SURVEY FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE VIRUSES, POULTRY – WILD BIRD INTERACTIONS, BIOSECURITY AND HUSBANDRY PRACTICES IN POULTRY PRODUCTION IN KOGI STATE, NIGERIA

BY

Negedu Onogu AMEJI, *DVM, M.Sc* (A.B.U)
(Ph.D/VET.MED/11142/2010-2011)

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DECLARATION

I declare that this research work titled "**SURVEY FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE VIRUSES, POULTRY – WILD BIRD INTERACTIONS, BIOSECURITY AND HUSBANDRY PRACTICES IN POULTRY PRODUCTION IN KOGI STATE, NIGERIA"** was carried out by me in the Department of Veterinary Medicine, Ahmadu Bello University, Zaria under the supervision of Professors L. Sa'idu., P. A. Abdu and J. Kabir.

The information derived from relevant literature and the works of other researchers were duly acknowledged in the references. No part of this thesis has been previously presented elsewhere for the award of a degree or diploma.

Negedu Onogu AMEJI	_____	_____
Student	Signature	Date

CERTIFICATION

This thesis titled "**Survey for Avian Influenza and Newcastle Disease Viruses, Poultry – Wild Bird Interactions, Biosecurity and Husbandry Practices In Poultry Production In Kogi State, Nigeria" by** Negedu Onogu AMEJI meets the regulations governing the award of the degree of Doctor of philosophy of Ahmadu Bello University, Zaria and it is approved for its contribution to scientific knowledge and literary presentation.

Prof. L. Sa'idu Chairman, Supervisory Committee	Signature	Date
Prof. P.A. Abdu Member, Supervisory Committee	Signature	Date
Prof. J. Kabir Member, Supervisory Committee	Signature	Date
Prof. L.B. Tekdek Head of Department, Veterinary Medicine	Signature	Date
Prof. K. Bala Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This work is dedicated to the Almighty God, Who paid a price for my life; my parents, late Pa Negedu and Mrs Iyone Negedu Ameji who taught me never to turn back from the battle of life.

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Abstract

Avian influenza (AI) and Newcastle disease (ND) are important poultry diseases in Nigeria affecting commercial poultry productivity and might be the cause of low commercial poultry production in Kogi State. The study was carried out in 5 phases to survey AI and ND viruses in poultry and wild birds, poultry – wild bird interactions, biosecurity and husbandry practices in poultry production in twelve Local Government Areas (LGAs) of Kogi State, Nigeria. The first phase surveyed AI and ND viruses antibodies in poultry where a total of 779 sera were analyzed for AI (H5) antibodies using haemagglutination inhibition (HI) test which gave an overall seroprevalence of 4.2% with mean antibody titre of 8.42 ± 0.254$\log_2$. Using enzyme linked immunosorbent assay (ELISA), an overall seroprevalence of AI nucleoprotein (NP) antibodies in poultry was 30.8% with mean antibody titre of 16.588 ± 0.882 EU. Similarly, 779 poultry sera were analyzed by HI test for ND antibodies which gave an overall seroprevalence of 75.9% with mean antibody titre of 6.77 ± 0.141 $\log_2$. Using ELISA, an overall seroprevalence of ND in poultry was 68.1% with mean antibody titre of 34.487 ± 1.444 EU. The second phase also surveyed AI and ND viruses' antibodies in wild birds where sera of 50 species of free flying wild birds from 8 families were analyzed by HI test for AI (H5) antibodies but the results were negative. However, using ELISA an overall AI (NP) seroprevalence of 4.5% and mean antibody titre of 6.61 ± 0.78 EU were obtained in wild birds. The overall seroprevalence of ND antibodies in wild birds using HI test was 29.2% with mean antibody titre of 2.69 ± 0.51 $\log_2$. Using ELISA, the overall seroprevalence of ND in wild birds was 27.1% with mean antibody titre of 11.06 ± 1.28 EU. The third phase was a molecular survey for AI viruses in poultry and wild birds where a total of 810 cloacal and oropharyngeal swabs were tested using reverse transcription-polymerase

chain reaction (RT-PCR) with set of primers targeting the AI matrix proteins. All the tested samples were negative for AI viruses. The fourth phase was a molecular survey for ND viruses in poultry and wild birds where a total of 810 cloacal and oropharyngeal swabs were tested using RT-PCR with set of primers targeting the avian paramyxovirus-1 (APMV-1) matrix protein with 60.5% being positive for the viruses. Groups were compared with each other using χ^2 and the relative risk of infection of ND viruses between groups were calculated as odd ratio. The χ^2 values of backyard poultry sampling unit against other sampling units (OR = 0.70, 95% CI = 0.28 – 1.76), LGAs without surveyed wild bird against LGAs with surveyed wild birds (OR = 0.99, 95% CI = 0.40 – 2.46) and poultry species against wild bird species (OR = 0.98, 95% CI = 0.25 – 3.77) were not significant but that of contiguous areas A against contiguous areas B were significant (χ^2 =6.59, p≤ 0.01, OR = 4.10 at 95% CI = 1.34 – 12.28). The fifth phase assessed poultry – wild bird interactions, biosecurity and husbandry practices in poultry production in Kogi State. Questionnaire was administered to a total of 108 respondents by interview, 77.8% of the respondents affirmed to wild birds scavenging for food around their farms, households and LBMs; 66.7% of the respondents kept poultry on free range; 63.9% of the respondents had trees where wild birds could roost around their farms, households or LBMs; 8.3% of the respondents had water body/fish pond around their farms or households; 85.2% of the respondents would dispose litter and dead birds in refuse dumps, while 50.0% of the poultry around major rivers or wetlands. From this work it was concluded that there were AI (H5) and AI (NP) antibodies in poultry. There were no AI (H5) antibodies but there were AI (NP) antibodies in wild birds. There were antibodies to ND viruses in poultry and wild birds in the study area. There were no AI viruses but there were ND viruses in poultry and wild birds in the study area. Poor biosecurity

and management practices in poultry production encouraged direct and indirect contact of poultry and wild birds in the study area. It is recommended that further surveillance should be carried out for AIVs and to characterize the APMV-1 circulating in poultry and wild birds in Kogi State. Poultry farmers should avoid or dislodge existent risk factors that encourage poultry – wild bird interactions around their farms and households as well as undertake biosecurity in poultry production.

