) who did a similar study in the Middle Belt Geo-political zone of Nigeria to which Plateau State belongs. The figure is however much lower than the figure of 10 chickens per household reported by the National Bureau for Statistics (NBS). It is possible that the NBS may have restricted its sampling to adult chickens only, because the average household flock size for most parts of Nigeria has been reported by agricultural professionals to be between 15-30 (Sonaiya and Olori, 1990). This study shows that plans for massive vaccination in any given area of Plateau State and the Northern Guinea Savanna Agro Ecological zone of Nigeria in general can be based on an estimate of 20 chickens per household. This can be used in combination with the number of households / village listing estimates that have been listed by the

Agricultural Development Programs which exist in all States of Nigeria to arrive at total poultry estimates.

The common livestock and poultry raised by farmers in rural areas in addition to domestic chickens, include goats, sheep, pigs cattle, rabbits and pigeons. The goat was the most common livestock raised. Iyayi and Tona (2004) reported that, goat keeping is more common amongst the rural livestock farmers probably due to their ability to thrive and survive on their own with little or no care. This was followed by pigs, sheep and cattle. However, it was observed that farmers raised these animals in combination of two or more animals per household. When considering the different types of livestock raised with rural poultry, goats, sheep and pig were the most prevalent. Although religious aspects on biodata of the respondents were not taken, it was observed that majority of the respondents in the study area, with the exception of Kanam LGA, were Christians. In Islam, pork consumption is not only forbidden but pig production is also forbidden to Muslims (Eusebio, 1980). Keeping different species of livestock and poultry together may encourage the maintanance and spread of diseases from non susceptible to highly susceptible birds. All the ND viruses isolated from dead and apparently heathy free roaming birds by Echeonwu *et al.* (1993) in Plateau State were VVND. This is of epizoological importance as these may serve as reservoirs of infection to the intensively raised flocks on commercial and backyard farms (Majiyagbe and Nawathe, 1981).

In this survey, the reason that there were more hens than cocks is that most farmers sell their cocks and growers while keeping hens for breeding. Selling cocks will generate income to the household and reduce excess cocks among the hens. Riise *et al.* (2005) has reported that income from rural poultry has a positive impact on the livelihood of rural poultry owners, in terms of improving the household diet, improving the living conditions of the family, household assets and educational expenditure on children. Rural chickens also fetch a premium price in Nigeria and also play a major part in the diet of rural population (Ogundipe, 1989). In the majority of rural households surveyed, most of the birds encountered were chickens. This is because of their relative ability to adapt to low or zero level of husbandry and environmental stress than the exotic strains as confirmed by Hassan *et al.* (1989).

Major strain of chickens encoutered were black, white, mottled, long legged, barred, dwarf, naked neck and frizzled chickens. Although there was a higher percentage of naked necks in the hotter ecological zone (9.5 versus 6.6%), their overall presence which constitutes only 8.3% of the total population indicates that they are not a dominant strain in any of the zones. Consequently, the speculation that the cool and hot climatic conditions may have resulted in the higher abundance of naked neck and white chickens in the hotter areas by natural selection was not confirmed in this study. The observation that there were much more mottled feathered strains in the cooler climate (24%) in contrast to only 11.2% in the hotter climate may have been because of a greater presence of commercial poultry in the Jos area. It is common knowledge that a lot farmers buy small numbers of commercial pullets and layers which they mix with the rural poultry. The higher prepondence of long legged chickens in the hotter climate of 17.2% in contrast to only 5.1% in the cooler climate may be due to the higher presence of nomadic Fulani cattle rearers in the southern region of the State. The long legged chicken is a strain that has been found to be the most dominant amongst the cattle Fulani (Sonaiya and Olori, 1990). Further studies will need to be conducted to establish a stronger basis for the preferential adoption of Naked Neck chicken for improvement of poultry production in the hotter areas of Nigeria and the Mottled feathered chicken in the Plateau regions of Nigeria with a cooler climate. The adoption of the long legged chicken for rural poultry improvement may be recommended because of its higher average body weight.

The study showed that the main source for foundation stock was open livebird market (62.5%) and gift (17.2%), while only 12.3% of the respondents inherited their stock. This may be attributed to the small unit cost of the stock which makes acquisition by buying from the live bird market affordable and most popular as reported by Hassan *et al.* (1989). In this study, open market sourcing of birds as foundation stock or for restocking is a very risky method as various reports have shown that outbreak of diseases for example avian influenza, ND and fowl cholera have been known to occur locally either through live bird markets or contaminated materials and surfaces (Martin, 1992; Nwanta *et al.*, 2006).

Though, men were the heads of households visited, the wives/mothers were responsible for most of the daily management routines, such as cleaning the rural household chicken house, letting the birds out in the morning, shutting the birds in the house in the night and checking the birds if they are sick. Various reports have shown that the poultry industry in rural areas is largely in the hands of women and children as they own or manage or both the rural poultry (Ogundipe, 1989; Dafwang, 1990; Kitalyi, 1998; Nwanta *et al.*, 2006; Sonaiya, 2007). Although women and men own poultry, it was found that women ensure that these birds are tended (Kitalyi, 1998). It is also the women that decide which birds are to be sold or slaughtered (Nwanta, 2003). This study also revealed that for other non specified activities such as building chicken house it was men’s responsiblity.

Majority of the farmers provided housing for their chickens which is mainly for night roosting to protect them against diseases and predators. This indicates that farmers were aware of the importance of housing and hence there is need to educate them to build proper housing so that they can realize more benefits. There was a wide variety of housing in the areas of study but, mud brick walls type of housing was the most common. These houses as observed are in some cases not actually meant for chicken but uncompleted or collapsed buildings meant for grain storage or sleeping room for childrens. Mapiye and Sibanda (2005) reported that the poled and raised housing yielded highest flock sizes and was attributed to the ability to provide adequate ventilation and protection from diseases, predators and parasites and they concluded that the type of housing had significant effect on the flock size, hen and chick numbers.

Provision of housing, improved feeding and general management have been reported by Mavale (2000) to reduce the incidence of and severity of ND. Houses are also reported by Harun and Massango (2000) and Nwanta *et al.* (2006) to protect the chickens and chicks from predators, disease contamination and also provide warmth to birds during cold weather, thereby reducing the stress of the extreme cold which could aggravate ND being incubated by chickens. The construction of proper housing using cheap, durable, locally available resources and skills can improve rural household chicken production. Lack of adequate housing can partly explain mortalities and thus good housing is therefore, a prerequisite for any viable and sustainable chicken project. Those chickens that are not provided with housing and roost on tree tops, the incidence of ND can be said to be reduced, as the contact of birds with nasal discharges and faecal droppings of infected birds may be absent or minimal.

Well and river/stream are the most popular sources of drinking water for rural poultry. These two main sources are actually the main sources of drinking water for rural poultry farmers in the study area. The implication of this is that, wild birds and other domestic poultry may also use the same source of drinking water which increases the chances of infection among infected or carrier birds to a susceptible bird. The provision of clean drinking water has been reported by Nwanta (2003) to reduce the chances of infection, particularly when it is conducted along with feed immediately the birds are released in the morning to scavenge.

Improved feeding as part of husbandry management system for poultry improves the disease resistance of birds to infection (Mavale, 2000). Majority of the farmers provided supplementary feed twice a day and mostly in the morning and evening. It was observed that these periods of supplementary feeding coincided with the period when most farmers were at home before going to the farms (morning) or when they were back from the farms (evening). The advantages of strategic feeding which is based on scavenging and supplementary feeding are contained in the reports of Bessei (1990a). Studies on the feeding habits of scavenging birds have shown that they consume large amount of invertebrates (Savory, 1989). Therefore, their basic ration has been found to be rich in protein and any supplement of grains or other energy rich by- products will result in a better balanced diet and increased production (Bessei, 1990a). The results obtained in this study indicated that, common supplementary feed given to rural chickens by rural farmers included maize, millet, maize offal, millet offal and kitchen waste. These feed stuffs were observed to be the most common staple food, cheapest and most eaten by humans in the study area. The results obtained in this study indicated that feeds and feeding systems were potential for intervention since majority of the farmers gave locally produced feedstuff as supplementary feeding to the chickens.

Majority of the farmers reported that diseases and birds of prey were the main causes of losses in rural household chickens. This finding is in agreement with studies carried out by Ibrahim and Abdu (1992) and Nwanta (2003) that diseases and predators were the main causes of stock attrition in rural household poultry. High disease levels were probably due to exposure of chickens to the immediate environment, interaction of different entities within and among flocks such as flock contacts during scavenging, uncontrolled introduction of new stock and contact through exchange or sale of live chickens or movement between households and villages. Also, Bourdin (1980) and ILRI (1995) cited by Fabusoro *et al.* (2007) reported that attack on farm animals by pest and diseases militate against the expansion of livestock production and could be costly in terms of death, reduced performance and treatment. The control of diseases in rural poultry is made difficult because of the multi age structure of the flocks, small size of the flocks, the labour in catching individual birds for vaccination and the inefficient use of the vaccines (Olabode *et al.*, 1992; Alders and Spradbrow, 2001). Although the present study showed that many farmers (62.4%) were aware of ND, various reports have shown that ND was the major cause of low productivity and survival of rural chickens (Philips 1973; Nawathe *et al.*, 1975; Fatumbi and Adene, 1979; Adene, 1990; Nwanta *et al.*, 2007). In many countries, seasonal outbreaks of ND are recognized. Attempts were often made to attribute these outbreaks to the weather conditions prevalent at the time. There may, however, be more realistic explanations based on patterns of movement in chickens and changes in the volume of chicken markets (Alders and Spradbrow, 2001).

Season of outbreaks of ND in rural chickens in the study area revealed that ND occurs all year round with more outbreaks occurring from November to March (Dry season). Abdu *et al.* (1985) and Nwanta (2006) reported that increased movement of sick and healthy chickens to markets in anticipation of various festivities particularly during Sallah, Christmas and New Year festivities may have been responsible for the peaks of ND outbreaks in November to March. Also, movement of infected chickens which may mix with healthy ones is probably the main source of ND. In many countries, veterinary workers, extension workers and indeed rural household people, recognize that the introduction of new birds to a flock is often associated with an outbreak of ND (Alders and Spradbrow, 2001). Although it is not possible to restrict the entry of new chickens into rural household flocks, this lesson needs to be spread by extension workers. The windy harmattan also encourages the spread of the ND. This agrees with the reports of Ibrahim and Abdu, 1992; Halle, *et al.*, 1999; Alders and Spradbrow, 2001; Sa'idu *et al.,* 2006). Other reports by Dao and Pham (1985) and Okeke and Lamorde (1988) have shown that cold weather induces stress on chickens and subsequently lowers their immunity to ND.

Although, farmers could not give the scientific name for common diseases affecting their flocks, they were able to describe the symptoms of these diseases as basis on which the highlighted diseases were identified. The major clinical signs observed by farmers for sick rural household chickens suspected of having ND were nervous signs, weakness, watery whitish/greenish stool and coughing/sneezing. These signs are indicative of very velogenic Newcastle disease virus (VVNDV) that was in circulation in the study area. Echeonwu *et al.* (1993) isolated VVND named "kudu" and "VG1" from free roaming scavenging ducks and apparently healthy guinea fowls respectively in Plateau State. The virus (VVND) and other pathotypes might have been responsible for the severity of infection and clinical signs observed. This is in agreement with reports of Alexander (1997) that clinical signs and the speed at which the signs appear vary widely and depend upon infectivity and dose of virus, the specie, age and immune status of the host, environmental conditions and the route of exposure.

Although, rural poultry farmers are aware of the need to keep birds in good health and when they are sick to source for prescription and procure medicaments for treating rural poultry suffering from ND, they often do so through the use of traditional (indigenous) method of medicare. Mahogany bark/roots (*Khaya senegalensis*), wild garden egg (*Solanum nodiflorum*) and bitter leaf (*Vernonia amygdalina*) were the main remedies used for the health management of the rural chicken suffering from ND. Traditional remedies are reported to be much easily and readily obtained and affordable (Abdu *et al*., 2000). Antihelminthic and antiprotozoan properties of *Khaya senegalensis, Vernonia amygdalina* and *Solanum nodiflorum* have been reported by Abdu and Faya (2000) and Atawodi *et al.* (2000). The practice of sourcing for medicament through the use of traditional method of medicare for treatment of birds is not enough to ensure a good health of the birds due to poor knowledge and understanding of the etiology and pathology of poultry diseases by the farmers (Nwanta, 2003). Hence, they may succeed in treating the observable symptoms of the disease without dealing with the actual cause. These remedies used by rural farmers in the study areas may or may not have effect on ND virus but have effect on protozoan and helthminth of rural poultry, thereby reducing the parasites burden and boosting immunity of the birds against ND infection. This implies that extension services are required in educating rural farmers in matters relating to use appropiate medicaments and adopting new technologies for improving rural chicken production and disease control. Alders and Spradbrow (2001) have recommended extension services and community participation for the successful adoption of the oral feed vaccine for the control of ND in rural areas.

The results in this study indicating wider use of traditional medicine may also be due to its low cost, local availability and ease of application. A study by Mapiye and Sibanda (2005) has shown that large flock sizes were obtained with farmers that gave traditional medicines to their chickens. This indicates that traditional medicines do work in some instances and have potential to improve the health status of rural household flocks. Hence, there is a need for researchers to take inventory of common medicaments used by local farmers in treating rural household chickens and determine their chemical properties, concentrations and routes of application.

The study revealed that majority of the farmers disposed off their birds by selling, as a gift or slaughter for consumption. This constitutes a danger to other susceptible birds and to humans. The introductions of sick birds to healthy susceptible flocks in the households have been reported to encourage the spread of the ND (Ibrahim and Abdu, 1992; Alders and Spradbrow, 2001). Likewise, deadly zoonotic diseases like Avian Influenza are reported to affect humans through handling of diseased poultry.

Rural householders have become astute at recognizing the early signs of Newcastle disease especially when their birds are sick. And when they recognise that their birds have ND, Spradbrow (2005) reported that, two options are usually explored by farmers. One is salvage of sick or even dead birds for consumption and another option is market place. The market was a common source of ND infection, sometimes through the random sale of infected birds, but more commonly through salvage sales which encouraged the maintainance and spread of ND virus in the study area. There is a need for enlightment of the farmers by extension agents to discourage this habit and to ensure that infected entrails are buried, and not discarded to be scavenged by other predators.

Despite the fact that most farmers are aware of ND, majority of farmers claimed ignorance of the existence of ND vaccines. This may be due to lack of or inadequte extension services by the vaccines manufacturing agency (NVRI, Vom) and the Plateau State Livestock Extension Service. The high cost of ND vaccines may also lead to low patronage. Private veterinary practioners may not like to penetrate the rural areas to create awareness and promote the use of the ND vaccines. This observation is similar to that reported by Alders and Spradbrow (2001) and Musa (2002) that the use of conventional ND vaccines in rural household chickens was limited by cost, dose format and thermostability. It was therefore, advocated by Olabode (1996) and Alders and Spradbrow (2001) that suitable ND vaccines that have been found to be relatively simple, cheap, safe and thermostable without reliance on refrigeration, should be adopted for use in rural households for protecting rural poultry.

It was concluded that:

- Disease and predators (birds of prey) were major cause of losses among rural household chickens in Plateau State.
- Majority of the farmers in the study area used traditional remedies for treating sick rural household chickens suspected of having ND.
- Mahogany bark/roots (*Khaya senegalensis*), wild garden egg (*Solanum nodiflorum*) and bitter leaf (*Vernonia amygdalina*) were the main remedies used by rural household chicken farmers in treating sick rural household chickens suspected of suffering from ND in four Local Government Areas of Plateau State.
- Newcastle disease occurs all year round among rural household chicken reared in Plateau State, but is most common during dry season (November to March).

- Some of the management practices such as mixing different species of livestock, sourcing for foundation stock from the open livebird market and selling of the sick chickens suffering from ND by farmers in managing rural household chicken flocks in the study area might, have contributed to the maintenance, spread and high prevalence of diseases in the study area.

Recommendatins were given as follows:

- Vaccination as a means of protecting chickens against ND before the anticipated period of high incidence should be practiced.
- Create awareness through extension services to identify and avoid management practices that could encourage the spread and maintenance of diseases such as ND in rural household chickens.
- Awareness services should be focused on women who are responsible for most of the daily routines of managing rural household chickens.

CHAPTER FOUR

4.0 NEWCASTLE DISEASE SEROPREVALENCE AND FIELD VACCINATION WITH NEWCASTLE DISEASE VACCINE STRAIN I_2 IN RURAL CHICKENS

4.1 ABSTRACT

A seroprevalence study for Newcastle disease virus and field vaccination with $NDVI_2$ on 1,208 chickens raised under traditional management system in rural chickens in four LGAs of Plateau State was conducted. The objectives of the study were to determine the seroprevalence of ND, determine the potency of $NDVI_2$ vaccine in rural chickens and to compare the potency of the NDV I_2 vaccine when administered to rural chickens via intraocular, intramuscular or drinking water routes. The seroprevalence of ND virus antibodies in the four local government areas in Plateau State showed that differences beteween the four LGAs were not statistically significant ($P \leq 0.05$). Of the 1,208 sera tested, 627(51.9%) had detectable antibodies to NDV but only 170 (14.1%) of the chickens had protective HI antibody titre of $\geq 4Log_2$. About 1,041(86.2%) of the chickens sampled were therefore at risk of suffering from clinical ND. Field vaccination showed that at three weeks postvaccination (pv) with $NDVI_2$ by i/o gave high geometric mean titre (GMT) of 8.3 ± 1.6 Log_2 and 92% protection rate than when $NDVI_2$ vaccine was administered through drinking water which gave GMT of 3.4 ± 0.6 Log_2 and only 46% protection rate. It was therefore concluded that majority of the rural chickens in Plateau State were at risk of suffering from ND when exposed to velogenic ND strain. Vaccination using $NDVI_2$ administered to rural chickens via intraocular route is therefore highly recommended to reduce the incidence and severity of ND.

4.2 INTRODUCTION

Serological studies in rural chickens were made in several countries. In the Khon Kaen province in Thailand, a health and productivity study in native rural chickens was carried out in two villages from September 1987 to August 1988 (Thitissak *et al*., 1989). Monthly blood samples were taken from 920 unvaccinated wing tagged birds and 448 offspring. The mean HI titre for ND was high in newly hatched chicks and declined as maternal antibodies disappeared at about 90 days. Thereafter, mean titres rose steadily as the age of birds increased, peaking in birds 3 years of age. However, the proportion of seropositive samples was not stated. ND occurred during the study period, but no information on the pattern of occurrence was provided. In Morocco, rural chicken flocks in six different regions were studied for the prevalence of ND (Bell & Moulodi, 1988). Serum samples were taken from 100 unvaccinated chickens from different regions. Antibodies against NDV were found in each region ranging from 5 to 83% (average 35%) of the chickens sampled.

In Tanzania, 13.3% of the chickens were seropositive when HI test was performed prior to vaccination against ND from 120 chickens greater than 4 months from five villages (Minga *et al*., 1989). In Mauritania, serum samples were taken from 80 chickens in rural poultry flocks in each of three different regions. Antibodies against NDV were detected in 4.6% of chickens. A serological study was also conducted in Benin in three ecologically different regions in the south, centre and north. Seroposivity of chickens for NDV was 56%, 75%, and 69%, respectively (Bell, 1992).

In Nigeria, a study carried out in chickens under various production systems by Ezeokoli *et al.* (1984), showed a 73% prevalence of antibodies against NDV in traditionally managed backyard flocks in Zaria, while 63% seroprevalence was reopred by Orajaka *et al.* (1999) in south eastern Nigeria. And in south western Nigeria around Ibadan 38% seroprevalence was reported by Oyewola *et al.* (1996).

These observed regional differences in ND seroprevalence showed ecological area variations in NDV activity and may perhaps a reflection of the impact of environment on the viability of NDV, spread and its epidemiology (Orajaka *et al*., 1999).

The presence of specific antibodies to ND virus in the serum of birds gives little information on the infecting strain of the virus and threfore has limited diagnostic value in relation to NDV strain. Nevertheless, in certain circumstances the demonstration of the presence of infection is sufficient for the needs of a diagnostician. Post vaccinal serology can be used to confirm succesful application of vaccine and an adequate immune response by the birds (Alexander *et al.,* 1984).

The objectives of the study were:

1. To determine the seroprevalence of ND in rural chickens in Plateau state.
2. To determine the potency of NDV I_2 vaccine in rural chickens.
3. To compare the potency of the NDV I_2 vaccine when administered to rural chickens via intraocular, intramuscular or drinking water routes.

4.3 MATERIALS AND METHODS

4.3.1 Experimental sites

Field trials were conducted in Jos South, Barkin Ladi, Kanam and Shendam LGAs of Plateau State. Sample analysis and challenged experiments were conducted in the Virology Laboratory, Viral Research and Poultry Division of National Veterinary Research Institute (NVRI), Vom, Plateau State.

4.3.2 Sample size and distribution

Cluster sampling method was used for calculating sample size and design for this study because the individual sampling frame is not known (Bennette *et al*., 1991; Otte and Cum, 1997). Due to the characteristic of backyard poultry, each household was considered as a flock within a cluster.

The sample size was calculated from an expected prevalence of 41% based on the highest prevalence obtained from a previous study in Plateau State by Adu *et al.* (1986) at 5% accuracy using the formula by Martin *et al.* (1987).

$$\text{Sample size (N)} = \frac{Z^2 p\, q}{L^2}$$

where $Z^2 = 1.96$

p = Prevalence = 0.41

q = (1-p) = 0.59

L = Allowable error 0.05

$$= \frac{1.96^2 \times 0.41 \times 0.59}{0.05^2}$$

= 371 samples.

To obtain an accuracy compared to that of random sampling the sample size was quadrupled (Martin *et al*., 1987). This gave a total of sample size of 1,484.

The number of clusters (villages), to be sampled was limited by availability of resources. Thirty two clusters were included in the study. The aim was to sample 46 birds per cluster resulting in 1,472 samples.

The prevalence rate was determined by dividing the total number of positive samples by the total number of samples tested (Bennette *et al.*, 1991).

The estimated 32 clusters were randomly apportioned into four LGAs chosen for the study. Each LGA had eight clusters. Cluster inhabitants having greater than 50 households were grouped and selected on random basis as clusters in proportion required for each LGA. While for those with less than the required number of households more samples were taken from available households to make up for the required number of samples. With the selected cluster, blood samples were obtained from rural chickens on either side of the major road dividing the rural into two.

Five samples per flock of every fifth household were taken, when volunteered. In case the houses were not many, samples were taken from all the houses that volunteered. About 77.4% (1,139) of the samples were sampled this way and about 22.6% (333) of the samples were obtained from rural chickens by random sampling of the major live bird markets in Jos South and Shendam LGAs.

4.3.3 Vaccines

Newcastle vaccine strain I_2 (Batch 1/2005) with a titre of $10^{10.2}$ EID_{50} per dose was obtained from the Virology Division of NVRI, Vom.

4.3.4 Field vaccination trials

Birds were identified using individually with numbered wing tags and blood samples were collected from each bird and vaccinated during serological survey. A different route of administration was used in the respective LGAs were intramuscular route (i.m)(Jos South), Barkin Ladi (control), intraocular route(i/o) (Kanam) and drinking water (dw) (Shendam). For im and i/o routes, 100 dose vial was diluted with 20 mls of sterile physiological saline (PBS), 0.2 ml of the diluted vaccine represented one field dose of the vaccine, each bird recieved 0.2 ml of the vaccine through i/o or im route. For birds recieving vaccine through dw, a 100 dose bial was diluted with one litre

(1000 mls) of drinking water, representing 10 mls containing one field dose per bird. The routes used in administering the vaccine, in this experiment, were based on common practice in the poultry industry in Plateau State and Nigeria in general.The birds were vaccinated once with $10^{10.2}$ EID_{50} of $NDVI_2$ per bird. The control group was not vaccinated. The status of individually tagged birds and the number of birds per household were recorded.

4.3.5 Blood collection

Blood samples were collected prevaccination (on the day of vaccination), three weeks post vaccination and two weeks post challenge. Two millilitres of blood were collected through the jugular or wing vein using sterile two ml syringe and 21 G needle. For prevaccination sero monitoring, blood samples were collected from chickens of different ages that had no history of previous vaccination against ND. The blood samples were deposited into sterile test tubes. Sera were separated by allowing the blood samples in the test tubes slant in racks at room temperature for one to two hours to clot. The sera were then decanted leaving the blood clot. The decanted sera were transported immediately to the laboratory and stored in cryovials at -20^{o}C freezer until tested after one to seven days.

4.3.6 Challenge studies

The ND virus strain used for challenge experiment was Kudu 113 isolated locally from ducks, characterized by Echeonwu *et al*. (1993) and with a titre of $10^{7.5}$ EID_{50} per ml. The virus was obtained from the Virology Division of NVRI, Vom. An ampoule stock containing 0.5 ml of lyophilized virus was reconstituted with 49.5 ml of PBS. Each bird received a dose of 0.20 ml containing $10^{6.5}$ EID_{50} of the virus intramuscularly. All birds were observed for clinical signs during challenge periods and the number of deaths in each group was recorded.

4.3.7 Buy back challenge

From each experimental group, 15 birds from each LGA were purchased and transported to NVRI, Vom and challenged with Kudu 113 ND virus.

4.3.8 Serology

Sera collected from vaccinated and unvaccinated rural chickens were tested for NDV specific antibody by the haemagglutination inhibition test (HI) as described by Allan and Gough (1974). The HI titre of each bird was determined and expressed on Log 2 and the mean for each bird was calculated. The HI test was performed using beta technique (constant virus and varying serum) against 4 HA units of the virus computed from the result of HA titration. Doubling dilutions (50 *u*l) of the sera was reacted with 50 *u*l of the 4 HA unit of the antigen (ND La Sota virus was used as antigen) per well. This was incubated for 30 minutes at room temperature for antigen antibody to reaction to take place and 50 *u*l of 0.25% suspension of washed chicken RBC was added to all the wells and shaken gently to mix and incubated at room temperature for 45minutes. Negative controls wells were also included. The HI antibody titres were expressed as the reciprocal of the highest dilution giving a complete inhibition of the agglutination of the RBC. Sera with HI antibody of ≥ 4 Log_2 were considered protective based on OIE recommendation of 2000.

4.3.9 Data Analysis

Geometric mean HI antibody titre (GMT) and percentages of detectable NDV HI antibody titre were calculated. The Statistical Package for Social Sciences (SPSS) Programme (version 13) was used to compare if there was any significant difference between the geometric means of the HI antibody titre. Morbidity, mortality, case fatality and protection rates were also calculated as follows:-

$$\text{Morbidity rate} = \frac{\text{Number of birds that are sick due to the disease}}{\text{Total number of birds in the group}} \times 100$$

$$\text{Mortality rate} = \frac{\text{Number of birds that died due to the disease}}{\text{Total number of birds in the group}} \times 100$$

$$\text{Case fatality rate} = \frac{\text{Number of deaths due to disease (sick)}}{\text{Total number of birds with the disease (dead)}} \times 100$$

$$\text{Protection rate} = \frac{\text{\% dead control - \% dead vaccinated}}{\text{\% dead control}}$$

4.4 RESULTS

Seroprevalence study showed that 51.9% of the rural chicken sera sampled had detectable antibodies to NDV which indicated evidence of ND virus infection (Table 4.1). The LGA with the least number of chickens with detectable antibodies was Kanam, while Jos South had the highest number of chickens at risk (93.6%) (Table 4.1).

Out of the 1,208 sera analysed only 170, (14.1%) were presumed to be protected (birds with NDV HI antibody titre of ≥ 4 Log_2). The overall percentage of rural chickens at risk in the LGAs sampled was high (86.2%).

Results of field vaccination showed that birds responded positively to vaccination (Table 4.2). Chickens vaccinated via intramuscular, intraocular or drinking water routes produced high NDV HI antibody titres three weeks post vaccination. Birds vaccinated with $NDVI_2$ through intramuscular or intraocular routes produced high HI antibody titre levels of GMT ± SD were 7.6 ± 2.8 Log_2 and GMT ± SD 8.3 ± 1.6 Log_2, respectively.

Table 4.3 shows the morbidity, mortality, case fatality and protection rates. Chickens vaccinated with $NDVI_2$ via intraocular or intramuscular routes were better (92% and 85%) protected against challenge with velogenic NDV than those vaccinated via drinking water routes (46%). Morbidity and mortality rates were higher in the control group (100% and 87%) and in the group vaccinated via drinking water route (60% and 47%).

Table 4.4 shows the clinical signs and postmortem lesions of rural chickens vaccinated with $NDVI_2$ vaccine and challenged with a local strain of velogenic NDV (Kudu 113 ND virus). The clinical signs exhibited by chickens vaccinated through the im route were weakness, inappetance, yellowish/whitish/greenish diarrhoea. Postmortem lesions were necrotic foci in the ileum, congestion of the spleen haemorrhages in the proventriculus. The birds in the control group exhibited, wings and legs paralysis, were moribound, colonic spasm, greyish yellow diarrhoea, sudden death and torticollis.

Severe postmortem lesions such as haemorrhages in the breast muscles, haemorrhagic enteritis, haemorrhages in the proventriculus, ileocecal haemorrhages and haemorrhages in the thigh muscles were observed in the control group. While in the group that were vaccinated via drinking water route, the clinical signs observed were somnolence, greenish diarrhoea, legs and wings paralysis, torticollis and colonic spasm and postmortem lesions were haemorrhagic enteritis, proventricular haemorrhages, skeletal haemorrhages and ballooned caeca.

Table 4.1: Distribution of Newcastle disease virus antibodies in chickens in households and live bird markets in four Local Government Areas of Plateau State.

LGA	Number of serum samples tested	Number of sera with detectable antibodies		Mean ± S.D	Number of chickens protective for ND		Number of rural chickens at risk	
		F	%		F	%	F	%
Shendam	230	114	49.6	1.4±2.0	32	13.9	198	86.1
Kanam	226	97	42.9	1.1±1.8	24	10.6	202	89.4
Jos South	283	165	58.0	0.6±1.6	18	6.4	265	93.6
Barkin Ladi	223	121	54.3	1.3±1.7	22	10.0	204	91.5
Jos South (market)	198	103	52.0	2.2±3.3	58	29.3	140	70.7
Shendam (market)	48	27	56.3	2.5±3.7	16	33.3	32	66.7
Total	**1,208**	**627**	**51.9**		**170**	**14.1**	**1,041**	**86.2**

Antibody titres (Log_2) of $\geq 4Log_2$ were considered positive to ND based on OIE recommendation of 2000, F = frequency, % = percentage
LGA = Local Government Area

Table 4.2: Geometric mean antibody titre (GMT) in sera of rural chickens vaccinated in the field with Newcastle disease vaccine strain I_2 administered through different routes.