TABLE OF CONTENTS

Title Page ----------i

Declaration ----------ii

Certification ----------iii

Dedication ----------iv

Acknowledgements ----------v

Abstract ----------vii

Table of Contents ----------ix

List of Tables ----------xvi

List of Figures ----------xviii

List of Plates ----------xix

List of Appendices ----------xxi

List of Abbreviations ----------xxii

CHAPTER 1: INTRODUCTION----------1

1.1 General Introduction----------1

1.2 Statement of ResearchProblems----------5

1.3 Justifications of the Study----------7

1.4 Aim of the Study----------9

1.5 Objectives of the Study----------9

1.6 Research Questions----------9

CHAPTER 2: LITERATURE REVIEW----------10

2.1 Historical background of avian influenza----------10

2.1.1 Avian influenza virus classification----------12

2.1.2 Avian influenza virus morphology and composition ----------13

2.1.3 Avian influenza virus pathogenicity and virulence----------13

2.1.4 Antigenic drift and shift of avian influenza----------15

2.1.5 Avian influenza viruses replication and pathogenesis----------16

2.1.6 Clinical signs and pathology of avian influenza----------20

2.1.7 Epidemiology of avian influenza----------21

2.1.8 Avian influenza in Nigeria----------24

2.1.9 Avian influenza in Africa----------26

2.1.10 Avian influenza in wild birds----------27

2.1.11 Diagnosis and detection of avian influenza----------29

2.1.12 Prevention and control of avian influenza ----------35

2.1.13 Public health importance of avian influenza----------41

2.2 Historical Perspective of Newcastle Disease----------.43

2.2.1 Aetiology of Newcastle disease ----------44

2.2.2 Pathotypes of Newcastle disease ----------44

2.2.3 Transmission of Newcastle disease ----------45

2.2.4 Hosts of Newcastle disease ----------46

2.2.5 Public health importance of Newcastle disease----------47

2.2.6 Diagnosis of Newcastle disease----------47

2.2.7 Prevention and control of Newcastle disease ----------47

2.3 Wild Bird – Poultry Interactions----------48

2.4 Biosecurity and Husbandry Practices in Poultry----------50

CHAPTER 3: SERO – PREVALENCE OF AVIAN INFLUENZA AND NEWCASTLE DISEASE IN POULTRY----------53

3.1 Introduction----------55

3.2 Statement of the Research Problems----------56

3.3 Justifications of the Study----------57

3.4 Aim of the Study----------57

3.5 Objectives of the Study----------57

3.6 Research Questions----------57

3.7 Materials and Methods----------58

3.7.1 Definitions----------58

3.7.2 Study area----------59

3.7.3 Sample size----------61

3.7.4 Sampling methods for poultry from rural, backyard farm, and live bird markets----------61

3.7.5 Collection of blood sample ----------62

3.7.6 Detection of avian influenza antibodies----------62

3.7.6.1 Haemagglutination inhibition test----------62

3.7.6.2 Enzyme link immunosorbent Assay ----------64

3.7.7 Detection of Newcastle disease antibodies----------65

3.7.7.1 Haemmagglutination inhibition test----------65

3.7.7.2 Enzyme Link Immunosorbent Assay ----------67

3.8 Results of Serological Tests----------68

3.8.1 Avian influenza antibodies detection by haemagglutination inhibition test and enzyme link immunosorbent assay ----------68

3.8.2 Newcastle disease antibodies by haemagglutination inhibition test and enzyme link immunosorbent assay ----------69

3.9 Discussion----------84

CHAPTER 4: SERO-PREVALENCE OF AVIAN INFLUENZA AND NEWCASTLE DISEASE IN FREE FLYING WILD BIRDS----------89

4.1 Introduction----------90

4.2 Statement of the Research Problems----------92

4.3 Justifications of the Study----------92

4.4 Aim of the Study----------92

4.5 Objectives of the Study----------93

4.6 Research Questions----------93

4.7 Materials and Methods----------93

4.7.1 Study area----------93

4.7.2 Sample size for wild birds----------93

4.7.3 Sampling method for wild birds----------93

4.7.4 Collection of blood sample----------94

4.7.5 Detection of avian influenza antibodies----------94

4.7.5.1 Haemagglutination innhibition test----------94

4.7.5.2 Enzyme link immunosorbent assay----------94

4.7.6 Detection of Newcastle disease antibodies----------94

4.7.6.1 Haemagglutination inhibition test----------94

4.7.6.2 Enzyme link immunosorbent assay----------94

4.8 Results of Serological Tests----------95

4.8.1 Avian influenza antibodies detection by haemagglutination test and enzyme link immunosorbent assay----------95

4.8.2 Newcastle disease antibodies detection by haemagglutination test and enzyme link immunosorbent assay ----------95

4.9 Discussion----------106

CHAPTER 5: SURVEY FOR AVIAN INFLUENZA VIRUSES IN POULTRY AND FREE FLYING WILD BIRDS----------111

5.1 Introduction----------112

5.2 Statement of the Research Problems----------112

5.3 Justifications of the Study----------113

5.4 Aim of the Study----------114

5.5 Objectives of the Study----------114

5.6 Research Questions----------114

5.7 Materials and methods----------114

5.7.1 Definitions----------114

5.7.2 Study area----------114

5.7.3 Sample size----------114

5.7.4 Sampling method for poultry from rural, backyard, live bird market and wild birds----------115

5.7.5 Collection of cloacal and oropharyngeal swabs----------115

5.7.6 Detection of avian influenza viruses in poultry and wild birds----------115

5.7.6.1 Preparation of samples----------116

5.7.6.2 Extraction of nucleic acid----------116

5.7.6.3 Preparation of polymerase chain reaction master mix----------117

5.7.6.4 Reverse transcription-polymerase chain reaction----------117

5.7.6.5 Preparation of tris-borate ethylene diamine tetra-acetic acid buffer----------118

5.7.6.6 Preparation of 1.5% 100 ml agarose gel ----------118

5.7.6.7 Agarose gel electrophoresis of the amplicons ----------118

5.8 Results of Molecular Survey for Avian Influenza Viruses in Poultry and Wild Birds ----------118

5.9 Discussion----------124

CHAPTER 6: SURVEY FOR NEWCASTLE DISEASE VIRUSES IN POULTRY AND WILD BIRDS ----------127

6.1 Introduction ----------128

6.2 Statement of the Research Problems ----------130

6.3 Justifications of the Study ----------130

6.4 Aim of the Study----------131

6.5 Objectives of the Study----------131

6.6 Research Questions----------131

6.7 Materials and methods----------131

6.7.1 Definitions----------131

6.7.2 Study area----------131

6.7.3 Sample size----------132

6.7.4 Sampling method for poultry from rural, backyard, live bird market and wild birds----------132

6.7.5 Collection of cloacal and oropharyngeal swabs ----------132

6.7.6 Detection of Newcastle disease viruses in poultry and wild birds----------133

6.7.6.1 Preparation of samples----------133

6.7.6.2 Extraction of nucleic acid----------133

6.7.6.3 Preparation of polymerase chain reaction master mix----------133

6.7.6.4 Reverse transcription-polymerase chain reaction----------133

6.7.6.5 Preparation of tris-borate ethylenediamine tetracetic acid buffer----------134

6.7.6.6 Preparation of 1.5% 100 ml agarose gel ----------134

6.7.6.7 Agarose gel electrophoresis of the amplicons ----------134

6.8 **Results of Molecular Survey for Newcastle Disease Virus in Poultry and Wild Birds ----------134**

6.9 Discussion ----------142

CHAPTER 7: ASSESSMENT OF POULTRY-WILD BIRD INTERACTIONS BIOSECURITY AND HUSBANDRY PRACTICES IN POULTRY PRODUCTION IN KOGI STATE, NIGERIA----------148

7.1 Introduction ----------149

7.2 Statement of the Research Problems ----------150

7.3 Justifications of the Study----------151

7.4 Aim of the Study----------152

7.5 Objectives of the study----------152

7.6 Research questions----------153

7.7 Materials and methods----------153

7.7.1 Study area----------153

7.7.2 Sample size and sampling method ----------153

7.7.3 Administration of questionnaire----------153

7.7.3.1 Assessment of wild bird – poultry interactions----------154

7.7.3.2 Assessment of biosecurity and husbandry practices that may favor the spread of avian influenza and Newcastle disease----------155