			GMT $\pm$ SD		
Local government area	Route of vaccination	Number of birds	Pre-vaccination (n)	3 weeks post vaccination (n)	2 weeks post challenge (n)
Jos south	Intramuscular	15	0.1 $\pm$ 0.3 (15)	7.6 $\pm$ 2.8 (15)	10.0 $\pm$ 0.8 (13)
Barkin Ladi	Control	15	0. 2 $\pm$ 0.1 (15)	0.2 $\pm$ 0.2 (15)	7. 0 $\pm$ 0.2 (2)
Kanam	Intraocular	15	0.1 $\pm$ 0.2 (15)	8.3 $\pm$ 1.6 (15)	10.3 $\pm$ 1.0 (14)
Shendam	Drinking water	15	0.1 $\pm$ 0.0 (15)	3.4 $\pm$ 0.6 (15)	7.0 $\pm$ 0.6 (8)

(n) = number of chickens

Table 4.3: Morbidity, mortality, case fatality and protection rates in rural chickens vaccinated with Newcastle disease vaccine strain I_2 in the field in four Local Government Areas of Plateau State and later challenged with a local strain of velogenic Newcastle disease virus intramuscularly.

Local government area	Number of chickens	Route of vaccination	Morbidity rate	Mortality rate	Case fatality rate	Protection rate
Jos South	15	Intramuscular	20.0	13.0	67.0	85.0
Barkin Ladi	15	Control	100	87.0	87.0	13.0
Kanam	15	Intraocular	7.0	7.0	100	92.0
Shendam	15	Drinking water	60.0	47.0	78.0	46.0

Table 4.4: Clinical signs and postmortem lesions in birds vaccinated with Newcastle disease vaccine strain I_2 vaccine and challenge with local strain of velogenic Newcastle disease virus.

Group	Clinical signs	Postmortem lesions
Jos South (intra muscular)	Weakness, inappetance yellowish/whitish/greenish diarrhea	Necrotic foci in the ileum, congestion of the spleen and hemorrhages in the proventriculus
Barkin Ladi (control)	Wings and legs paralysis, moribound, colonic spasm, greyish yellow diarrhea sudden death and torticollis	Haemorrhages in the breast muscle, hemorrhagic enteritis, haemorrhages in the proventriculus, ileocecal haemorrhages and haemorrhages in the thigh muscle
Kanam (intraocular)	None	None
Shendam (drinking water)	Somnolence, greenish diarrhea, legs and wings paralysis, torticollis and colonic spasm	Haemorrhagic enteritis, proventricular haemorrhages, skeletal hemorrhages and ballooned ceca

4.5 DISCUSSION

The present study showed a relatively higher (51.9%) seroprevalence rate of ND virus antibodies in rural chickens in the four LGAs, compared to what has been reported (41%) earlier by Adu *et al.* (1986). Although there was no significant differences (P>0.05) between NDV seroprevalence rate in the different LGAs, the observed differences in the rates of NDV HI antibodies in the four local government areas may be as a result of ecological variations in ND activity or sampling methods and may perhaps be a reflection of the impact of environment on the viability of NDV and epidemiology (Orajaka *et al.*,1999). Other factors responsible for differences in NDV HI antibody may be as result of concentration of commercial poultry in some of the LGA sampled. About 80% of the commercial poultry were located in Jos South and Barkin Ladi LGAs. Commercial poultry are routinely vaccinated against ND (Saidu *et al.,* 2006) which rural chicken may come in contact with especially during sales or processing.

The observation that birds in Plateau State had NDV antibodies indicated that ND was wide spread in the areas studied. The implication of the spread and the carrier status of the rural chicken could be of importance considering the fact that the rural chickens were reported to constitute over 90% of chicken population in Nigeria and are capable of scavenging around the environment spreading the NDV to vaccinated and unvaccinated healthy birds (RIM, 1993; Alders and Spradbrow, 2001). Newcastle disease infection occur year round in most rural poultry populations, but is most common and severe at times of climatic stress. Outbreaks are often associated with change of season, particularly the start of the wet season (Ronohardjo *et al.,* 1988; Thitisak *et al.,* 1988). Cold and hot weather have been cited as contributory factors in ND outbreaks (Dao and Pham, 1985; Bell *et al.,* 1990; Abdu *et al.,* 1992). The disease was also reported to be more common during the dry harmattan (November - March) and cold stress has been known to worsen the outcome of ND (Abdu *et al.*, 1992; Sa'idu *et al.*, 1994; Halle *et al.*, 1999).

None of the chickens sampled had a history of previous vaccination against ND. It could therefore, be deduced that antibodies detected in the rural chickens before vaccination were as a result of natural infection by NDV. Therefore, the 51% seroprevalence rate of ND antibodies in the four LGAs can be attributed to a number of factors.

- The management system in traditional production may serves as stress factor and favour infection.
- Continued exposure to array of infectious agents and wild birds.
- Nutritional deficiencies.
- The absence of disease control through vaccination
- Contact of birds of one rural with those in other villages through gift and sale of rural chickens which in some cases are diseased or serve as carriers of some diseases which may facilitate the spread of diseases like ND among flocks.

The high percentage of the chickens sampled that were at risk (86.2%) of being infected with NDV may depend on several factors including the virulence of the strain causing the outbreak (Alexander, 1997). In the neighboring state of Kaduna, Nwanta (2003) reported a prevalence of 73.3%. In most cases rural chickens are kept together with other breeds of poultry like guinea fowls and ducks, these species of poultry may serve as carriers of diseases like ND. For example, a study conducted by Mai *et al.* (2006) in Plateau State indicated that, out of 165 serum samples collected from live birds market, ducks 6.7% had antibodies against the ND virus. Similarly, out of the 205 serum samples collected from guinea fowls, 13.6% had antibodies against the ND virus and all sera from the ducks and guinea fowls were negative for IBD antibodies. It was concluded that both local ducks and guinea fowls had been exposed to the NDV but not to the infectious bursal disease virus.

Only 14.1% of chickens had antibody titres ≥ 4 Log_2 – the protective base line. This may probaly due to exposure of the birds to the virus as a result of ND outbreak or contact with infected or vaccinated birds. This is in accordance with the reports of Martin (1992) and Adu *et al.* (1985) who reported that velogenic NDV was responsible for the majority of ND outbreaks in rural poultry and that velogenic strains of NDV were prevalent in traditionally managed poultry.

The results obtained in the field vaccination trials showed that the vaccine can be successfully administered via different routes. The highest mean antibody titre was achieved in the group where the vaccine was administered via i/o. The vaccination regime employed in the $NDVI_2$ vaccine field trial was unusual in that the vaccine was given once only for each route of administration. This was done to determine the most economically viable strategy for vaccination.

Bell *et al.* (1995) found that a single intraocular administration of NDV4 heat resistant (HR) vaccine provided acceptable protection to rural chickens in Cameroon. Although intraocular administration requires that each bird be caught, this is the route known to provokes the highest HI antibody response and better protection. As farmers gain confidence in the ND control activities, they should be encouraged to improve the housing provided for their chickens. Improved housing will facilitate the catching of birds for intraocular administration of the ND vaccine and should reduce losses due to predation. Bell *et al.* (1995) also recommended improved housing for rural chickens, especially for young chicks up to four weeks of age, as this was the group that suffered the highest losses from predation.

About 46 % that recieved $NDVI_2$ vaccine through drinking water despite having mean titre of 3.4 $\pm$ 0.6 Log_2 failed to resist challenged, this showed that titre of 3 Log_2 may not be sufficinent to offer protection against challenge with velogenic ND. Similar observation was made by Schmidt and Schmidt (1955) that reported a relationship between HI titre and the protection capacity for birds for a 10-month period following vaccination. They found that a variable percentage (85- 95%) of birds having titres up to $3Log_2$ failed to resist the challenge infection against virulent NDV, whereas those having titre $4Log_2$ and above, resisted the challenge infection, and thus considered HI titre Log_2 4 and above to be protective.

CONCLUSIONS

- This study has shown that the prevalence of NDV in the four LGAs of Plateau State was high.
- That over 80% of rural chickens in Plateau State were at risk of dying of ND when exposed to a virulent NDV.
- That $NDVI_2$ administered to rural chickens by i/o, or im routes gave high HI antibody titre and protection against a local virulent isolate of NDV.
- Administration of $NDVI_2$ to rural chickens by i/o route provoked a greater immune response than administration via i.m or drinking water routes.
- Newcastle Disease vaccine strain I_2 when administered to chickens through intraocular or intramuscular routes would be suitable for use in the control of ND in rural chickens in Plateau state.

RECOMMENDATIONS

(a) Newcastle vaccine strain I_2 should be adopted as a vaccine of choice for protection against ND in rural chickens.

(b) The administration of $NDVI_2$ vaccine to rural chickens in the field should be through i/o or i.m routes. However, because of easyness of application i/o route is most recommended.

(c) Vaccination as a means of protecting birds against ND before the period of outbreaks should be carriedout.

(d) Extension services on how to administer the $NDVI_2$ vaccine to rural chickens should be pursued.

CHAPTER FIVE

5.0 THERMOSTABILITY AND POTENCY OF NEWCASTLE DISEASE VACCINE STRAIN I_2

5.1 ABSTRACT

Thermostabilty and immunogenicity studies on an Australian Newcastle disease vaccine strain I_2 (NDVI$_{2)}$ stored at different periods and temperatures were conducted in Plateau State. The storage of the NDVI$_2$ vaccine was through refrigeration at +4 ^oC for 365 days, room temperature ranging between 23-29^oC for 1 to 14 days and incubation in a water bath at +45^oC for 24 h. The vaccines were titrated in nine day old embryonated chicken eggs to determine their viability after storage. Reconstituted and lyophilized NDVI$_2$ vaccines maintained a minimum infective dose of 6.8 Log_{10} and 8.4 Log_{10} for 3 and 35 days, respectively from an initial titre of 10.2 $Log_{10.}$ On the basis of Log_{10} loss of titre, reconstituted NDVI$_2$ lost 3.4 Log_{10} titre after exposure at room temperature for 3 days. Lyophilized NDVI$_2$ seems to be more stable as it lost only 1.8 Log_{10} titre after exposure at room temperature for 35 days. The study further showed that reconstituted and lyophilized NDVI$_2$ vaccines were immunogenic and protective against challenge with a virulent local isolate of ND virus when administered via drinking water or intraocular route under laboratory conditions.It was recommended that NDVI$_2$ should be adopted for vaccination of rural chickens.

5.2 INTRODUCTION

The stability of the ND virus is crucial to its value as a vaccine strain (Allan *et al.*, 1978). Recent developments on ND vaccines have been focused on the thermostability of vaccine strains and suitability for rural operational settings (Nssiens and Adene, 2002).

The avirulent, thermostable ND vaccine strains NDVI$_2$ was developed by researchers at The University of Queensland in Australia to provide rural poultry farmers with an effective, affordable means of controlling ND in their flocks (Bensink and Spradbrow, 1999). The NDV I$_2$ vaccine has been used successfully in rural chicken populations in many countries in Asia and Africa. The control of ND in rural chickens would allow greater production of chicken meat and eggs as well as provide an incentive for controlling other diseases and improving husbandry (Ideris *et al.*, 1990). Although called 'heat-stable', no quantified definition has been applied to this term, and it is unclear how many hours and at what temperatures the vaccine can be kept before its potency deteriorates (Jackson, 1992). Vaccine performance is also affected by excessive vibration and extended exposure to direct sunlight (Spradbrow, 1992b). This necessitated the need to evaluate the thermostability of locally produced NDVI$_2$ vaccine in Nigeria.The objective of the study was to determine the immunogenicity and potency of locally produced NDVI$_2$ vaccine stored at different temperatures and forms.

5.3 MATERIALS AND METHODS

5.3.1 Experimental sites

The experiments were conducted in the Virology, Viral Research and Poultry Divisions of the National Veterinary Research Institute (NVRI), Vom for the study.

5.3.2 Experimental birds

One hundred and twenty day old unsexed Shika Brown chicks were obtained from the Poultry Research Farm, NVRI, Vom.

5.3.3 Housing and feeding of birds

The chicks were housed in a room previously cleaned, washed, disinfected and fumigated. They were fed chicks mash containing maize 55%, soy bean cake 27%, wheat offal 13.50%, bone meal 1.5%, limestone 1.5%, fish meal 0.5%, chick premix (GCOML PLC, Jos) 0.25%, salt 0.25%) and water *ad libitum.*

5.3.4 Vaccines

Ten vials of $NDVI_2$ vaccine containing 100 doses of $10^{10.2}$ EID_{50} per vial in a lyophilized form were obtained from the Virology Division of the NVRI, Vom.

5.3.5 Vaccine carriers

Chick mash as described in section 5.7.3 of this dissertation was obtained from the NVRI, Vom feed mill and used as vaccine carrier.

5.3.6 Challenge virus

The virus strain used for the challenge experiment was the NDV Kudu 113 strain isolated locally from duck and characterized by Echeonwu *et al.* (1993) with a titre of $10^{7.5}$ EID_{50}. The virus was obtained from the Virology Division of the NVRI, Vom.

5.3.7 Antigen for HI test

ND La Sota virus obtained from virology division of NVRI, Vom was used as antigen.

5.3.8 Storage and titration of Newcastle vaccine strain I_2

A vial of NDV I_2 vaccine was reconstituted with 20 ml of PBS (pH 7.4) and aliquot in 2 ml into 10 bottles marked A-J. The aliquots were stored at room temperature (23 – 29 ^{o}C) on shelve and at 45^{o}C in a water bath. The lyophilized NDVI_2 was stored and titrated at 7, 14, 21, 28 and 35 days at stotorage temperature of 29 – 30oC. The reconstituted and lyophilized NDVI_2 vaccines were titrated as described by Young *et al.* (2002). Nine to ten day old embryonated chicken eggs were used to titrate the vaccine and ten fold dilutions (10^{-5} to 10^{-10}) of the suspension were inoculated into five eggs per dilution. About 0.2 ml each of the aliquot was inoculated at 8.0 am, 2.0 pm and 8.0 pm for day one, two and three respectively. Infected eggs were detected by examining allantoic fluid for haemagglutinin activity. The 50% EID was determined by the method of Reed and Muench (Villegas, 1998).

5.3.9 Vaccination of birds

Birds were vaccinated via dw, i/o or feed as shown in Table 5.1. Non-vaccinated control birds were housed separately and attended to by a different poultry attendant. All birds were observed for clinical signs after vaccination and challenge and the number of dead birds in each group were recorded. Sera were collected at two and three weeks post vaccination and at two weeks post challenge.

5.3.10 Challenge of experimental birds

An ampoule containing 0.5 ml of lyophilized ND virus Kudu 113 strain was reconstituted with 49.5 ml of PBS (pH 7.4). Each bird received a dose of 0.20 ml containing $10^{6.5}$ EID_{50} of the virus im. All birds were observed daily for clinical signs during the vaccination and challenge periods and the number of deaths in each group was recorded.

5.3.11 Collection of blood

Blood was collected as described in 4.7.5.

5.3.12 Serology

Serology was conducted as described in 4.7.8.

5.3.13 Experimental design

Two experiments were conducted (1) Reconstituted and lyophilized $NDVI_2$ vaccines were kept at room temperatutre for 3 and 35 days respectively and titrated to determine their EID_{50}. (2) In the second experiment, birds at three weeks of age were tagged individually with numbered wing tags and randomly allocated into twelve experimental groups of ten chicks each and vaccinated via dw, i/o or feed as shown in Table 5.1.

Table 5.1: Experimental design on studies of thermostability, storage and routes of administration of $NDVI_2$ vaccine.

Group	Number of birds	Storage temperature (°C)	Duration of storage (days)	Route of administration
A1	10	+4°C	365	Drinking water
A2	10	+4°C	365	Intraocular
B1	10	Room temperature(23-29°C) **	3	Drinking water
B2	10	Room temperature **	3	Intraocular
C1	10	Room temperature	14	Drinking water
C2	10	Room temperature	14	Intraocular
D1	10	Room temperature	1	Drinking water
D2	10	Room temperature	1	Intraocular
E 1	10	+45°C	1	Drinking water
E2	10	+45°C	1	Intraocular
E3	10	Room temperature	1	Feed
Control	10	None	None	None

** Reconstituted $NDVI_2$ vaccine

5.3.14 Data analysis

Data was anaysed as described in 4.3.9.

5.4 RESULTS

Figure 5.1 shows the effect of storage temperature on reconstituted $NDVI_2$ vaccine titre. There was steady decline of virus titre from the initial titre of 10.2 Log_{10} EID_{50} to 6.8 Log_{10} EID_{50} in 72 h. The highest (0.6 Log_{10} $EID_{50)}$ decline of titre was observed after 24 h and 48 h of storage. The cumulative difference in drop of titre after 72 h was 3.4 Log_{10} EID_{50}.

The effect of storage temperature on lyophilized vaccine is shown in Figure 5.2. The cumulative difference in titre from the initial titre of 10.2 Log_{10} EID_{50} was 1.8 Log_{10} EID_{50}. There was also steady decline in titre from seven days post storage to 35 days with the highest titre loss of 1.2 Log_{10} EID_{50}.

Table 5.2 shows the HI antibody response of chickens vaccinated with $NDVI_2$ stored under different temperatures and challenged with a local velogenic NDV Kudu 113 strain. The result indicated that, the chickens responded positively to the vaccination. All groups produced detectable H1 antibodies two and three weeks post vaccination. Also, detectable HI antibody titre was produced by all groups two weeks post challenge. There was no significant difference in the levels of the HI antibody titres in all the groups post vaccination. Furthermore, no significant difference in the HI antibody titres of birds that received $NDVI_2$ through drinking water and the eyes. However, significant differences were observed in HI antibody titres in group E3 that received $NDVI_2$ vaccine through feed and the control group.

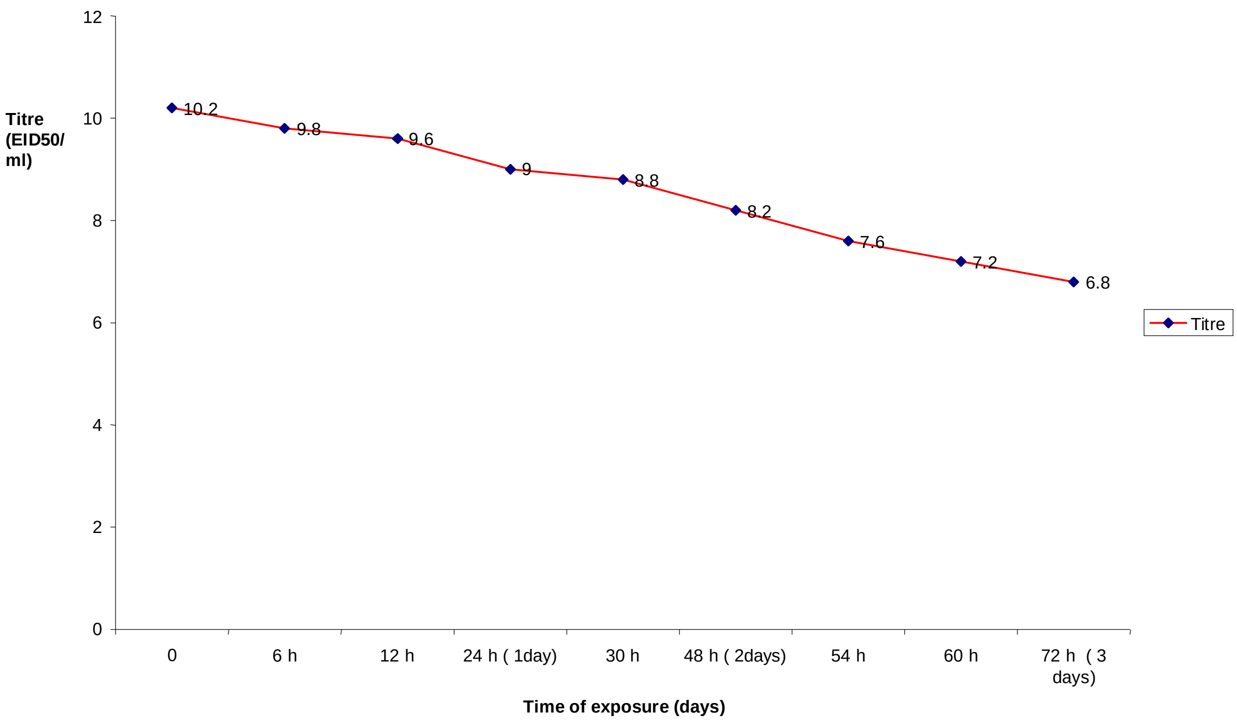

Figure 5.1: Effect of storage on reconstituted NDVI$_2$ stored at 29 to 30^{o}C for 3 days

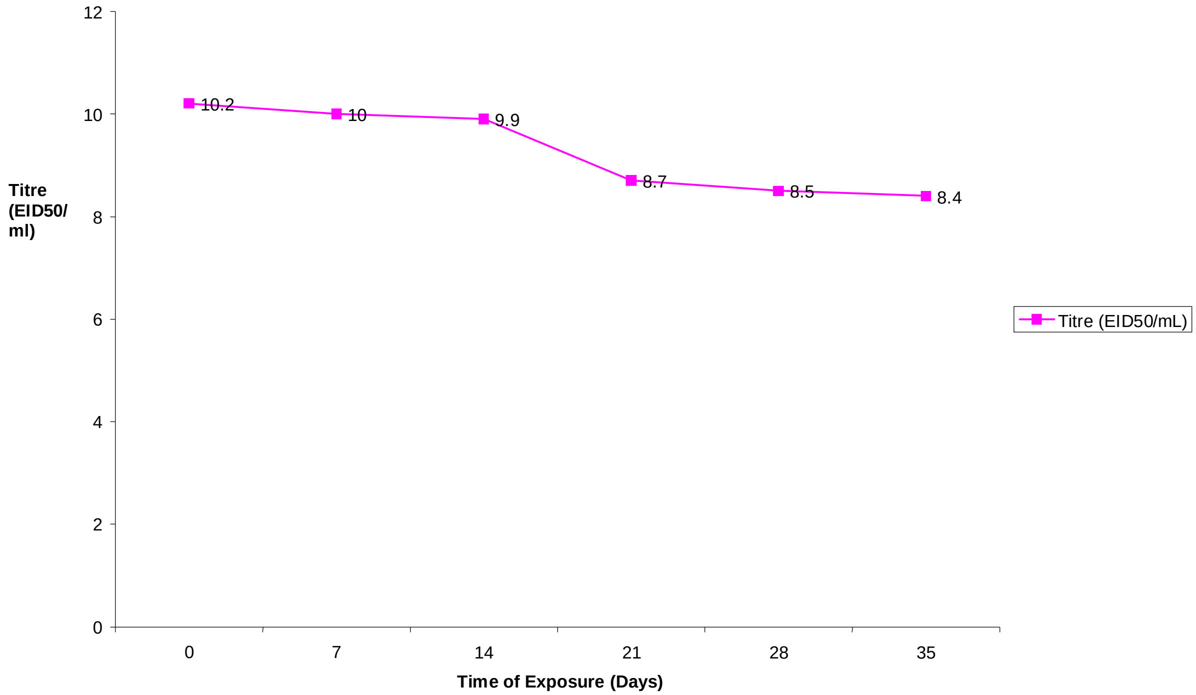

Figure 5 2: Effect of storage on lyophilized $NDVI_2$ vaccine titre stored at 29 to 30°C for 35 days.

Table 5.3 shows serological profile of birds with a titre of $\geq$ 4.0 Log_2 at 3 weeks post vaccination with $NDVI_2$ vaccine stored under different temperatures. The high (GMT $\pm$ SD Log_2 7.9 $\pm$2.0) level of the protective (titre of $\geq$ 4.0 Log_2) H1 antibody titre 3 weeks post vaccination was observed in birds that received $NDVI_2$ stored at +4 ^oC (group A2) and at room temperature for 24 h (group D1) administrated to chickens through the eyes and drinking water, respectively. The lowest antibody titre (GMT $\pm$ SD $Log_2$1.9 $\pm$ 1.7, 0.2 $\pm$ 1.6) at three weeks post vaccination was observed in birds vaccinated through feed (group E3) and birds in control group.

Table 5.4 shows the morbidity, mortality, case fatality and protection rates of birds vaccinated with $NDVI_2$ vaccine stored under different temperatures and challenged with a velogenic local strain of NDV Kudu 113 strain intramuscularly. The mortality rate due to ND occurred in vaccinated and unvaccinated birds, but was significantly higher ($p<0.05$) in the unvaccinated birds and birds that received $NDVI_2$ through feed and control group compared to the vaccinated groups (Table 2). The highest (100%) morbidity rate was observed in groups E3 that received $NDVI_2$ through feed and control group and the lowest was in groups A2 (+4^{o}C, 365 days, i/o), D2 (room temperature, 1day, i/o), E1 (+45^{o}C,1 day, dw) and E2 (+45^{o}C,1 day, i/o). The highest mortality rate (80%) was observed in control group and the lowest mortality rate (0%) was in groups A1 (+4^{o}C, 365 days, i/o), A2, B2 (room temperature, 35 days, i/o), D2 (room temperature, 1 day, i/o), E1, E2 and E3 (room temperature, 1 day, feed).

Two weeks after challenge, 100% case fatality rate was observed in group C2 (room temperature, 14 days, i/o) and D2 100% protection rate was observed in groups A2, B2, D2, E1 and E2. The protection rates in the control and group E3 were 20% and 22.2%, respectively.

Table 5.5 summarized the clinical signs and postmortem findings in birds vaccinated and those in control group after challenge. The common clinical signs observed were greenish /yellow /whitish diarrhoea, torticollis, wings and legs paralysis. These signs were more severe in group E3 and control groups. No clinical signs were observed in group A1, B1, B2, C1, D2, E1 and E2. The postmortem lesions were also more severe in those birds vaccinated with $NDVI_2$ through feed. The most obvious lesions observed in the proventriculus, skeletal muscles, intestines, caecal tonsils include necrotic foci in the ileum, congestion of the spleen, haemorrhages in the breast muscles, haemorrhagic enteritis, haemorrhages in the proventriculus, ileocecal haemorrhages and haemorrhages in the thigh muscle.

Table 5.2: Geometric mean antibody titre (GMT) and standard deviation (SD) in sera of chickens vaccinated with NDVI$_2$ vaccine stored under different temperatures and challenged with Newcastle disease virus 113 Kudu 113 strain.

			GMT $\pm$ SD (Log_2)			
Group	Storage	Route of administration	Prevaccination (n)	2 weeks post vaccination (n)	3 weeks post vaccination (n)	2 weeks post challenge(n)
A1	+4 ^oC (365 days)	Drinking water	0.1 $\pm$ 0.3 (10)	3.3 $\pm$ 1.2 ^{a} (10)	5.1 $\pm$ 2.1^c (10)	8.0 $\pm$ 0.8 ^{e}(10)
A2	+4 ^oC (365 days)	Intraocular	0.0 $\pm$ 0.0 (10)	5.1 $\pm$ 0.7 ^{a} (10)	7.9 $\pm$ 2.0 ^{c} (10)	8.8 $\pm$ 2.0 ^{e} (10)
B1**	+23-29 ^oC (3 days)	Drinking water	0.1 $\pm$ 0.3 (10)	5.1 $\pm$ 0.2 ^{a} (9)	6.1 $\pm$ 1.3^c (9)	8.0 $\pm$ 1.3 ^{e} (9)
B2**	+23-29 ^oC (3 days)	Intraocular	0.1 $\pm$ 0.3 (10)	4.5 $\pm$ 1.0 ^{a} (9)	5.9 $\pm$ 0.9^c (9)	8.5 $\pm$ 2.7 ^{e} (9)
C1	On bench for 2 weeks	Drinking water	0.0 $\pm$ 0.0 (10)	4.4 $\pm$ 1.3 ^{a} (10)	6.1 $\pm$ 2.1^c (10)	6.9 $\pm$ 0.8 ^{e} (9)
C2	On bench for 2 weeks	Intraocular	0.1 $\pm$ 0.3 (10)	4.6 $\pm$ 1.2 ^{a} (9)	6.1 $\pm$ 2.0^c (9)	9.0 $\pm$ 1.6 ^{e} (9)
D1	On bench for 24 h	Drinking water	0.1 $\pm$ 0.3 (10)	4.0 $\pm$ 1.8 ^{a} (9)	7.0 $\pm$ 2.4^c (9)	9.3 $\pm$ 1.8 ^{e} (9)
D2	On bench for 2 4 h	Intraocular	0.1 $\pm$ 0.3 (10)	5.2 $\pm$ 0.9 ^{a} (9)	6.2 $\pm$ 1.3 ^{c} (9)	8.0 $\pm$ 2.5 ^{e} (9)
E1	+45 ^oC for 24 h	Drinking water	0.0 $\pm$ 0.0 (10)	4.3 $\pm$ 0.8 ^{a} (9)	5.0 $\pm$ 0.0 ^{c} (9)	6.6 $\pm$ 0.5 ^{e} (9)
E2	+45 ^oC for 24 h	Intraocular	0.0 $\pm$ 0.0 (10)	4.2 $\pm$ 1.0^a (8)	5.0 $\pm$ 1.0 ^{c} (8)	6.8 $\pm$ 0.5 ^{e} (8)
E3	Bench 24 hs. In feed	Commercial Feed	0.0 $\pm$ 0.0 (10)	1.1 $\pm$ 04 ^{b} (9)	0.2 $\pm$ 1.6 ^{d} (9)	5.3 $\pm$ 1.6 ^{e} (3)
Control	None	None	0.3 $\pm$ 0.1 (10)	0.0$\pm$ 0.0 (10)	0.0 $\pm$ 0.0 (10)	6.3 $\pm$ 1.5 (3)

(n)* = Number of chicks
** = Reconstituted NDVI$_2$
$^{a,b,\ c,\ d\ and\ e}$ = Means with the same letters in the same column are not significantly different at 0.05 level of significance

Table 5.3: Variation in haemagglutination inhibition antibody titres of chickens three weeks post vaccination with $NDVI_2$ stored at different temperatures and forms.

Group	Titre (Log_2)											No. ≥ 4.0 (Log_2)	Mean titre (Log_2)
	0	1	2	3	4	5	6	7	8	9	10		
A 1	0	0	1	0	0	1	4	2	0	0	0	7	5.1±2.1
A 2	0	0	0	0	0	1	3	0	1	2	3	10	7.9±2.0
B 1	0	0	0	0	1	2	2	3	1	0	0	9	6.1±1.3
B 2	0	0	0	0	0	4	3	3	0	0	0	10	5.9±0.9
C 1	0	0	1	0	1	0	4	2	0	1	1	9	6.1±2.1
C 2	0	0	1	0	1	0	4	2	1	1	0	9	6.1±2.0
D 1	0	0	1	0	0	1	3	0	0	5	0	9	7.0±2.4
D 2	0	0	0	0	2	0	3	3	1	0	0	9	6.2±1.3
E 1	0	0	0	0	4	2	4	0	0	0	0	10	5.0±0.9
E 2	0	0	0	1	3	2	3	1	0	0	0	9	5.0±1.0
E 3	3	3	2	2	0	0	0	0	0	0	0	0	1.3±1.6
Control	8	1	0	0	0	0	0	0	0	0	0	0	0.2±0.7

Table 5.4: Morbidity, mortality, case fatality and protection rates of chickens vaccinated with $NDVI_2$ vaccine stored under different temperatures and challenged with velogenic Newcastle disease virus Kudu 113 local strain intramuscularly.