7.7.4 Data analyses----------156

7.8 Results----------156

7.8.1 Results of assessment of wild bird – poultry interactions----------156

7.8.2 Results of assessment of biosecurity and husbandry practices----------167

7.9 Discussion ----------175

CHAPTER 8: CONCLUSION AND RECOMMENDATIONS----------181

8.1 Conclusion----------181

8.2 Recommendations----------183

REFERENCES----------184

APPENDICES----------196

LIST OF TABLES

Table	Title	Page
3.1	Seroprevalence of avian influenza (H5) antibodies in different poultry species using haemagglutination inhibition test in Kogi State	70
3.2	Seroprevalence of avian influenza (H5) antibodies in poultry using haemagglutination inhibition test in the twelve Local Government Areas of Kogi State	71
3.3	Seroprevalence of avian influenza (H5) antibodies in poultry using haemagglutination inhibition test in the surveyed sampling units of Kogi State	72
3.4	Seroprevalence of avian influenza (NP) antibodies in different poultry species surveyed using Enzyme Link Immunosorbent Assay in Kogi State	73
3.5	Seroprevalence of avian influenza (NP) antibodies in poultry using Enzyme Link Immunosorbent Assay in the surveyed twelve Local Government Areas of Kogi State	74
3.6	Seroprevalence of avian influenza (NP) antibodies in poultry using Enzyme Link Immunosorbent Assay in the surveyed sampling units from Kogi State	75
3.7	Seroprevalence of Newcastle disease antibodies in different poultry species using haemagglutination inhibition test test from Kogi State	76
3.8	Seroprevalence of Newcastle disease antibodies in poultry using haemagglutination inhibition test in the twelve Local Government Areas of Kogi State	78
3.9	Seroprevalence of Newcastle disease antibodies in poultry using haemagglutination inhibition test in the surveyed sampling units in Kogi State	79
3.10	Seroprevalence of Newcastle disease antibodies in different poultry Species using Enzyme Link Immunosorbent Assay in Kogi State	80
3.11	Seroprevalence of Newcastle disease antibodies in poultry using Enzyme Link Immunosorbent Assay in the surveyed twelve Local Government Areas of Kogi State.	81
3.12	Seroprevalence of Newcastle disease antibodies in poultry using Enzyme Link Immunosorbent Assay in the surveyed sampling units	82
3.13	The odd of reactors of poultry sera to Newcastle disease antibodies by Enzyme Link Immunosorbent Assay and haemagglutination test in the surveyed twelve Local Government Areas of Kogi State	83
4.1	The families, species and migratory status of the wild birds that were surveyed for avian influenza and Newcastle disease viruses in Kogi State.	96
4.2	Seroprevalence of avian influenza (NP) antibodies in wild bird using Enzyme Link Immunosorbent Assay in the surveyed Local Government Areas of Kogi State	97

LIST OF TABLES

Table	Title	Page
4.3	Seroprevalence of avian influenza (NP) antibodies using Enzyme Link Immunosorbent Assay in different wild bird species surveyed in Kogi State	98
4.4	Seroprevalence of Newcastle disease antibodies using haemagglutination inhibition test in sera of wild birds from the surveyed Local Government Areas of Kogi State	99
4.5	Seroprevalence of Newcastle disease antibodies using haemagglutination inhibition test in different wild bird species from Kogi State	100
4.6	Seroprevalence of Newcastle disease antibodies in wild birds using Enzyme Link Immunosorbent Assay from the surveyed Local Government Areas of Kogi State	101
4.7	Seroprevalence of Newcastle disease antibodies in wild bird species using Enzyme Link Immunosorbent Assay from Kogi State	103
4.8	Distribution of positive and negative sera to Newcastle disease antibodies by Enzyme Link Immunosorbent Assay and haemagglutination inhibition test in the wild birds surveyed in Kogi State, Nigeria	104
4.9	The odd of reactors of wild bird sera to Newcastle disease antibodies by Enzyme Link Immunosorbent Assay and haemagglutination inhibition tests in the surveyed Local Government Areas of Kogi State	111
6.1	Prevalence of Newcastle disease virus in the surveyed sampling units and Local Government Areas of Kogi State	139
6.2	Prevalence of Newcastle disease virus among avian species surveyed in the various Local Government Areas of Kogi State	140
6.3	The relative risk of Newcastle disease virus infection in different categories of birds in the surveyed areas of Kogi State	141
7.1	Scoring of possible risk factors given by respondents that are indicative of likely wild bird-poultry interactions in the surveyed Local Government Areas of Kogi State	157
7.2	Scoring of possible risk factors given by respondents that are indicative of likely wild bird-poultry interactions in the surveyed sampling units of Kogi State	158
7.3	Wild birds frequently seen around households, poultry farms and wetlands with their local names in Igala, Ebira and Okun, the three major Languages spoken by the people of Kogi State, Nigeria	160
7.4	Assessment of risk factors that enhance the spread of avian influenza, Newcastle disease and other avian pathogens in the surveyed poultry sampling units in Kogi State	169
7.5	Univariate analysis of risk factors for possible poultry – wild bird interactions, spread of avian influenza, Newcastle disease and other avian pathogens in Kogi State	172
7.6	Production system used by poultry farmers in Kogi State, Nigeria	173

LIST OF FIGURES

Figure	Title	Page
3.1	Map of Kogi State showing the 21 Local Government Areas grouped into western (blue area), central (yellow area) and eastern (pink area) senatorial districts	60
5.1	Distribution of cloacal and oropharyngeal swabs collected from the various sampling units in the twelve Local Government Areas of Kogi State, Nigeria	122
5.2	Distribution of cloacal and oropharyngeal swabs collected from the various species of poultry and wild birds in the surveyed twelve Local Government Areas of Kogi State, Nigeria	123
7.1	Wild bird – poultry interaction pathways and infection cycle based on favorable risk factors in Kogi State.	161
7.2	Assessment of risk factors that enhance the spread of avian influenza, Newcastle disease and other avian pathogens in the surveyed Local Government Areas of Kogi State, Nigeria	168
7.3	Types of poultry found in the various sampling units surveyed in Kogi State, Nigeria	174

LIST OF PLATES

Plate	Title	Page
I	Avian influenza virus reverse transcription polymerase chain reaction results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 1 – 18 with all being negative for avian influenza viruses, M = molecular marker of 250 bp, C^+ = positive control)----------	120
II	Avian influenza virus reverse transcription polymerase chain reaction results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 20 – 37 with all being negative for avian influenza viruses, M = molecular marker of 250 bp, C^+ = positive control)----------	121
III	Newcastle disease virus reverse transcription polymerase chain reaction results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 1 – 17 with 1, 2, 3, 7, 8, 10, 11, 12, 15, and 16 being positive for Newcastle diseaseviruses, M = molecular marker of 250 bp, C^+ = positive control) ----------	135
IV	Newcastle disease virus reverse transcription polymerase chain reaction results of oropharyngeal and cloacal swabs from poultry and wild birdsin the surveyed areas of Kogi State (Pooled samples 39 - 55 with 39, 41, 42, 45, 46, 47, 48, 49, 50, 51, 52 and 53 being positive for Newcastle disease viruses, M = molecular Marker of 250 bp, C^+ = positive control)----------	136
V	Newcastle disease virus reverse transcription polymerase chain reaction results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 56 – 73 with 58, 59, 60, 61, 63, 65, 66, 69, 70, 71, 71, 72 and 73 being positive for Newcastle disease viruses, M = molecular marker of 250 bp, C^+ = positive control)----------	137
VI	Backyard poultry in cages with wild birds (arrows) scavenging on feed spillage (**A**) around backyard poultry farm. The arrival of day-old chicks from hatcheries in Ibadan (south-west Nigeria) just before collection by farmers (**B**)----------	162
VII	River Niger in Lokoja (**A)** where rural poultry and wild birds often mix together at the bank in search of food and water. A vast area of wetland in Ajaokuta LGA cultivated year round with rice and often visited by rural poultry and wild birds (**B**)----------	163
VIII	Wild birds (pigeons and doves) roosting on rooftop (**A)** and scavenging around household where rural and backyard poultry are kept (**B**)----------	164
IX	Rural poultry on free range scavenging in the same area with piglets (arrow) (**A**). Rural poultry scavenging around a refuse dump (arrow) where poultry litters are discarded (**B**)----------	165

LIST OF PLATES

Plate	Title	Page
X	The nature of live bird markets in Kogi State with poultry kept in baskets, iron and wooden cages (A and B) adjoining other commodities in the market with no fence for movement restriction. Transportation of poultry inside locally made baskets to live bird markets using commuter vehicle and motocycle (C and D)----------	166

LIST OF APPENDICES

Appendix	Title	Page
I	Sample of the structured questionnaires used for observation and to collect data from poultry farmers and poultry sellers in the surveyed Local Government Areas of Kogi State	196
II	Preparation of 20 µl master mix for avian influenza antigens detection polymerase chain reaction	199
III	Preparation of 20 µl master mix for Newcastle disease antigens detection polymerase chain reaction	200
IV	Photographs of some wild birds surveyed in Kogi State	201

LIST OF ABBREVIATIONS

%	Percentage
˚C	Degree Centigrade
AGID	Agar Gel Immunodiffusion
AGPT	Agar Gel Preciptin Test
AI	Avian Influenza
AIVs	Avian Influenza Viruses
AICP	Avian Influenza Control Programme
APMV-1	Avian Paramyxovirus-1
ARD	Acute Respiratory Disease
B-cells	Bursa derived cells
CZC	Country, Zone or Compartment
DIVA	Differentiate Infected from Vaccinated Animals
DNA	Deoxyribonucleic Acid
cDNA	Complementary Deoxyribonucleic Acid
DTT	Dithiothreitol
dNTP	Deoxynucleoside triphosphate
EFSA	European Food Safety Agency
EID_{50}	Mean Embryo Infective Dose
ELISA	Enzyme-linked Immunosorbent Assay
EMA	Glutamic acid (E) change to lysine (K) at 627 of Polymerase Protein-2 for Mammalian Adaptation
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agricultural Organization
FCT	Federal Capital Territory
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FLDPC	Federal Livestock Department and Pest Control
g	Centrifugal or gravitational force
g	Gramme
GD	Gumboro disease

GDP	Gross Domestic Product
GF-TADs	Global Framework for the Progressive Control of Transboundary Animal Diseases
GIT	Gastro-intestinal tract
H_2O_2	Hydrogen Peroxide
HA	Haemagglutination/Haemagglutinin
HAO	Haemagglutinin polyprotein
HAU	Haemagglutination Unit
HI	Haemagglutination Inhibition
HPAI	Highly Pathogenic Avian Influenza
i/v	Intravenous
IDSR	Integrated Disease Surveillance Response
IgG	Gamma Immunoglobulin
IgM	Micro Immunoglobulin
ILI	Influenza-like illness
i/m	Intramuscular
IVPI	Intravenous Pathogenicity Index
KGSADP	Kogi State Agricultural Development Project
KMnO4	Potassium Permanganate
KPMs	Potassium Peroxymonosulfate
L	Litre
LAMP	Loop Mediated Isothermal Amplification
LBM	Live Bird Market
LGAs	Local Government Areas
LPAI	Low Pathogenic Avian Influenza
M	Molar concentration
ml	Millilitre
mRNA	Messenger Ribonucleic Acid
NA	Neuraminidase
NaCl	Sodium Chloride
NADIS INFO	National Animal Disease Information System
NaOH	Sodium Hydroxide

NASBA	Nucleic Acid Sequence Based Amplification
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NDV4	Newcastle disease virus Australian strain
nm	Nanometre
NP	Nucleic Protein
NVRI	National Veterinary Research Institute
OD	Optical Density
OIE	Office International des Epizootics (World Organization for Animal Health)
OR	Odd Ratio
PAN	Poultry Association of Nigeria
PBS	Phosphate Buffered Saline
PCS	Proteolytic cleavage site
pH	Hydrogen ion Concentration
QACs	Quaternary Ammonium Compounds
RBCs	Red Blood Cells
rFPV	Recombinant Fowl Pox Vaccine
RNA	Riboxynucleic Acid
-ssRNA	Negative sensed single stranded RNA
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
rRT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
SEM	Standard Error of Mean
SAN	Specific Antibody Negative
SAR	Special Administrative Region of the People's Republic of China
SPF	Specific Pathogen Free
SPSS	Statistical Package for Social Sciences
TADs	Transboundary Animal Diseases
TNF-∞	Alpha Tumor Necrotic Factor
ug	Microgramme
ul	Microlitre
USA	United State of America

USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UV	Ultra Violet
w/v	Weight per volume
WHO	World Health Organization
WPRO	Western Pacific Regional Office of WHO

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Poultry and wild birds are important in the transmission of several avian diseases avian influenza (AI), Newcastle disease (ND), Salmonellosis, Chlamydiosis, Arizonosis, Campylobacteriosis, Cryptosporidiosis and avian tuberculosis among themselves as well as to humans (Jacquie *et al.,* 2011). These diseases except for cryptosporidiosis are on the World organization for Animal Health (OIE) list of notifiable diseases. It is necessary to know these diseases and their level of threats as well as the precautions to take when coming in contact with or handling birds (Jacquie *et al.,* 2011).

Surveillance is a key tool in the prevention and control of the emergence of these avian diseases especially AI and ND by providing early warning of disease incidence as well as identifying possible hosts and modes of disease transmission (Swayne, 2008). Hence, surveillance helps to inform timely intervention in disease prevention and control. In addition, surveillance helps in certifying a country free of reportable diseases for the benefit of international trade as well as in identifying an infected zone or compartment (Dufour, 2001).

Over a long period of time, avian influenza (AI) and Newcastle disease (ND) were thought to be the same disease (Alexander, 1987). Formerly, they were both referred to as fowl plague due to the high virulence and mortality associated with both diseases (Swayne, 2008). This misconception started at the onset after Doyle in 1927 described ND as a different disease and this lasted until 1955 when Schaffer described the viral cause of fowl plague as influenza A (Kaleta and Catherine, 2008).

The long disagreement in accepting AI and ND as two disease entities was due to their epidemiological and clinico-pathological similarities. They both have seasonal prevalence tending to be high within the cold months of the year (Sa'idu *et al.,* 2004; Swayne, 2008). Wild birds often serve as means of introduction and spread of both viruses to susceptible hosts over long distances (Abdu, 2007; Swayne, 2007).

Based on clinical signs alone one cannot distinguish avian influenza from ND as they often present with the same clinical signs with difficulty in differential diagnosis (Easterday *et al.*, 1997). Concurrent infection of ND with low pathogenic AI (LPAI) is associated with severe clinical disease and mortality (Easteday *et al*., 1997; Wakawa *et al*., 2009). In addition, both diseases have highly pathogenic pathotypes which are able to cause up to 100% mortality in affected poultry on their own as well as low pathogenic pathotypes that cause production losses thereby making them of high economic importance (Abdu, 2007; Musa *et al*., 2010).