Group	Storage temperature (^oC)	Duration of storage (days)	Vaccination route	Morbidity rate	Mortality rate	Case fatality rate	Protection rate
A1	+4^oC	365	Drinking water	20	0	0	80
A2	+4^oC	365	Intraoccular	0	0	0	100
B1	Room temperature	3	Drinking water	22.20	11.10	50	50
B2	Room temperature	3	Intraoccular	11.10	0	0	100
C1	Room temperature	14	Drinking water	20	10	50	50
C2	Room temperature	14	Intraoccular	11.10	11.10	100	88.90
D1	Room temperature	1	Drinking water	11.10	11.10	100	88.90
D2	Room temperature	1	Intraoccular	0	0	0	100
E1	+45^oC	1	Drinking water	0	0	0	100
E2	+45^oC	1	Intraoccular	0	0	0	100
E3	Room temperature	1	Commercial feed	100	77.80	77.8	22.2
Control	None	None	NA*	100	80	70	20

* NA means not applicable

Table 5.5: Clinical signs and postmortem lesions in birds vaccinated with $NDVI_2$ vaccine stored under different temperatures and challenged with a local velogenic NDV strain.

Group	Clinical signs	Postmortem lesions
A1	Somnolence, yellowish/whitish diarrhoea and leg paralysis	Necrotic foci in the ileum, congestion of the spleen haemorrhages in the proventriculus
A2	None	None
C2	Recumbent, moribund, legs and wings paralysis	Greenish haemorrhagic enteritis, congested liver and spleen
D1	Recumbent and moribund, leg paralysis wing paralysis	Skeletal haemorrhages, necrotic foci in the ileum and haemorrhagic enteritis
E3	Somnolence, head shaking, legs and wings paralysis, torticollis and colonic spasm	Haemorrhagic enteritis, proventricular haemorrhages, skeletal hemorrhages and ballooned caeca
Control	Wings and legs paralysis, moribound, colonic spasm, greyish yellow diarrhea sudden death and torticollis.	Haemorrhages in the breast muscle, haemorrhagic enteritis, haemorrhages in the proventriculus, ileo-cecal haemorrhages and haemorrhages in the thigh muscle

5.5 DISCUSSION

This work shows that, there was persistent decline of titre of reconstituted and lyophilized $NDVI_2$ vaccines following storage at different temperatures. This indicates that $NDVI_2$ vaccine in either reconstituted or lyophilized form when stored at room temperature for three days or 35 days contains minimum infective dose to immunize birds. This is in agreement with similar studies conducted by Tu *et al.* (1998) who reported that the infectivity of lyophilized $NDVI_2$ vaccine fell to less than 1.0 Log_{10} unit on storage for 6 days at 26-32 ^oC and vaccines stored at similar temperatures induced high levels of immunity in laboratory chickens after storage for 17 days and in rural chickens after storage for 21 days. Furthermore, in the same study vaccine reconstituted after storage for 24 days at 30-35 ^oC still produced substantial protection in vaccinated chickens. The significance of this finding is that it is possible for $NDVI_2$ vaccines to remain potent and reach rural chickens in any part of Nigeria, without maintaining a cold chain. Uruakpa (1997) reported that $NDVI_2$ vaccine had similar thermostability to NDV4 vaccine and when kept in a favourable diluent (with lactose, sodium glutamate and bovine serum albumin) $NDVI_2$ lost only 0.3 Log_{10} units on storage for one month at 22 ^oC. In an experiment conducted by Hussein (1987), $NDVI_2$ vaccine stored for 6 or 7 weeks at room temperature still gave substantial protection to vaccinated chickens and concluded that there was no advantage in storing the vaccine at 4 ^oC. In Nigeria, NDV4 a similar vaccine to $NDVI_{2,}$ was found to remain viable for over 3 months on storage at 20 ^oC when the residue from the production of maize porridge was used as a carrier (Olabode, 1996).

Blood samples collected prevaccination and tested for HI antibody titre to NDV for all treatments including birds in the control group were low or negative for HI antibody titre suggesting that maternally derived antibodies (MA) to NDV acquired from parent stock may have weaned at the time (five weeks of age) of bleeding prior to vaccination. This is in agreement with similar observation by Spradbrow (1987) who reported that MA in newly hatched chicks may only last for five weeks and that chicks are also fully capable of mounting immune response at about the same age.

The HI antibody response of chickens vaccinated with $NDVI_2$ stored under different temperature and challenged with a local velogenic NDV Kudu 113 strain indicates that, chickens respond adequately to the vaccine. All groups produced detectable HI antibodies two and three weeks post vaccination. Also, detectable HI antibody titre were produced by all groups two weeks post challenge. Furthermore, non significant difference in HI antibody titre of those birds that received the $NDVI_2$ through drinking water and intraocular showed that both the reconstituted and lyophilized $NDVI_2$ vaccines are immunogenic and protects birds against challenge with a local velogenic NDV Kudu 113 strain when administered via these routes.

Vaccinated birds with HI anti body titre of $\geq 4\ Log_2$ were found to be protected when challenge with NDV Kudu 113 strain, while those with a titre of $\leq 3\ Log_2$ were not protected. Birds are considered protected if 80% of the birds resisted challenge with virulent strain of the NDV. The above correlation between achieved mean titres due vaccination and level of protection against challenge closely agreed with the earlier study of Schmidt and Schmidt (1955) and Westbury (1984) who studied the relationship of HI antibody titre and protection capacity and reported that birds having HI titre up to $3\ Log_2$ failed to resist the challenge infection against virulent ND virus and those having HI titre of $4\ Log_2$ and above resisted the challenge infection and this is also in agreement with OIE (2000) recommendation of HI antibody titres of ≥ 4 Log_2 to be considered as protective.

Therefore, in this study, it was not surprising that two weeks after challenging the birds with the velogenic NDV, 100% case fatality rate was observed in group C2 and D2. Complete protection (100%) from challenge with velogenic NDV was observed in groups that received $NDVI_2$ through intraocular route and drinking water. The least protected after challenged with velogenic NDV virus were control group (20%) and group E3 (22.2%) This result confirms earlier reports by Alders and Spradbrow (2001) and Musa (2002) that reported low level of HI antibody titre and very low level of protection of birds vaccinated with $NDVI_2$ or NDV4 via feed. The chick mash used in this study as a vaccine carrier did not receive any special treatment prior to mixing with the vaccines. Therefore, it is possible that the ingredients it contains may have some antiviral substances which inactivate the $NDVI_2$ vaccine virus, hence the production of low HI antibody titre (Echeonwu *et al.*, 2008b). This is in agreement with Jackson (1992) and Echeonwu *et al.* (2008b) who reported that to avoid the inactivation of the vaccine virus by antiviral agents in feed particle various treatments need to be done for some potential carrier foods to sustain the infectivity of the vaccine virus. Among these washing before coating or soaking overnight followed by washing, boiling or heating of food type before coating with the vaccine virus.

It was observed in this study that, inspite of vaccination; mortality was recorded in both the vaccinated (birds in group B1, C2 and D1) and unvaccinated group. This collaborates the previous findings by Giambrone and Close (1990) who reported that inspite of the significant advances in poultry vaccinations programmes and efficacy, outbreaks of infectious diseases, particularly ND are still being recorded in many areas in both vaccinated and unvaccinated flocks. The clinical signs and postmortem findings of vaccinated and the control group that died as a result of challenge with velogenic NDV have shown that, the most obvious lesions were observed in the proventriculus, skeletal muscles, intestines and caecal tonsils. These lesions are indicative of the velogenic viscerotropic nature of the challenge virus.

It was concluded that:

- Newcastle disease vaccine strain I_2 is thermostable, immunogenic and protective when stored at room temperature ranging from 23- 29^o C for up to 35 days or when stored at temperature of 45^{o}C for 24 h.
- Newcastle disease vaccine strain I_2 admnisterd through i/o or drinking water routes gave higher HI antibody titre and protection rate than when the vaccine is administered through commercial feed.

It is recommended that

(a) Newcastle vaccine strain I_2 can be adopted as a vaccine of choice for protection of rural chickens against ND in Plateau state.

(e) The route of administration of ND Vaccine strain I_2 to birds in the laboratory can be through i/o or dw.

(f) There is a need for NVRI, Vom to establish facilities in each of it's Zonal Laboratories to produce and deliver NDVI$_2$ to rural chicken farmers.

CHAPTER SIX

6.0 FEED BASED VACCINATION WITH NEWCASTLE DISEASE VACCINE STRAIN I_2 IN COMMERCIAL AND RURAL CHICKENS

6.1 ABSTRACT

Vaccination trials and comparative immunogenicity study using $NDVI_2$ and ND La Sota vaccines administered to commercial and rural chickens through untreated sorghum, parboiled sorghum, sorghum coated with gum Arabic and a commercial chick mash feed as carriers was conducted. Newcastle vaccine strain I_2 and ND La Sota vaccines provided protection to commercial and rural chickens vaccinated through intraocular, intramuscular or drinking water. No significant difference ($P<0.05$) was observed in the antibody titre of commercial or rural chickens vaccinated with either $NDVI_2$ or ND La Sota vaccines administered through commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and untreated sorghum. Newcastle vaccine strain I_2 or ND La Sota vaccines administered through commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and untreated sorghum gave no or limited protection (0-22%) to the birds when challenged with a local strain of velogenic viscerotropic Newcastle disease virus Kudu 113 strain. It was concluded that untreated sorghum, parboiled sorghum, sorghum coated with gum Arabic and commercial feed mash when used as feed carriers for $NDVI_2$ vaccine gave low antibody titre and less protection following challenge. However, $NDVI_2$ and ND La Sota vaccine when administered to chickens through intraocular, intramuscular or drinking water would be suitable for use in the control of ND in rural chickens in Plateau state.

6.2 INTRODUCTION

The choice of vaccine is governed by three main considerations namely (a) the immunogenecity of the vaccine (b) the type of vaccine (inactivated or live) and (c) virulence and spreading potential of the vaccine strains, not only for the recipient host but also for other birds which may become infected due to lateral spread of the vaccine virus (Alexander, 1995). The method of application of vaccines is also important (Alexander, 1995). For live vaccines, the likely exacerbation of infection with other organisms or vaccines strains must be considered (Alexander, 1995).

With modern techniques, considerable antigenic diversity has been detected in ND isolates and strains (Russell *et al*., 1990). However, the vaccine commonly use are capable of producing antibodies that protect against those NDV isolates showing a high degree of antigenic diversity from the live vaccine strain (Alexander *et al*., 1992). Therefore, antigenic variation is not an important consideration in the choice of ND vaccine (Alexander, 1995). Though the most popular live vaccines evolved from field isolates of low virulence, one difficulty associated with these live vaccines is that the immune response appears to be related to the virulence of the virus strains (Reeve *et al*., 1974). La Sota for example gives better protective antibody titres than Hitchner B1 but is more likely to cause adverse reactions.

In an attempt to make delivery of ND vaccine easier to rural chickens different types of feed stuffs have already been tested as carriers for the vaccine. Not all feed stuffs were found to be suitable and some such as sorghum, millet and other small grains have not yet been studied in detail (Spradbrow, 1992b; Musa, 2002). As sorghum is one of the staple foods produced in many areas of Nigeria, a laboratory vaccination trial was conducted using sorghum coated with gum Arabic, parboiled sorghum, untreated sorghum and a commercial feed mash as vaccine carriers of NDVI$_2$ vaccine. The objectives of the study were:

1. To compare the potency of the NDV I$_2$ vaccine strain and ND La Sota vaccine.
2. To compare the immunogenecity of NDV I$_2$ vaccine strain and ND La Sota vaccine administered to rural and commercial chickens via intraocular, intramuscular, drinking water, untreated sorghum, parboiled sorghum and parboiled sorghum coated with gum Arabic.

6.3 MATERIALS AND METHODS

6.3.1 Experimental site

The experiments were conducted in the Viral Research and Poultry Divisions of the NVRI, Vom.

6.3.2 Commercial chickens

One hundred and twenty day old unsexed Shika Brown chicks were obtained from the Poultry Research Farm, NVRI, Vom. The chicks were housed in a room previously cleaned, washed, disinfected and fumigated. They were fed chick mash and provided with water *ad libitum.* At three weeks of age, the birds were tagged individually with numbered wing tags and randomly allocated into twelve experimental groups of ten chicks each and vaccinated with NDV La Sota or NDVI$_2$ vaccines via drinking water, intraocular, intramuscular, sorghum coated with gum Arabic, parboiled sorghum, untreated sorghum and a commercial chick mash feed.

6.3.3 Rural chickens

One thousand rural chickens eggs were purchased from the open market in Jos South and Kanam Local Government Areas of Plateau State. After selection, 876 eggs were found to be suitable for incubation. The eggs were candled on the 18th day of incubation. One hundred and seventy eight chicks were hatched (20.3% hatchability) and harvested between the 21st and 22nd day of incubation.

The hatched chicks were selected on the basis of fitness, uniformity and body weight. At three weeks of age, one hundred and twenty chicks were tagged individually with numbered wing tags and bled to determine their pre-vaccination HI antibody titre and then the chicks were randomly allocated into 12 experimental groups of ten chicks each and vaccinated with NDV La Sota or $NDVI_2$ vaccines via drinking water, intraocular, intramuscular, sorghum coated with gum Arabic, parboiled sorghum, untreated sorghum or a commercial feed.

6.3.4 Vaccine food carriers

Commercial chick mash feed was obtained from NVRI, Vom Feed Mill. Five kilogram of sorghum and two kilogram of gum Arabic were purchased from a local market in Bukuru, Jos South LGA of Plateau State.

6.3.5 Vaccines

Newcastle disease vaccine strain I_2 with a titre of $10^{10.2}$ EID_{50} per vial and ND La Sota vaccine with a titre of $10^{9.8}$ EID_{50} /vial in lyophilized form was obtained from the Virology Division of NVRI, Vom.

6.3.6 Phytochemical and proximate analysis of the food carrier

Proximate and phytochemical analysis of sorghum and gum arabic was carried out as described by A.O.A.C (1995) in the Biochemistry and Molecular Biology Division of the NVRI, Vom.

6.3.7 Preparation and coating of food carrier with vaccine virus

Two kilogram of the sorghum was washed with clean water, sieved and dried. The sorghum was packed in polythene bags and stored at room temperature until used for coating with vaccine virus. The method described by Alders and Spradbrow (2001), with slight modification was used for coating the sorghum with vaccine virus. About 100 g of gum Arabic was dissolved in 1,000 ml of distilled water and boiled for 30 min and allowed to cool. One vial of the freeze-dried $NDVI_2$ vaccine containing 100 doses was reconstituted in 10 ml of PBS (pH 7.4). Then 40 ml of diluted gum Arabic was thoroughly mixed with the reconstituted vaccine (100 doses), mixed with one kilogram of sorghum and the mixture of the vaccine, gum Arabic and sorghum was thoroughly mixed manually. After mixing, the coated food vaccine was then spread on metal trays and kept at room temperature (RT) to dry under gentle air current for 30 min. About 10 g of the sorghum was given to 10 birds.

6.3.8 Challenge of experimental chickens

The challenge virus and challenge study was conducted as described in sections 5.7.6 and 5.7.10.

6.3.9 Blood collection

Blood was collected as stated in section 4.7.5.

6.3.10 Serology

Sera were tested for NDV antibodies as outlined in section 4.7.8.

6.3.1 Data Analysis

Data was analysed as stated in section 4.7.9.

6.4 RESULTS

Table 6.1 shows that tannins and saponins were detected in gum Arabic, while anthraquinone and alkaloids are contained in sorghum. Steroids and flavonoids are absent in sorghum and gum Arabic, while cardiac glycosides were present in sorghum and gum Arabic. Sorghum contained a higher percentage of crude protein (12.0.4%) and crude fibre (8.97%) than gum Arabic (4.29% C.P and 5.09% crude fibre) (Table 6.2).

There were no significant differences ($P \geq 0.05$) in the prevaccination HI antibody titres. Furthermore, no significant difference ($P \geq 0.05$) in HI antibody titres in chicks vaccinated with $NDVI_2$ or ND La Sota vaccines administered through im route or via dw at two and three week post vaccination (Table 6.3). The highest HI antibody titre (6.3 ± 1.3 Log_2 and 9.8 ± 1.2 Log_2) were observed in chicks vaccinated with ND La Sota via intraocular route two and three weeks post vaccination. The lowest (0.0 ± 0.0 Log_2) HI antibody titre was observed in unvaccinated control group. All the groups responded to challenge with velogenic NDV and no significant difference in HI titre was observed two weeks post challenge.

Table 6.4 shows commercial chickens with HI antibody titre of ≥ 4.0 Log_2 at three weeks post vaccination with $NDVI_2$ or La Sota vaccines administered through different routes and vaccine carriers. About 90% of the birds in CCi/o group produced HI antibody titre of $\geq$ 5.0 Log_2 (the cut off titre considered for this study was $\geq$ 4.0 Log_2), while 90% of the birds in CCF2a that received the $NDVI_2$ in feed produced HI antibody titre of $\leq$ 1.0 Log_2.

Commercial chickens vaccinated with $NDVI_2$ via i/o and im routes and were vaccinated with NDV La Sota via the same routes were all protected (100%) against challenge (Table 6.5). The least protected group were chicks vaccinated with $NDVI_2$ via parboiled sorghum coated with gum Arabic.

Proventricular haemorrhages, haemorrhagic enteritis and skeletal haemorrhages were observed in the groups vaccinated via commercial feed, sorghum coated with gum Arabic, parboiled sorghum and unvaccinated controls (Table 6.10). Clinical signs and postmortem lesions in rural chickens after challenge were similar to those observed in commercial chickens (Table 6.10).

Somnolence, legs and wings paralysis, torticollis and colonic spasm and haemorrhagic enteritis, proventricular haemorrhages, skeletal haemorrhages and ballooned ceca were observed in commercial chicks after challenge with NDV (Table 6.6). The clinical signs and postmortem lesions were similar in all the groups.

The highest (7.8$\pm$ 0.9 Log_2 and 9.3 $\pm$ 1.4 Log_2) HI antibody titre of rural chicks vaccinated with $NDVI_2$ or ND La Sota vaccine via different routes and vaccine carriers was observed two and three PV, respectively in group LCdw that were vaccinated with $NDVI_2$ via drinking water. The lowest (0.0 $\pm$ 0.0 Log_2) HI antibody titre was observed in the unvaccinated control group. There was no significant difference in HI antibody titre of chicks vaccinated via i/o, im, dw and commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic with $NDVI_2$ and ND La Sota vaccines. HI antibody titres were also not significantly different in birds vaccinated two weeks post challenge (Table 6.7).

About 80% of the birds in LCi/m and LCdw groups produced HI antibody titre of $\geq$ 4.0 Log_2 while 80% of the birds in LCF1b that received the ND vaccine strain I_2 in feed produced HI antibody titre of $\leq$ 1.0 Log_2 (Table 6.8).

Commercial chickens in groups vaccinated with $NDVI_2$ through i/o and im routes were 100% protected. Similarly, those chicks vaccinated with ND La Sota vaccine via i/o or dw had complete (100%) protection against challenge with velogenic NDV. The least protected were chicks vaccinated with $NDVI_2$ via commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and ND La Sota vaccine via commercial feed.

For the rural chickens 100% protection against challenge with local strain of velogenic NDV was observed among birds vaccinated with $NDVI_2$ or ND La Sota vaccine via i/o, im or dw (Table 6.9). High morbidity (100%), mortality and case fatality rates were observed in groups that were either vaccinated with $NVDI_2$ or La Sota via parboiled sorghum, sorghum coated with gum Arabic, untreated sorghum and commercial feed (Table 6.9).

Table 6.1: Phytochemical parameters of sorghum and gum Arabic.

Parameter	Sorghum	Gum Arabic
Saponins	Negative	Positive
Anthraquinone	Positive	Negative
Cardiac glycosides	Positive	Positive
Steroids	Negative	Negative
Flavonoids	Negative	Negative
Tannins	Postive	Positive
Alkaloids	Positive	Negative

Table 6.2: Proximate composition of sorghum and gum Arabic per 100 g dry matter.

Sample	Moisture	Crude Protein	Crude Fiber	Lipids	Ash	NFE	Calcium	Phosphorus
Sorghum	9.04	12.04	8.97	4.43	1.41	73.15	0.05	0.30
Gum Arabic	10.36	4.29	5.09	1.29	1.86	87.51	0.03	0.28

Key: NFE = Nitrogen free extract

Table 6.3: Geometric mean antibody titres (GMT) in sera of commercial chickens vaccinated with NDVI$_2$ or NDV La Sota vaccine administered through different routes and vaccine carriers.

Group	Vaccine	Route/vehicle of administration	GMT $\pm$ SD (Log_2) Pre-vaccination (n)	2 weeks post vaccination (n)	3 weeks post vaccination (n)	2 weeks post challenge (n)
CCIi/o	NDVI$_2$	Intraocular	0.0 $\pm$ 0.0 (10)	5.2 $\pm$ 1.4^a (9)	7.9 $\pm$ 2.1^c(9)	10.8 $\pm$ 1.6^e(9)
CCIi/m	NDVI$_2$	Intramuscular	0.0 $\pm$ 0.0 (10)	6.7 $\pm$ 1.6^a (9)	8.6 $\pm$ 2.2^c (8)	11.3 $\pm$ 0.9^e (8)
CCIdw	NDVI$_2$	Drinking water	0.0 $\pm$ 0.0 (10)	4.8$\pm$ 2.2^a (9)	8.8 $\pm$ 3.2^c (8)	11.4 $\pm$ 0.8^e (7)
CCIF1a	NDVI$_2$	Commercial feed	0.0 $\pm$ 0.0 (10)	0.3 $\pm$ 0.5^b (9)	1.0 $\pm$ 0.7 ^{d}(9)	11.7 $\pm$ 0.6^e (2)
CCIF2a	NDVI$_2$	Parboiled sorghum	0.3 $\pm$ 0.2 (10)	0.4 $\pm$ 0.7^b (9)	1.7 $\pm$ 2.0^d (9)	12.0 $\pm$ 0.0^e (2)
CCIF3a	NDVI$_2$	Parboiled sorghum coated with gum Arabic	0.2 $\pm$ 0.4 (10)	0.4 $\pm$ 0.7^b (9)	0.4 $\pm$ 0.7^d (9)	12 $\pm$ 0.0^e (1)
CCIF5a	NDVI$_2$	Untreated sorghum	0.2 $\pm$ 0.4 (10)	0.5 $\pm$ .7^b (10)	0.5 $\pm$ 1.0 ^{d} (10)	12$\pm$ 0.0^e (1)
CCLi/o	NDV La Sota	Intraocular	0.0 $\pm$ 0.0 (10)	6.3$\pm$1.3^a(10)	9.8 $\pm$ 1.2^c (10)	11.4 $\pm$ 0.7^e (10)
CCLi/m	NDV La Sota	Intramuscular	0.0 $\pm$ 0.0 (10)	4.9 $\pm$ 1.1^a(8)	6.3 $\pm$ 1.6^c (8)	10.6 $\pm$ 1.0^e (8)
CCLdw	NDV La Sota	Drinking water	0.0 $\pm$ 0.0 (10)	5.3$\pm$ 2.3^a (10)	8.1 $\pm$ 3.1^c (10)	10.6 $\pm$ 1.9^e (10)
CCLF1a	NDV La Sota	Commercial feed	0.0 $\pm$ 0.0 (10)	0.1 $\pm$ 0.3^b (9)	1.2 $\pm$ 1.1^d (9)	8.0$\pm$ 0.0^e (1)
Control	None	None	0.0 $\pm$ 0.0 (10)	0.0 $\pm$ .0^b (10)	0.0 $\pm$ 0.0 (10)	12$\pm$ 0.0 (1)

(n)* = number of chicks in the group, CC = commercial chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= NDV La Sota, I = NDVI$_2$ 1a= commercial feed, 2a Parboiled sorghum, 3a = Parboiled sorghum coated with gum Arabic, 5a = Untreated sorghum

$^{a,b,\ c,\ d\ \text{and}\ e}$ = Means with the same letters in the same column are not significantly different at 0.05 confidence level.

Table 6.4: The H1 antibody titre of commercial chickens post vaccination with $NDVI_2$ or NDV La Sota vaccines administered through different routes and vaccine carriers.

Group	Titre (Log_2)/number with titre												% with ≥4.0 Log_2	Mean titre Log_2
	0	1	2	3	4	5	6	7	8	9	10	11		
CCIi/o	0	0	0	0	0	1	3	0	0	3	1	1	100	7.9±2.1
CCI i/m	0	0	0	0	1	0	0	0	4	1	2	1	90	8.6±2.2
CCIdw	0	1	0	0	0	0	0	0	0	1	6	0	70	8.8±3.2
CCIFla	2	5	2	0	0	0	0	0	0	0	0	0	00	1.0±0.7
CCIF2a	4	0	3	1	0	0	1	0	0	0	0	0	0	1.7±2.0
CCIF3a	6	2	1	0	0	0	0	0	0	0	0	0	0	0.4±0.7
CCiF5a	7	2	0	1	0	0	0	0	0	0	0	0	0	0.5±1.0
CCLi/o	0	0	0	0	0	0	0	0	0	0	2	8	100	9.8±1.2
CCLi/m	0	0	0	0	0	4	0	0	4	0	0	0	80	6.3±1.6
CCLdw	0	0	0	0	0	1	0	1	1	2	3	1	90	8.1±3.1
CCLFla	1	7	0	0	1	0	0	0	0	0	0	0	0	1.2±1.1
Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0.0±0.0

CC = commercial chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_2$
1a = commercial feed, 2a Parboiled sorghum, 3a = Parboiled sorghum coated with gum Arabic, 5a = Untreated sorghum

Table 6.5: Morbidity, mortality case fatality and protection rates in commercial chickens vaccinated with $NDVI_2$ or NDV La Sota vaccine administered through different routes and vaccine carriers and challenged with a local strain of velogenic NDV intramuscularly.

Group	Vaccine	Route/vehicle of administration	Morbidity rate	Mortality rate	Case fatality rate	Protection rate
CCIi/o	$NDVI_2$	Intraocular	0	0	0	100
CCIi/m	$NDVI_2$	Intramuscular	0	0	0	100
CCIdw	$NDVI_2$	Drinking water	30.0	20.0	67.0	78.0
CCIF1a	$NDVI_2$	Commercial feed	100	80.0	80.0	11.0
CCIF2a	$NDVI_2$	Parboiled sorghum	100	80	80	11.0
CCIF3a	$NDVI_2$	Parboiled sorghum coated with gum Arabic	100	90.0	90.0	0.0
CCIF5a	$NDVI_2$	Untreated sorghum	100	90.0	90.0	0.0
CCLi/o	NDV La Sota	Intraocular	0	0	0	100
CCLi/m	NDV La Sota	Intramuscular	20.0	20.0	67.0	78.0
CCLdw	NDV La Sota	Drinking water	0	0	0	100
CCLF1a	NDV La Sota	Commercial feed	100	90.0	80.0	11.0
Unvaccinated Control	None	None	100	90.0	90.0	10.0

CC = commercial chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= LaSota, I = $NDVI_2$
1a = commercial feed, 2a Parboiled sorghum, 3a = Parboiled sorghum coated with gum Arabic, 5a = Untreated sorghum

Table 6.6: Clinical signs and postmortem lesions in commercial chicks vaccinated with $NVDI_2$ or La Sota administered through different routes and challenged with a local strain of velogenic NDV virus Kudu 113 strain.

Group	Clinical signs	Post mortem lesions
CCIdw	Depression, anorexia, whitish diarrhoea	Haemorrhagic enteritis,haemmorhages in the cecal tonsils
CCIF1a	Somnolence, legs and wings paralysis, torticollis and colonic spasm	Haemorrhagic enteritis, proventricular haemorrhages, skeletal haemorrhages and ballooned ceca
CCIF2a	Depression, anorexia, whitish/greyish diarrhoea	Congested liver, lungs and whitish diarrhea
CCIF3a	Recumbent, moribund, legs and wings paralysis	Greenish haemorrhagic enteritis congested liver and spleen
CCIF5a	Recumbent and moribund, leg paralysis wing paralysis	Skeletal haemorrhages, necrotic foci in the ileum and haemorrhagic enteritis
CCLdw	Depression, anorexia, whitish diarrhoea	Congestion of liver and lungs, proventricular haemorhages
CCLF1a	Somnolence, legs and wings paralysis, torticollis and colonic spasm	Haemorrhagic enteritis, proventricular haemorrhages, skeletal haemorrhages and ballooned ceca
Control	Wings and legs paralysis, moribund, colonic spasm, greyish yellow diarrhoea, sudden death and torticollis.	Haemorrhages in the breast muscle, haemorrhagic enteritis, haemorrhages in the proventriculus, ileocecal haemorrhages and haemorrhages in the thigh muscle.

CC = commercial chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_{2,}$1a = commercial feed, 2a Parboiled sorghum, 3a = Parboiled sorghum coated with gum Arabic, 5a = Untreated sorghum

Table 6.7: Geometric mean antibody titre (GMT) Log_2 of rural chickens vaccinated with $NDVI_2$ or NDV La Sota vaccine administered through different routes and vaccine carriers.

Group	Vaccine	Route/vehicle of administration	GMT $\pm$ SD (Log_2)			
			Pre-vaccination (n)	2 weeks post vaccination(n)	3 weeks post vaccination(n)	2 weeks post challenge (n)
LCIi/o	$NDVI_2$	Intraocular	0.0 $\pm$ 0.0 (10)	6.4 $\pm$ 1.5^a (9)	8.7 $\pm$ 2.8^c(9)	11.0 $\pm$ 0.8^e (9)
LCIi/m	$NDVI_2$	Intramuscular	0.0 $\pm$ 0.0 (10)	5.9 $\pm$ 1.1^a (8)	8.0 $\pm$ 1.4^c(8)	10.9 $\pm$ 0.8^e (8)
LCIdw	$NDVI_2$	Drinking water	0.0 $\pm$ 0.0 (10)	7.8$\pm$ 0.9^a (8)	9.3 $\pm$ 1.4^c(8)	10.3 $\pm$ 1.0^e (8)
LCIF1b	$NDVI_2$	Commercial feed	0.0 $\pm$ 0.0 (10)	0.3 $\pm$ 0.5^b (8)	2.6 $\pm$ 0.7^d(8)	11$\pm$ 0.0^e (1)
LCIF2b	$NDVI_2$	Parboiled sorghum	0.0 $\pm$ 0.0 (10)	0.4 $\pm$ 0.5^b (7)	1.3 $\pm$ 0.8^d(7)	12 $\pm$ 0.0^e (1)
LCIF3b	$NDVI_2$	Parboiled sorghum coated with gum Arabic	0.0 $\pm$ 0.0 (10)	0.8 $\pm$ 0.4^b (8)	1.7 $\pm$ 0.4^d(8)	12 $\pm$ 0.0^e (1)
LCIF5b	$NDVI_2$	Untreated sorghum	0.0 $\pm$ 0.0 (10)	0.3 $\pm$ 0.4^b (8)	1.2 $\pm$ 0.4^d(8)	12 $\pm$ 0.0^e (2)
LCLi/o	NDV La Sota	Intraocular	0.0 $\pm$ 0.0 (10)	6.6$\pm$ 1.8^a (9)	7.3 $\pm$ 1.7^c(9)	9.8 $\pm$ 2.1^e (9)
LCLi/m	NDV La Sota	Intramuscular	0.0 $\pm$ 0.0 (10)	5.1 $\pm$ 1.2^a (9)	5.7 $\pm$ 3.9^c(9)	10.3 $\pm$ 1.3^e (9)
LCLdw	NDV La Sota	Drinking water	0.0 $\pm$ 0.0 (10)	5.3$\pm$ 1.0^a(9)	6.4$\pm$ 2.4^c(9)	10.3$\pm$ 1.7^e(9)
LCLF1b	NDV La Sota	Commercial feed	0.0 $\pm$ 0.0 (10)	0.8 $\pm$ 0.8^b (9)	0.7 $\pm$ 0.7^d(9)	12 $\pm$ 0.0^e (1)
Control	None	None	0.0 $\pm$ 0.0 (10)	0.0 $\pm$ 0.0 (9)	0.0 $\pm$ 0.0 (9)	12.0 $\pm$ 0.0 (2)

LC = rural chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_2$, 1b = commercial feed, 2b = Parboiled sorghum, 3b = Parboiled sorghum coated with gum Arabic, 5b = Untreated sorghum

[a,b, c, d and e] = Means with the same letters in the same column are not significantly different at 0.05 level of significance

Table 6.8: The HI antibody titre of rural chickens post vaccination with $NDVI_2$ or NDV La Sota vaccine administered through different routes and vaccine carriers.