Avian influenza is caused by influenza A virus belonging to the family *Orthomyxoviridae.* The AI virus nuclear core is segmented, single stranded RNA made of eight different genes that encode at least ten viral proteins with high rates of mutation and reassortment (Suarez, 2008). The nucleocapsid or matrix antigen is conserved for all influenza A but the haemagglutinins and neuraminidase, which are surface proteins, are highly variable and are used to differentiate subtypes (Suarez, 2008). Based on the haemagglutinin, there are 16 subtypes (H1-H16) and 9 subtypes (N1-N9) based on the neuraminidase (Swayne, 2008).

Avian influenza is not limited to birds alone but it affects humans and other mammals with fatal consequences (Suarez, 2008). The likelihood adaptation of AI in humans to be able to transmit from human to human is the basis for the fear of an influenza

pandemic as occurred previously (Suarez, 2008). Three major influenza pandemics that occurred in the past were that of 1918, caused by H1N1 derived from swine, termed Spanish flu; Asian flu of 1957 caused by H2N2, derived from avian species, and Hong Kong flu of 1968 caused by H2N3, also derived from avian species (Swayne, 2008).

Migratory wild birds especially, waterfowls are the main reservoirs of AI that pose constant threat and often serve as uncontrollable sources of fatal infection to susceptible poultry (Swayne, 2008). The adaption of AI in poultry results into two main pathotypes relating to variable forms of pathogenicity. The two pathotypes are low pathogenic avian influenza (LPAI) resulting in decreased egg production and highly pathogenic avian influenza (HPAI) causing severe clinical signs and up to 100% mortality (Capua and Morangon, 2003).

On the other hand, ND is caused by Paramyxovirus-1 of the genus Avulavirus and family, *Paramyxoviridae* (Abdu, 2007). The ND virus is an enveloped single stranded RNA virus that is not segmented but with three surface glycoproteins which are the F–protein and haemagglutinin - neuraminidase proteins (Jordan, 1990). The ND virus was initially thought to be the only member of the Paramyxovirus but isolations from other avian species showed distinct characteristics from the ND virus, which led to their grouping into serotypes ranging from PMV-1 to PMV-9 (Jordan, 1990).

In addition, the ND virus has different strains with ability to cause disease of variable signs and severity even in the same host (Jordan, 1990). The very virulent strain of ND virus causes severe clinical signs and mortality of up to 100% (Easterday *et al.*, 1997; Abdu, 2007). The very virulent strain of ND virus had equally caused three major panzootic in poultry in the past. The first panzootic coincided with the onset of

the disease in the mid 1920s, which led to the adoption of compulsory slaughter policy for control until around 1963 when vaccination policy was adopted (Miller and West, 1972).

The first report of ND in Nigeria was around Ibadan in 1953 which was laterfollowed by reports from other parts of the country both rural and commercial poultry (Hill *et al.,* 1953; Ezeokoli *et al*., 1984; Abdu *et al.*, 1992; Sa'idu *et al*., 1994). Sa'idu *et al.* (1994) reported the disease to be more common and severe during the cold harmattan season while farms where mixed species, rural and exotic breeds are kept experience more outbreaks (Abdu *et al*., 1992).

In countries where ND is endemic, vaccination is the main stay of prevention and control but it is not an alternative to good biosecurity practices (Musa *et al.,* 2010). Outbreaks of ND with mortality ranging up to 100% have been known to occur even after vaccinations hence, it is suggested that under field conditions, its prevention should not be based only on vaccination (Sa'idu *et al*., 2008; Musa *et al*., 2010). Factors responsible for ND outbreaks in the face of vaccination vary but of most importance is that relating to the immunogenicity of the vaccinal virus strain with the endemic field virus (Sa'idu *et al*., 2008).

In Nigeria and may be most part of Africa, smallholder rural poultry farmers source their stock for rearing from the LBMs (Ameji, 2010). The live bird market is an area of high risk of disease and has been reported to play a role in AI transmission (Ducatez *et al.,* 2007; Aye, 2010). The high risk is associated with the mixing of poultry from different places in the LBM where pathogens may be shared before they are sold and carried to different places for household slaughter, sacrifice or rearing (Ameji, 2010). Although, ND is mostly pathogenic for the domestic chickens, turkeys

and guinea fowls, other poultry and wild birds may not show clinical disease but do develop antibodies to the virus (Jordan, 1990; Mohammed *et al*., 1998; Abdu, 2007). The LBM provides the platform for interaction among different poultry species and some wild birds before they are sold. Hence, the live LBM can be a niche and disseminating point for contagious poultry diseases.

In a study reported by Ameji (2010), seroprevalence of AI, ND and Gumboro disease (GD) were found in chickens in the LBMs in six LGAs of Kogi State. From that previous study, it becomes imperative to design a further study to determine the presence of AI and ND viruses in poultry and wild birds from twelve LGAs of Kogi State. The study will also assess wild birds – poultry interactions, biosecurity and husbandry practices to determine the epidemiological relationship between poultry and wild birds in the introduction/spread of contagious avian diseases of economic importance.

1.2 Statement of the Research Problem

Majority of poultry in Nigeria are rural poultry kept on free range (FLDPCS, 2003; Adene and Oguntade, 2006). Wild birds and rural poultry interact freely in most farms, rural communities and around wetlands where they contaminate food and water, air and the soil with fecal materials that may pose danger as sources of infections to rural and commercial poultry where there is compromised biosecurity in management practices (Echeonwu *et al.,* 1993; Ibu *et al.,* 2009). Wild birds were implicated as the source of introduction of HPAI to Nigeria and other parts of Africa in 2006 probably due to wild birds abundance and diversity, the presence of migratory birds fly ways, predominance of rural poultry and poor management practice (Brown, 2006; Ducatez *et al.,* 2006).

Avian influenza and ND are among the most important viral diseases of poultry in Nigeria requiring the adoption of appropriate prevention and control strategies (Abdu, 2007). The advent of HPAI in Nigeria in the 2006 outbreaks led to huge socio-economic impacts on poultry production and human livelihood. Directly, there were severe economic losses arising from reduction in sales of eggs and poultry, production losses and culling of over one million poultry in affected and non-affected farms (AICP, 2009; Abdu, 2010). Indirectly, many people were thrown out of farm work in both affected and non-affected farms as well as reduction in intake of animal protein (Abdu, 2010).

Highly pathogenic avian influenza is zoonotic and has caused over 300 human deaths since the 2006 outbreaks in Asia, Europe and Africa (WHO, 2010). Equally, ND is zoonotic and it is known to cause mild conjunctivitis in poultry farmers and veterinarians (Abdu, 2007). The husbandry and management practices encourage contact between poultry and humans which may lead to the risk of human infections with AI and ND (Adene and Oguntade, 2006).

Kogi State is strategic in location and is surrounded by about eight States including the Federal Capital Territory (FCT) where HPAI was earlier reported (AICP, 2008). Lokoja, the State capital serves as a transit route and resting point for travelers from the southwest, southeast and south to the FCT and for live rural poultry moving from the north to LBMs in the south. Similarly, the State receives table eggs, day old chicks and veterinary biologicals which may serve as sources of infection from the south west where HPAI had been reported (Adene and Oguntade, 2006).

Rivers Benue and Niger pass through Kogi State and converged at a point to form a confluence with abundant wetlands in Kogi, Lokoja, Bassa, Ibaji, Ajaokuta and Idah

LGAs that may serve as resting points for migratory wild birds (KGSADP, 2009). Wild birds, vehicles, formites and people are involved in the spread of AI, ND and other disease causing agents (Sawabe *et al.*, 2006; Abdu, 2007).

Moreover, antibodies against AI and ND were detected in chickens from rural, backyard and LBMs in six LGAs of Kogi State in an earlier study (Ameji *et al.*, 2011).