Group	Titre (Log_2)/number with titre													% with ≥ 4.0 Log_2	Mean titre (Log_2)
	0	1	2	3	4	5	6	7	8	9	10	11	12		
LCI i/o	0	0	0	0	1	0	1	0	2	0	2	0	2	80	8.7±2.8
LC Ii/m	0	0	0	0	0	0	1	2	3	0	2	0	0	80	8.0±1.4
LCIdw	0	0	0	0	0	0	0	1	0	5	1	0	1	80	9.3±1.4
LCIFla	0	1	1	6	0	0	0	0	0	0	0	0	0	0	2.6±0.7
LCIF2a	1	3	3	0	0	0	0	0	0	0	0	0	0	0	1.3±0.8
LCIF3a	0	5	1	0	0	0	0	0	0	0	0	0	0	0	1.7±0.4
LCIF5a	0	5	1	0	0	0	0	0	0	0	0	0	0	0	1.2±0.4
LCL i/o	0	0	0	0	0	0	1	0	1	1	1	1	2	70	9.8±2.1
LCL i/m	0	0	1	2	0	3	0	0	1	0	2	0	0	60	5.7±3.0
LCL dw	0	0	0	1	0	3	1	0	1	1	1	0	0	70	6.4±2.4
LCLF1a	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0.7±0.7
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0±0.0

LC = rural chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_{2,}$1b = commercial feed, 2b = Parboiled sorghum, 3b = Parboiled sorghum coated with gum Arabic, 5b = Untreated sorghum

Table 6.9 : Morbidity, mortality case fatality and protection rate of local chickens vaccinated with $NDVI_2$ or ND LaSota vaccine administered through different route and vaccine carrier and challenged with local strain of velogenic NDV intramuscularly.

Group	Vaccine	Route/vehicle of admnistration	Morbidity rate	Mortality rate	Case fatality rate	Protection rate
LCIi/o	$NDVI_2$	Intraocular	0	0	0	100
LCIi/m	$NDVI_2$	Intramuscular	0	0	0	100
LCIdw	$NDVI_2$	Drinking water	0	0	0	100
LCIF1b	$NDVI_2$	Commercial feed	100	70.0	87.5	22.0
LCIF2b	$NDVI_2$	Parboiled sorghum	100	70.0	87.5	22.0
LCIF3b	$NDVI_2$	Parboiled sorghum coated with gum Arabic	100	70.0	87.5	22.0
LCIF5b	$NDVI_2$	Untreated sorghum	100	80.0	75.0	11.0
LCLi/o	NDV La Sota	Intraocular	0	0	0	100
LCLi/m	NDV La Sota	Intramuscular	0	0	0	100
LCLdw	NDV La Sota	Drinking water	0	0	0	100
LCLF1a	NDV La Sota	Commercial feed	100	90.0	89.0	10.0
Control	None	None	100	90.0	100	10.0

LC = rural chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_{2}$, 1b = commercial feed, 2b = Parboiled sorghum, 3b = Parboiled sorghum coated with gum Arabic, 5b = Untreated sorghum

Table 6.10: Clinical signs and postmortem lesions in rural chicks vaccinated with $NVDI_2$ and La Sota administered through different routes and challenge with local velogenic NDV virus Kudu strain

Group	Clinical signs	Postmortem lesions
LCIF1b	Listeness, depression, anorexia, whitish/greyish diarrhoea	Congested liver, lungs and whitish diarrhoea
LCIF2b	Whitish diarrhoea , recumbent, moribund, legs and wings paralysis	Greenish haemorrhagic enteritis congested liver and spleen
LCIF3b	Recumbent and moribund, leg paralysis wing paralysis	Proventricular haemorrhages, necrotic foci in the ileum and haemorrhagic enteritis
LCIF5b	Anorexia, weakness, diarrhoea	Proventricular haemorrhages, necrotic foci in the ileum and haemorrhagic enteritis
LCIdw	Weakness, whitish diarrhoea, anorexia	Haemorrhagic enteritis, proventricular haemorrhages
LCIF1b	Wings and legs paralysis, moribound, colonic spasm, greyish yellow diarrhoea sudden death and torticollis	Hemorrhages in the breast muscle, haemorrhagic enteritis, haemorrhages in the proventriculus, ileo- cecal haemorrhages and haemorrhages in the thigh muscle
Control	Anorexia, somnelence, wings and legs paralysis, moribound, colonic spasm greyish yellow diarrhoea and torticollis.	Hemorrhages in the breast muscle, haemorrhagic enteritis, haemorrhages in the proventriculus, ileo- cecal haemorrhages and haemorrhages in the thigh muscle

LC = rural chicken, dw = drinking water, F= feed, I = $NDVI_{2,}$1b = commercial feed, 2b = Parboiled sorghum, 3b = Parboiled sorghum coated with gum Arabic, 5b = Untreated sorghum

6.5 DISCUSSION

Apart from depopulation to stamp out, the most effective and reliable control method for ND is by vaccination (Echeonwu *et al.,* 2008a). The vaccine used for ND control must be able to protect the target chickens to an acceptable level to be regarded as useful. Oral vaccination of chickens with thermostable vaccines requires that the virus be attached to the food, survives and released on ingestion by the chicken (Spradbrow, 1992a).

The efficacy of any vaccine is determined mainly by assessment of the level of antibody produced in the target animal and ability of the vaccinated animal to resist exposure to the virulent agent when compared with unvaccinated control (Spradbrow, 1993/1994). The result obtained in this study showed that at three weeks post vaccination, all commercial and rural chickens that received $NDVI_2$ or ND La Sota vaccines via i/o or dw, produced high level of HI antibody titre which was considered protective. The suggested and reported protective antibody titres for ND vaccines are HI $\geq$ 4 Log_2 (OIE, 2000). By implication, antibody titre less than $\geq$ 4 Log_2 may not be protective. The vaccine viruses ($NDVI_2$ and NDV La Sota) as observed in this study were immunogenic and protective only in commercial or rural chickens that were vaccinated via i/o, im or dw routes and not protective in birds vaccinated via commercial feed, parboiled sorghum, parboiled sorghum coated with gum arabic and untreated sorghum. Earlier reports on similar investigations showed varying outcomes in protecting the chickens against challenge with a virulent ND (Aini *et al.,* 1990a; Echeonwu *et al.,* 2008b). Failure of some of these trials using grains as vaccine carriers to protect the chickens was blamed on antiviral factors constituent in the seed or introduced as preservatives. The presence of tannins in sorghum and gum arabic as observed in this study may have been responsible for inactivating the vaccine virus.

As tannins are known to form chellates with ND vaccine virus making it unavailable to the birds, this effect of tannins on NDV may probably be associated with substances that inactivate the NDV and with binding to food lectins (Spradbrow, 1992b).

Bioactive chemical compounds like saponins present in gum Arabic are known to be immune pontentiators in birds and other mammals (Hughes *et al.*, 1958).The nutritional composition of sorghum showed that it contains high percentage of protein (12.0.4%) and crude fibre (8.97%) than gum Arabic (4.29% C.P, 5.09% crude fibre), in addition to being a vaccine carrier, the protein content in the sorghum and gum Arabic would therefore have been an additional source of nutrient for the birds.

Newcastle disease vaccines administered orally have been reported to primarily provoke mucosal immunity (Parry and Aitken, 1977; Jayawardane and Spradbrow, 1992a). It is thought that this is the first line of defense against NDV infection, which occurs either by inhalation or ingestion or both (Alexander, 1988). This arm of humoral immunity is reported to be responsible for protection of the chickens even before detectable HI antibody is found in the serum (Spradbrow, 1992b). Although OIE (2000) recommended HI (Log_2) titre of 4.0 as protective with reference to conventional ND vaccines designed for intensively reared commercial chickens, HI antibody titre of 3.0 Log_2 was considered to be adequate for food-based vaccines administered orally to scavenging chickens (Echeonwu *et al.*, 2007). This is more so since it has been found that even chickens with HI antibody titre of $\leq Log_2$ 3.0 resist challenge with velogenic ND virus as observed in both the commercial and rural chickens, indicating that serum antibody alone may not be responsible for the resistance to challenge.

Following challenge experiments to assess the potency of the vaccination method, clinical signs observed especially in those chickens that were administered vaccines via feed signs seen were similar to velogenic viscerotropic ND outbreaks. These signs were listlessness, increased respiration, weakness, prostration, greenish watery diarrhoea, and torticollis, paralysis of legs and wings, and death in that order within 3-5 days, ,haemorrhagic lesions in the small intestines, proventriculus and caecal tonsils. Other lesions observed were tracheal congestion and air sacculitis (McFerran and McCraken, 1988; Alexander 1997; Abdu *et al*., 2004).

The challenge experiments did not follow the natural routes of infection in the field, namely oral by drinking water in line with the suggestion of Spradbrow (1993/94) that the conventional intramuscular route would by-pass the natural route of infection in the field. Though, Iroegbu and Nchinda (1999) employed the drinking water route for challenge experiments with satisfactory results, it was however, considered in this experiment that for birds to receive equal doses of challenge virus and to assess the efficacy of vaccination on protection of the birds against velogenic ND virus in the laboratory, im route was considered the method of choice. It was concluded that

- The i/o, i.m, dw routes of $NDVI_2$ or NDV La Sota vaccines administration gave higher HI antibody titre and protection rate in commercial or rural chickens.
- Using untreated sorghum, parboiled sorghum, sorghum coated with gum Arabic and a commercial feed mash as feed carriers for $NDVI_2$ or ND La Sota vaccines gave low antibody titre and less protection following challenge with velogenic NDV.

It is recommended that

(a) Newcastle disease vaccine I_2 can be adopted as a vaccine of choice for protection against ND in rural chickens.

(b) Until a more effective vaccine carrier is developed $NDVI_2$ vaccine should be administered to rural chickens through i/o, im or dw.

(c) Other traditional feeds such as maize, millet, cassava or yam should be examined to determine the most suitable feed carrier for delivery of $NDVI_2$ vaccine in Nigeria.

CHAPTER SEVEN

7.0 COST IMPLICATIONS AND BENEFITS OF INTRAOCULAR VACCINATION OF RURAL CHICKENS, WITH NEWCASTLE DISEASE VACCINE STRAIN I_2

7.1 ABSTRACT

An estimate of cost benefit of ND control in rural chickens in four Local Government Areas in Plateau State using $NDVI_2$ via intraocular route and financial losses in the event of ND outbreaks in rural chickens at the prevalence and mortality rates of 40% and 28% respectively using static model was investigated. The estimates of direct financial losses due to ND outbreak in unvaccinated flocks in terms of value of birds and eggs amounted to ₦1,250,505.9 out of which the value of birds amounted to ₦799,195.5 and the value of eggs amounted to ₦451,310.4. The total cost estimates of vaccinating amounted to ₦187,669. The overall total annual financial implication of ND outbreak and vaccination with $NDVI_2$ through intraocular route was ₦1,577,592.4. The overall total financial losses avoided following vaccination was ₦10,296,264 while the benefit cost ratio arising from adopting $NDVI_2$ via intraocular route for control of ND in rural chickens is 7.2. It was concluded that the use of $NDVI_2$ via intraocular route for vaccination of rural household chickens in Plateau State was a good and an economically beneficial method for ND control. It is therefore strongly recommended as the most feasible, efficient and effective method of ND control in rural chickens.

7.2 INTRODUCTION

Rural household chickens rearing is the most widespread stock breeding bussiness in every household in rural areas (Nwanta, 2003). The bussiness contributes significantly to the improvement of Nigeria's rural and national economy. Wosu (1992) reported that rural poultry sector contributes 68.5% of the national poultry meat supply as against 11.39% from commercial chickens and 19.7% from other poultry, they provide scarce animal protein in the form of meat and eggs they are also for sale or barter in societies where cash is not abundant. Rural household poultry participates in pest control, manure provision and are used for special festivals and to meet social obligations (Alders and Spradbrow, 2001).

Newcastle disease has been described by Okeke and Lamorde (1988) as one of the most important poultry diseases of economic importance in Nigeria. Losses due to this disease have been recorded in many commercial farms and rural household poultry. Newcastle disease is reported from all parts of Nigeria (Ezeokoli *et al.,* 1984; Abdu *et al.,* 1985; Gomwalk *et al.,* 1985; Echeonwu *et al.,* 1993; Halle *et al.,* 1999). Studies carried out on prevalence and outbreaks of Newcastle disease in different parts of Nigeria showed varied results. In Plateau State, a prevalence of 40.0% was documented by Adu *et al.* (1986). In Ibadan, Oyo State seroprevalence of 38.0% was documented by Oyewola *et al.* (1996). In Maiduguri Borno State, Baba *et al.* (1998) recorded ND seroprevalence of 54.0%. While 63.0% seroprevalence was reported by Orajaka *et al.* (1999) in Southeast derived Savannah Zone of Nigeria. In Kaduna State 74.3% seroprevalence of NDV antibodies was documented by Nwanta (2003).

Any cost effective strategy like ND control that increases the productivity of rural household poultry will assist in poverty reduction, improve food security and increase access to protein intake, access to cash and other resources that culminate in improvement of the standard of living of the rural populace.

The objective of this study is aimed at determining the cost effectiveness of i/o vaccination using $NDVI_2$ versus no vaccination. The benefits that may be derived from these vaccinations will be determined and recommendations made where necessary.

7.3 MATERIALS AND METHODS

7.3.1 Questionnaire survey

The study area and sampling procedure used for the study were as described in section 3.3.1 and 3.3.2 of this dissertation. Data was collected using a structured questionnaire (Appendix II) on management, productivity, sales of birds and eggs through interviews with farmers were used. Representative flocks were chosen for the investigation using the simple random sampling technique of Putt *et al*. (1982). The flock structure was defined as chicks (0- 10 weeks old), cockerels (11-20 weeks old), pullets (11-20 weeks old), adult cocks ($\geq$ 21 weeks old) and adult hens ($\geq$ 21 weeks old). A market survey of the prices of chicks, growers, cocks, hens and eggs in Kanam and Barkin Ladi LGAs were made and used for the study.

7.3.2 Study design

Data on ND prevalence in ND affected flocks in Plateau State Adu *et al*. (1986) was used and average mortality rate in ND affected flocks in Kaduna State Abdu *et al.* (1992) was used because of the absence of available literature on average mortality rate in ND affected flocks in Plateau State.

The following information was obtained as shown in Table 7.1 to 7.3:

(i) Basic parameters for the analysis of the estimated financial losses and benefit cost ratio (Table 7.1).

(ii) Financial losses estimate due to ND outbreak in unvaccinated flocks (Table 7.2).

(iii) Cost of implementing vaccination against ND in the sampled flocks in Plateau State (Table 7.3).

Table 7.1: Basic parameters for the analysis of the estimated losses and benefit cost ratio

Discount rate	=	10.0
Prevalence rate of ND (Adu *et al.*, 1986)	=	40.0
Average mortality recorded in a flock (Abdu *et al.*, 1992)	=	28.0%
Percentage protection on using $NDVI_2$ in the field trial (Table 4.3)	=	92%
Chicken population in sampled households	=	27,078
Hens (31.4%)	=	8,498
Cocks (18.6%)	=	5,053
Chicks (29.5%)	=	7,987
Growers (20.5%)	=	5,540
Average number of chickens lost annually due to ND per household	=	18
Chicks (60%)	=	11
Mature chickens (40%)	=	7
Estimate of reproductive traits obtained through interview		
Annual average egg production per hen	=	40
Average number of chicks per hatch	=	9
Average number of eggs per clutch	=	12
Diluent per bird during vaccination	=	0.2 ml
Cost of diluent per bird per day during vaccination	=	₦ 0.14
Average cost of birds		
₦ / Hen	=	₦ 350
₦ /Cock	=	₦ 450
₦ /Chick	=	₦ 50
Cost of $NDVI_2$ (Subsidized rate) per dose	=	₦ 1.00
Transportation cost for the vaccination Programme		
₦ 600 per village per day x 32 x 4 days of visits	=	₦ 76,800.00
Labour for the vaccination		
20 Animal Health Officers @ ₦ 1,000 per day for 4 days	=	₦ 80, 000.00

7.4 RESULTS

Table 7.2 shows estimated financial losses in the event of ND outbreak in unvaccinated rural household chickens and costs of implementing vaccination against ND in the sampled flocks in Plateau State. At prevalence and mortality rates of 40% and 28%, respectively, the estimates of direct financial losses due to ND outbreak in unvaccinated flocks in terms of value of birds and eggs amounted to ₦ 1,250,505.9 out of which the value of birds amounted to ₦ 799,195.5 and the value of eggs amounted to ₦ 451,310.4 While the total cost estimates (cost of diluents, vaccines, transportation and labour) of financial cost of vaccinating 27,078 rural household chickens with $NDVI_2$ via intraocular route amounted to ₦ 187,669 .The overall Total annual financial implications of ND outbreak and vaccination with $NDVI_2$ through intraocular route was ₦ 1,577,592.4.

Table 7.3 shows an estimate of benefits of vaccinating rural household chickens with a $NDVI_2$ vaccine via intraocular route in four LGAs of Plateau State Nigeria. About 24,912 rural household chickens valued at ₦ 6,589,224 are expected to be protected from which 7,723 hens are expected to be saved. The saved chickens are expected to produce 308,920 eggs valued at ₦3,707,040. The overall total financial losses avoided following vaccination was ₦ 10,296,264 while the cost benefit ratio is 7.2.

Table 7.2: Estimates of financial losses to ND outbreak in unvaccinated flocks and costs of implementing vaccination against ND in the sampled flocks in Plateau State Nigeria.

(a).	Estimates of financial losses due to ND outbreak in unvaccinated flocks			
(i).	Direct losses due to mortality			
	-	Prevalence rate (morbidity rate)		= 0 .40 x 27,078 = 10,831
	-	Mortality rate in infected flocks		= 0.28 x 10,831 = 3,033
(ii).	Value of lost birds			
	-	Chicks	0.3 x 3,033 x ₦ 50	= ₦ 45,495
	-	Growers	0.2 x 3,033 x ₦ 250	= ₦ 151,650
	-	Cocks	0.2 x 3,033 x ₦ 450	= ₦ 272,970
	-	Hens	0.31 x 3,033 x ₦ 350	= ₦ 329,080.5
	-	Sub total		= ₦ 799,195.5
(iii).	Value of lost eggs (egg production losses) per annum			
	-	Hens	0.31 x 3,033 x 40 x ₦ 12	= ₦ 450,310.4
Sub total cost (a)				**= ₦ 1,250,505.9**

(b). Estimates of financial cost of vaccinating 27,078 rural household chickens with $NDVI_2$ via intraocular route

(i).	Cost of materials for the vaccination -		
	-	Cost of diluents for 27,078 birds @ ₦ 0.14	= ₦ 3,791
	-	Cost of vaccine for 27,078 birds x ₦ 1.00	= ₦ 27,078
(ii).	Cost of transportation during vaccination		= ₦ 76,800.00
(ii).	Labour for the vaccination		= ₦ 80,000.00
Sub total (b) =			**= ₦ 187,669**
(iv).	Total financial cost (losses in outbreak and cost of vaccination)		
	a + b (**₦ 1,250,505.9 + ₦ 187, 669**)		**= ₦ 1,438,174.9**
(v).	Discounted cost of vaccination		
	Future Value (FV) = Present Value (PV) $(1 + 0.1)^n$		
(vi).	Total annual financial implications of ND outbreak and vaccination with $NDVI_2$ through i/o = ₦ 1,438,174.9 $(1 + 0.1)^1$		**= ₦ 1,577,592.4**

Table 7.3: Estimate of benefits of vaccinating rural household chickens with a $NDVI_2$ vaccine via intraocular route in four local government areas of Plateau State Nigeria.

(i).	Protection offered by the vaccine			= 92 %
(ii).	Expected number of birds protected - 0.92 x 27,078			= ₦ 24,912
	-	Chicks	0.3 x 24,912 x ₦ 50	= ₦ 373,680
	-	Growers	0.2 x 24,912 x ₦ 250	= ₦ 1, 245,600
	-	Cocks	0.2 x 24,912 x ₦ 450	= ₦ 2,242,080
	-	Hens	0.31 x 24,912 x ₦ 350	= ₦ 2,702,952
(iii)	**Sub total (a)**			**= ₦ 6,589,224**

(iv) . Number of hens saved due to vaccination 0.31 x 24,912 = 7,723

(v). Annual egg production @ 40 eggs per hen x 7,723 = 308,920

(vi). Financial value of eggs @ ₦ 12 x 308,920 **(b)** = **₦3, 707,040**

(vii). Total financial losses avoided following vaccination (a + b) = **₦ 10,296,264**

(viii). Discounted benefits of vaccination

- Future Value (FV) = Present Value (PV) $(1 + 0.1)^n$

= $10,296,264(1 + 0.1)^1$ = **₦ 11,325,890.4**

Benefit Cost Ratio = $\dfrac{\sum \text{Benefits}}{\sum \text{Cost}} = \dfrac{11,325,890.4}{1,577,592.4}$

= **7.2**

7.5 DISCUSSION

It was observed from this study that the highest amount of money lost was from hens (₦ 329,080.5) and the least was from chicks (₦ 45,495). The low value of chicks unlike the hens may due to under valued as the chicks were not normally sold by rural chicken owners in Nigeria, however, the value was estimated based on the information supplied by farmers. The estimated financial losses and benefits due to ND outbreaks in the study area were based on direct and indirect chickens and egg losses. Nwanta (2003) reported that the direct losses are relatively easy to quantify as they vary with the prevalence of the disease. While indirect losses were based on the information supplied by the chicken owners and could vary with the prevalence and severity of the ND and also with the location of the area under consideration. The overall value of lost eggs and birds per annum amounted to ₦1,250,505.9, though the losses seems to be reticent but important if it is considered that rural household chickens constitute the majority of chicken population in the four LGAs under study. Nwanta (2003) reported that information on the exact financial losses due ND outbreak in rural household chickens in Nigeria is lacking. Similarly, Okeke and Lamorde (1988) reported that the implication of ND outbreak could be varied from many socio economic viewpoints from the lost of the cocks, hens, eggs and lack of growth of the flock population.

The estimated financial cost of vaccinating 27,078 rural household chickens with $NDVI_2$ via intraocular route seems to be modest compared to the benefits derived. Though, it is possible that some of the unit costs might have been under valued or overlooked, the use of $NDVI_2$ via intraocular route with benefit cost ratio of 7.2 showed it to be highly economically advantageous method for the control of ND in rural household chickens in the four LGAs in the study areas than the cost - benefit ratio of 2.0 that was reported by Nwanta *et al*. (2005) in Kaduna State when NDV4 HR was admnistered to chicken via feed. Costs like transportation and labour which make the bulk of the cost can be further cut down when farmers or extension agents are trained and involved in carrying out some of the activities. Earlier reports by Johnson and Cumming (1991) and Nwanta (2003) highlighted the resultant benefits of the cost effective method for controlling ND in rural household chickens. The increase in egg production and hatchability and the multiplier effect in relation to sales of chickens and eggs, income and welfare of rural farmers, particularly women and children are some of the benefits for effective ND control in rural household chickens (Johnson and Cumming, 1991).

It was concluded that the use of $NDVI_2$ via i/o route for vaccination of rural household chickens in Plateau State was better than when ND is allow to run its course in an unvaccinated rural chickens or when NDV4 HR was used in feed and an economically advantageous method for ND control. It is therefore highly recommended ($NDVI_{2)}$ as the most feasible, efficient and effective method of ND control in rural household chickens in Nigeria.

8.0 GENERAL CONCLUSIONS

1. At the time of the study about 86% of the rural household chickens in four local government areas (Jos South, Barkin Ladi, Kanam and Shendam) of Plateau State were at risk of dying of ND when exposed to a velogenic NDV.
2. Diseases such as ND are one of the most important causes of attrition of rural household chickens in Plateau State.
3. Sourcing for foundation stock from rural household market is the most popular means of starting or restocking a rural household chicken flock.
4. In the laboratory, $NDVI_2$ or NDV La Sota vaccines provided protection to commercial and rural poultry vaccinated through i/o, im or dw.
5. In the field ND, vaccination with $NDVI_2$ or NDV La Sota via drinking water may not be appropriate.
6. Commercial chick mash feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and untreated sorghum cannot be used as vaccine carriers.
7. The work has confirmed the thermostability of $NDVI_2$ vaccine. The vaccine can be stored for three days and 35 days at temperature of 29 to 30 ^{o}C in reconstituted and lyophilized form, respectively and still remain viable and potent.
8. The use of $NDVI_2$ via intraocular route for vaccination of rural household chickens in Plateau State has been found to be an economically advantageous method for ND control.

8.1 GENERAL RECOMMENDATIONS

- Newcastle disease vaccine I_2 should be adopted as a vaccine of choice for the control of rural household chickens against ND.
- Vaccination against ND before the period of high incidende (dry months) should be undertaken.
- The administration of $NDVI_2$ vaccine to rural household chickens in the field should be through i/o or i.m.
- Maize, millet, cassava or yam should be examined to determine the most suitable feed carrier for the delivery of $NDVI_2$ vaccine in Nigeria.
- Different processing methods of traditional feeds or their offal should be utilized to reduce or eliminate possible antiviral substances that may be present in feed grains.
- There is need to create awareness in form of extension on management practices that discourage spread and maintenance of ND in rural household chickens.
- Extension services should be focused on women who are responsible for most of the daily routines of managing rural household chickens.
- Extension services on the importance of ND control and how to administer the $NDVI_2$ vaccine to rural household chickens should be pursued.

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APPENDIX I: MAP OF PLATEAU STATE SHOWING THE 17 LOCAL GOVERNMENT AREAS.

Source : Anonymus (2006). Ministry of Information and Culture Plateau State.

APPENDIX II: LOCAL POULTRY PRODUCTION AND DISEASE CONTROL QUESTIONNAIRE.

NAME OF INTERVIEWER---

INTRODUCTION

Rural poultry are important part of rural life in Nigeria. Almost every household in rural communities keeps local poultry. One constraint to the production of these local poultry is disease. Therefore in order to assist rural poultry farmers reduce disease incidence in their flocks and increase production we would like to know more about the major problems associated with raising rural poultry. The response given by farmers to this questionnaire would be of assistance in making recommendation for improvement.

1. **Personal details**

Name	
District	
L.G.A	
Rural	
Male/Female	
Occupation	

2. **Number of chickens you have**

Cocks	Number
Hens	
Growers	
Chicks	

3. **Type of local chicken you have**

Type	Response (mark X)
Dwarf	
Naked Neck	
Frizzled	
White	,
Black	
Mottled	
Barred	
Long Legged	
Others specify	

4. **Which of these species of birds do you have?**

Species	Number
Ducks	
Turkeys	
Pigeons	
Guinea fowls	
Others (Specify)	

5. **Who owns the birds? (Mark the correct response with an "X")**

Husband	
Wife	
Sons	
Daughters	-
Others (Specify)	

6. **Are the chickens living with other animals?**

Species	Number
Goats	
Sheep	
Pigs	
Cattle's	
Rabbits	
Others (Specify)	

7. **Source of your birds (mark the correct response with an "X")**

Purchase from market	
As gift	
Inherited	
Others (Specify)	

8. **Do your birds have house**? Yes ( ) No ( )

9. **Where do your birds sleep?**

Location	Response
Tree	
Mud house on the ground	
Under the buildings	
Other (Specify)	

10. **Daily routine for rural poultry**

Activity	Who is responsible
Give water	
Give food	
Cleaning the house	,
Let the chicken out in the morning	
Shut the chicken in the night	
Check weather they are sick	
Others (specify)	

11. **Source of water?**

Well	
River/Stream	
Tap	
Pond	,
Rain water	
Bore hole	

12. **How many eggs do a bird produced per clutch?**

Species	Number of eggs,	Number hatched
Chicken		
G/fowl		
Duck		
Turkey		
Others (Specify)		

13. **How many chicken on average survive for first two months**

Species	Number
Chicken	
G/fowl	
Duck	
Turkey	
Others (Specify)	

14. **What food do you give your birds (tick)?**

Type of food	Response
Nothing	
Millet	
Maize	
Rice	
Maize offal	
Millet offal	
Kitchen waste	
Others (Specify)	

15. **How often do you feed the birds?**
1x 1 day (   )   2x / day (   )   3x / day (   ) Not at all (   )

16. **When do you feed them?**

Morning (   )   Afternoon (   )   Evening (   ) Any time (   )

17. **In your opinion what are the main causes of mortality (death) in your birds?**

Disease	
Theft	
Birds of prey	
Car accident	,
Dogs/Cats	
Snakes	
Lack of feed	
Others (specify)	

18. **If disease, do you isolate sick birds**   Yes (   )   No (   )

19. **What do you do with the death?** ___________________________

20. **How does the bird behave when sick?**

____

21. **How many of your birds have died in the last six months?**

From Disease (ND)			**Slaughter**		**Other causes**	
	Chicks	Adults	Chicks	Adults	Chicks	Adults
Chickens						
Ducks						
Turkeys						
G/Fowls						
Other						

22. **Are you aware of a disease called Newcastle Disease?**   Yes (   )   No (   )

23. **What is the local name for Newcastle disease?**

____

24. **In which month of the year is ND more likely to occur? Place X next to appropriate month(s)**

January	April	July	October
February	May	August	November
March	June	September	December

25. **What treatment do you give your birds when affected by ND? And how do you prepare the treatment.**

		Conventional	Traditional
a.	Treatment		
b.	How to prepare and Administer		

26. **Where do you get this treatment?**

Veterinary Service	
Traditional Medicine	
Pharmacy	
NGO/Project	,
Shops/ Open markets	
Others (specify)	

27. **Did you know that there are vaccines that can prevent ND Yes/ No?**

28. **Have you ever vaccinated your birds?** Yes ( ) No ( )

29. **If Yes ( ) what type of vaccine ________________________**

30. **Do you know there are vaccines that can prevent Newcastle disease?**
Yes ( ) No ( )

31. **Do you sell your sick birds?**
Yes ( ) No ( ) Sometime ( )

32. **Do you eat your sick birds?**
Yes ( ) No ( ) Sometime ( )

33. **Any other comments**