1.3 Justification of the Research

Although, HPAI was not reported in Kogi State during the 2006 – 2008 outbreaks in other parts of Nigeria, there is need for sustained active surveillance because of the presence of risk factors which make the State a "suspect nidus" for establishment and spread of the disease. There are wetlands, wild birds, established backyard poultry farms, live bird markets and vast number of rural poultry in the State (Adene and Oguntade, 2006; Abdu, 2010) which have informed the choice of Kogi State for the study.

The spread of AIVs, NDVs and other pathogens are known to occur through several routes such as trade in live poultry and wild birds as well as movement over long distance by migratory wild birds (Brown, 2006; Kilpatrick, *et al.*, 2006). Kogi State has a number of LBMs and it is involved in poultry trade with other States across Nigeria. The State also receives table eggs, day-old chicks and veterinary biological that may serve as sources of infection from some States in the South-west where HPAI was reported. Most rural poultry and some backyard (semi commercial) poultry find their ways to the LBM where they are offered for sale and the LBM had been shown to be one of the major routes of disease introduction and transmission

(Webster, 2004; Pagani *et al*., 2008). In Kogi State, most people sourced birds for rearing from the LBMs, a practice which needs to be discouraged based on facts.

As a result of similar epidemiological and clinical presentations, there is tendency of misdiagnosing AI for ND and vice-versa. Therefore, there is the need to increase the perspective of the people on the recognition of the diseases, their economic impacts and associated risk factors for disease transmission.

There are little or no previous studies done on AI and ND in Kogi State and those done elsewhere cannot be used to give conclusions on the activities of the viruses in the State due to varying geographical and epidemiological characteristics. The study will help to increase knowledge on the status of AI and ND in poultry and wild birds in Kogi State which will be useful for the design of prevention and control programmes against the diseases.

The studies will help to generate information to knowing the status of AI and ND in poultry and wild birds in Kogi State, useful for prevention and control. Equally, it will help in knowing and avoiding sources of risk to human infection with AI.

Prior to the outbreaks of AI in Asia and Africa, little was known about the roles of wild birds, rural poultry and live poultry markets in the transmission cycles of AIVs and NDV hence, were not considered in the development of preventive and control strategies in these areas. This gross oversight in the integration of rural poultry in the design of control programmes and their negative health impacts might be the bane of low commercial poultry production in Kogi State and else where due to the effects of diseases on poultry husbandry (Sonaiya, 1990).

1.4 Aim of the Study

The aim of the research was to conduct surveillance for AI and ND in poultry and wild birds, assess wild bird – poultry interactions, biosecurity and husbandry practices in poultry production in Kogi Stat, Nigeria.

1.5 Objectives of the Study

The objectives were to:

1. Determine the sero-prevalence of AI and ND in poultry and wild birds in Kogi State, Nigeria.
2. Detect by RT-PCR the presence of AI and ND viruses in poultry and wild birds in Kogi State, Nigeria.
3. Assess poultry-wild bird interactions, biosecurity and husbandry practices in poultry production in Kogi State, Nigeria.

1.6 Research questions

1. Are there evidence of AIVs and NDVs in poultry and wild birds in Kogi State?
2. Are there AI and ND viruses in poultry and wild birds in Kogi State?
3. Are there interactions between poultry and wild birds with the spread of AI and ND in Kogi State?

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical Background of Avian Influenza

Avian influenza is not a new disease. The earliest evidence of the disease in poultry dated back to 1878 in Italy when highly pathogenic avian influenza in chicken was termed as Fowl plague by Perroncito (Alexander, 1987). In 1880, Rivolta and Delprato in Italy provided epidemiological reports on fowl plague in chickens and the evidence of its transmission to turkeys, geese and ducks, while Centanni and Savonuzzi in 1901 demonstrated the agent to be a filterable virus (Alexander, 1987; Suarez, 2008).

However, it was not until 1955 in Germany that Schaffer characterized the agent of fowl plague as influenza A virus thereby changing the name of the disease to avian influenza (Alexander, 1987). Wild aquatic birds are the natural hosts of influenza A viruses where infection causes little or no disease in these reservoir hosts because the viruses live in perfect balance in their natural hosts (Alexander, 1987; Suarez, 2008).

Avian influenza is of a global concern due to its ability to cause devastating disease in domestic poultry and human populations (Alexander, 1987). In poultry, the disease occurs in two forms; low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) depending on the virulence and strains of the viruses (Suarez, 2008).

In humans, three major avian influenza pandemics had occurred in the past in which several million lives were lost. They include the “Spanish Flu” of 1918-1920; the “Asian Flu” of 1957; and the “Hong Kong Flu” of 1968 (Alexander, 1987). The Spanish Flu alone was responsible for 20-40 million deaths worldwide while the

Asian and Hong Kong pandemics resulted in 1.5 million deaths with an estimated loss of US$32 billion in productivity and medical bills (Alexander, 1987).

Thereafter, several human infections that have not caused a pandemic but raised global concerns were recorded. In 1976, H1N1 virus was isolated from an army recruit who died in the US. It was also reported that there was a massive influenza vaccination response of over 40 million people by the end of 1976 but the vaccination programme was later stopped because the infection was limited to the army recruit camp (WPRO, 1999).

Similarly, H5N1 virus was isolated in 1997 from a boy who later died in Hong Kong Special Administrative Region (SAR). That was the first case of H5N1 infection in humans and by the end of December, 1997, a total of 17 human infections with 5 additional deaths were reported (Swayne, 2008). This discovery led to the culling of over a million poultry in Hong Kong live poultry market thereby bringing the situation under control.

However, the H5N1 virus persisted to cause intermittent outbreaks in Hong Kong SAR in 2001 as well as several other Asian countries in late 2003 (Sims and Brown, 2008). Since 2003, over 63 countries have reported outbreaks of H5N1 HPAI that has killed or led to culling of over 400 million poultry with an estimated US$20 billion loss in economic damage globally (PHAC, 2012). A total of 608 human infections have occurred within this period resulting in 359 human deaths, a situation which showed a case fatality rate of about 60% (PHAC, 2012).

In April, 2009; a new strain of influenza A (H1N1) subtype was identified as the cause of “swine flu” from patients who suffered influenza-like illness in Southwest USA, a disease which was first reported in Mexico City in March, 2009 (WHO,

2009a). The disease quickly spread in the same month to Canada, New Zealand, United Kingdom, Spain and Israel and by the month of June, 2009, the WHO raised a pandemic alert with about 30,000 laboratory confirmed cases worldwide (WHO, 2009a). The virus was thought to be a reassortant variant of four known strains of influenza A H1N1 subtype derived from strains endemic in humans, birds and pigs (WHO, 2009a).

By November, 2009; WHO reported that over 206 countries and overseas territories recorded a total of 503,536 confirmed cases with 6,027 deaths worldwide (WHO, 2009b). However in 2010, WHO declared the "swine flu" pandemic to be over after much strategic efforts were made towards its prevention and control.

2.1.1 Avian influenza virus classification

Avian influenza viruses belong to the family *orthomyxoviridae* which are single stranded 8-segmented negative sensed riboxynucleic acid (-ssRNA) that encode at least 10 viral proteins (Capua and Alexander, 2004). The family is divided into three main genera of influenza type A, B, C and two other unknown genera: *Isavirus* and *Thogotovirus* (Suarez, 2008). Avian influenza viruses belong to the genus of influenza A which are most spread among other influenza viruses with members infecting avian and mammalian species (Capua and Alexander, 2004).

Influenza A viruses are further classified by strains or subtypes based on the envelope proteins, haemagglutinin (HA) and neuraminidase (NA) proteins. Presently, based on the HA, there are 16 subtypes (H1-H16) and 9 subtypes (N1-N9) based on NA (Capua and Alexander, 2004). The HA and NA are capable of eliciting subtype specific immune responses which protect within subtypes and partially protect across subtypes (Swayne, 2008).

2.1.2 Virus morphology and composition

Avian influenza viruses are morphologically variable with the variability being controlled by the matrix protein (M1) (Suarez, 2008). The viruses are pleomorphic, roughly spherical with a diameter of 80-120 nm to filamentous forms that can be several microns in length (Causey and Edwards, 2008). The virion in any morphological forms is encapsulated in a lipid envelop derived from the host cell with globular spikes or projections of membrane proteins (Suarez, 2008).

The virion is composed of a single negative stranded RNA genome of eight segments that encodes at least ten viral proteins. These encoded viral proteins include three envelop proteins, haemagglutinin (HA), neuraminidase (NA) and matrix protein (M2); the three polymerases, PB1, PB2 and PA; one nucleocapsid protein (NP); matrix protein (M1) and the two non structural proteins, NS1 and NS2 (Suarez, 2008). These proteins may be grouped based on their functions into mRNA synthesis genes, regulatory genes and structural genes (Perdue, 2008).

2.1.3 Avian influenza pathogenicity and virulence

The HA surface protein is the major antigenic determinant and determinant of virulence resulting in severity of clinical signs and immune response (Perdue, 2008). The HA is a polyprotein with a globular head that must be cleaved into two subunits (HA-1 and HA-2) before it can be functional. The globular head of the HA is used for viral attachment to host cell. Hence, antibodies are directed against the globular head to prevent binding of the viral receptor site to host cell but the globular head also changes rapidly as a result of mutation known as antigenic drift to avoid host cell antibodies (Perdue, 2008).

The fusion of the virus membrane with the host cell membrane to release the virion to begin viral replication in the host cell is also mediated by the fusion peptide on the HA. Uncleaved HA polyprotein is termed HAO which must be cleaved into HA-1 and HA-2 at the point of their disulfide linkages known as proteolytic cleavage site (PCS) before the virus can infect a cell. The basic amino acid sequence of the PCS on the HAO determines the pathogenicity of avian influenza viruses (Perdue, 2008).

Avian influenza viruses are classified based on pathogenicity in chickens and turkeys into low pathogenic avian influenza (LPAI) which causes mild respiratory disease or drop in egg production and highly pathogenic avian influenza (HPAI) which causes severe systemic disease with mortality up to 100% (OIE, 2006). The difference between the two lies in the ability of the proteolytic cleavage site (PCS) of the HA polyprotein of LPAI to be cleaved by trypsin-like endoproteases present only in the gut and lung epithelia while that of HPAI can be cleaved by numerous furin or subtilysin endoproteases present in most parts of the body (Perdue, 2008).

The OIE (2006) defines notifiable avian influenza (NAI) as any influenza A infection of poultry caused by H5 or H7 irrespective of pathogenicity. This definition became necessary as a result of the ability of LPAI H5 and H7 subtypes to mutate to HPAI after circulating for some time (Capua and Alexander, 2004).

However, the OIE (2006) further defines NAI HPAI on the following criteria:

1. An isolate that causes greater than 75% mortality or death of 4-8 week old chickens within 10 days following intravenous inoculation with 0.2ml of 1:10 dilution of bacteria- free, infective allantoic fluid, or
2. An isolate that has an intravenous pathogenicity index (IVPI) greater than 1.2 in 4-8 week old specific pathogen free chickens, or

3. An isolate of influenza A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the Proteolytic cleavage site of the HA.

The European Union (EU) and United State Department of Agriculture (USDA) have similar criteria to NAI HPAI with little variations.

The antigenicity of influenza A viruses changes gradually by point mutation (antigenic drift) and drastically by genetic reassortment (antigenic shift) as a means of adaptation to hosts and maintaining viral diversity in the population (Perdue, 2008). These antigenic diversities have major implications for vaccination and emergence of HPAI from LPAI as had occurred in Mexico and Italy (Capua and Alexander, 2004).

2.1.4 Antigenic drift and shift of avian influenza viruses

Antigenic drift is a minor change due to mutation in the gene segment or surface glycoproteins mainly, the haemagglutinin to produce a strain different from the original strain of a subtype virus (Suarez, 2008). Antigenic shift on the other hand, is a major change in the gene segments of AI viruses occurring in a mixed infection of the same host in which reassortment of genetic materials results in a novel virus of a different subtype (Bush *et al.,* 1999; Alexander *et al.,* 2004).

These are two primary mechanisms possessed by AIVs to provide diversity in the viral population owing to their high mutation rate and the ability to reassort gene segments (Turner, 2003). The mechanisms provide an opportunity for the virus to rapidly change and adapt thereby establishing virulent infections in a new host species (Turner, 2003).

Avian influenza viruses can differ in their amino acid sequence, particularly in the surface glycoproteins, HA and NA (Steinhauer, 1999). These differences in amino

acid sequence result in differences in antigenicity, such that antibodies to a subtype will only neutralize viruses of that subtype and not any other AI subtypes (Capua and Morangon, 2003). These antigenic differences have major drawbacks on vaccination because vaccine protection is mediated mainly by specific antibodies being produced to the HA protein and to a lesser extent, the NA protein (Lee and Suarez, 2005). Therefore, current vaccines are limited to providing only subtype protection and to attain complete protection from AI would need the addition of 16 different antigens of each HA subtype (Suarez, 2008).

Antigenic drift causes variable response and sometimes complete failure to vaccination in poultry and humans. Antigenic shift is the main reason for the global panic about pandemic influenza (Suarez, 2008).

2.1.5 Avian influenza replication and pathogenesis

Avian influenza viruses initiate infection and replicate in host cells by several mechanisms which are discussed under the following headings.

2.1.5.1 Avian influenza replication

Infection of the host is made possible by the virus attaching to host cell via the haemagglutinin (HA) glycoprotein to the cell surface sialic acid receptor. Sialic acid receptor molecules are classified as to how they are linked to the underlying sugars by the α-2 carbon. The most common linkages are the α-2, 3 and α-2, 6 linkages (Suzuki, 2005). The variations in conformations of the sialic acid receptors affect the binding of the virus to the cells of different hosts' species (Alexander *et al*., 2004).

The sialic acid receptors are abundantly found in ciliated respiratory epithelium and the intestinal tract. The α-2, 3 sialic acid receptors are expressed by avian hosts while the α-2, 6 linkages are expressed by human hosts which are used to define host

specificity and species barrier in AI infection (Perdue, 2008). However, there are exceptions to the presence of host specificity and species barrier in AIVs infection due to the expression of same sialic acid receptors by humans and some avian hosts such as quails which have the receptor linkages though at different cell locations (Swayne, 2008).

Based on the sialic acid receptor linkages, the pigs have been taken as a mixing vessel for both human and avian AIVs because they express high levels of α-2, 3 and α-2, 6 sialic acid receptors in their respiratory epithelium. This may lead to reassortment if the pig is infected with both human and avian AIVs at the same time resulting in a novel virus that can cause a pandemic (Van Reeth, 2007).

Following successful attachment of the AIVs to the sialic acid receptor of respiratory epithelium or digestive tract in the case of LPAI; systemic dissemination can occur in HPAI infection to various organs where the viral HA can be cleaved by ubiquitous endoproteases like furin. The virus is then taken into the host cell by endocytosis in an endosome with the endoprotease resulting in cleavage of the HAO into its subunits. The host cell also acidifies the endosome via the influx of H^+ through the M2- ion channels into the virus and causes fusion of the viral envelope (viral uncoating) with the cytoplasmic membrane of host and release of viral RNA (Steinhauer, 1999). The viral RNA-polymerase complex is then released into the cytoplasm and transported into the nucleus by the NP.

In the nucleus, the negative ssRNA is transcribed into anti-sense mRNA by the polymerase complex. The virus also uses host proteins to synthesize mRNA and RNA polymerase II. However, the 5' capped primer needed for mRNA synthesis is stolen by the PB2 from host mRNA in a process known as cap snatching (Steinhauer, 1999).

The anti-sense mRNA then moves to the cytoplasm where it acts as a template to produce the negative sense viral RNA as well as the production of other viral proteins using host cellular machinery (Steinhauer, 1999).

The NP and M1 proteins play important roles in the transportation of viral proteins to and from the nucleus but the M1 protein alone is involved in viral structure and assembly. The M1 protein bridges the three integral membrane proteins, HA, NA and M2 with the ribonucleoprotein and each of the eight viral gene segments before complete packaging of the virion. Some defective viral particles may be formed due to inefficient packagings which are lost as a result of selection pressure (Enami *et al*., 1991). The enzymatic activity of the NA protein to remove sialic acid from the HA glycoprotein results in successful budding of progeny viral particles from cell surfaces to begin another infective cycle.

2.1.5.2 Pathogenesis of avian influenza

Following entry through the respiratory tract in aerosolized droplets or the gastro-intestinal tract via faecal-oral route, the AIV initiates infection and replication in the host cell. Highly pathogenic avian influenza has been found to cause systemic infection in both humans and animals through the blood stream, lymphatics and may be the nervous system (Perkins and Swayne, 2003).

The HPAI H5N1 viruses have been isolated from the vascular endothelium, heart, pancreas, brain and adrenal glands of chickens and other gallinaceous birds. This event may lead to cardiovascular damage due to endothelial activation and disruption, leukocyte activation with systemic release of cytokines, cardiopulmonary failure and multi-organs damage (Perkins and Swayne, 2003). These are similar to findings in humans that deaths were as a result of progressive respiratory failure with associated

decrease in leukocytes, platelets and lymphocytes counts. Other pathways that HPAI can cause disease in humans are by pneumonia, acute respiratory distress syndrome (ARDS), multi-organs failure, renal dysfunction, and cardiac dilatation with tachyarrhythmias (WHO, 2005).

Several virulence factors have been reported to be responsible for the pathogenicity of HPAI infection. The major determinant of virulence is the HA glycoprotein which contains the globular head for viral attachment to host cell as well as the fusion peptide that helps to fuse the viral envelope to the host cytoplasmic membrane to release viral RNA. Antibodies directed at the globular head of the HA effectively prevent attachment to host cell but the HA can change rapidly due to mutation (antigenic drift) to produce novel structures that are no longer recognized by the antibodies. This rapid change in HA conformation gives the AIV an escape mechanism from host cell antibodies to again allow efficient attachment and entry into new cells (Wright and Webster, 2001).

Moreover, the HA of HPAI has the ability to be cleaved by ubiquitous proteases which helps in the wide spread dissemination of the AIV to several organs. The cleavage of the HA polyprotein exposes the fusion peptide which helps to initiate infection in the various organs resulting in multi-organ failure and mortality of up to 100% (Skehel *et al.,* 2001).

Additional adaptations that enhance pathogenesis is a substitution in the amino acids sequence of the PB2 with lysine (K) instead of glutamic acid (E) at position 627 often called mammalian signature (E627K). This mammalian signature promotes the replication of the HPAI H5N1 viruses in poultry and mammals (Subbarao and Katz, 2000). Moreover, a substitution in the NS-1 protein with glutamic acid (E) instead of

asparagine at position 92 helps to increase the virus resistance to interferons and tumor necrotic factor α (TNF-α). The NS gene has been reported to confer resistance to the antiviral effects of interferons and TNF-α thereby enhancing viral pathogenesis by disarming the cytokines activated responses in many ways (Seo *et al*., 2002).

2.1.6 Clinical signs and pathology of avian influenza

Pathological lesions and clinical signs vary depending on influenza pathotype, species and age of the host. In LPAI infection in chickens and turkeys, clinical signs are often mild respiratory distress and reduced egg production. Lesions of tracheitis, sinusitis, airsacculitis, involvement of the ovaries and oviduct with yolk peritonitis in layers are sometimes observed (Adene *et al.*, 2006; Suarez, 2008; Wakawa *et al.*, 2008).

Clinical signs may be absent in peracute HPAI disease. The following clinical signs may be observed in some cases: respiratory distress, coughing, sneezing, cyanosis of combs and wattle, haemorrhages on the shank, bleeding from the nares, diarrhea, circling, incordination and death (Easterday *et al*., 1997; Adene *et al*., 2006; Wakawa *et al.*, 2008). Lesions seen may include hydropericardium, haemorrhages in the mesentery and pericardial serosa, pulmonary edema, mucus in the trachea, pinpoint haemorrhages in the proventriculus, serous exudates in the body cavities and non-suppurative brain lesions (Perkins and Swayne, 2003; Adene et al., 2006).

2.1.7 Epidemiology of avian influenza viruses

The epidemiology of AIVs in their natural hosts and man made systems is complex and much is still unknownabout the diversity.

2.1.7.1 Natural hosts and reservoirs

The natural hosts and reservoirs for all subtypes of influenza A viruses are wild aquatic birds of the orders Anseriformes such as ducks, swan and geese; and

Charadriiformes such as terns, gulls and shorebirds (Webster *et al*., 1992; Suarez, 2008). The natural State of influenza A viruses is the LPAI form in their natural hosts, the viruses do not cause disease but live in evolutionary stasis and appear to be evolving slowly with most internal genes, NP and M1 being conserved at amino acid level (Suarez, 2000). In their natural hosts, influenza A viruses replicate mostly in the intestinal tract which result in the excretion of viruses in the faeces in high titre resulting in efficient dissemination of the viruses through the faecal oral route (Webster *et al*., 1992; Suarez, 2000).

There have been reports of isolation of influenza A viruses from other species that utilize aquatic habitats in the orders Ciconiiformes (heron, egret and stork), Gaviiformes (Arctic loon), Gruiformes (American coot), Pelecaniformes (Great cormorant), Podicipediformes (grebe), Procellariformes (shearwater) as well as Columbiformes (Collard dove), Piciformes (woodpecker) and Passeriformes (swallow, warbler) associated with terrestrial habitats (Nettles *et al.,* 1985; Stallknecht and Brown, 2008).

2.1.7.2 Transmission of avian influenza

Transmission of AI viruses in their natural LPAI State from their natural reservoirs to other susceptible hosts such as poultry is dependent on the faecal-oral route. This is achieved either directly via contact from host to host or indirectly, via virus contaminated water, feed and formites (Suarez, 2008). The transmission cycle is made possible and maintained by activities that bring carrier wild birds and susceptible poultry together like free range poultry husbandry and sales of birds in live poultry markets (Brown *et al.,* 2007; Suarez, 2008).

In the environment, the excreted viruses are protected from physical and chemical inactivation by the accompanying poultry manure and other organic materials (Easterday *et al*., 1997). For instance, HPAI H5N1 viruses can be viable in liquid manure for 105 days in the winter under freezing conditions, 30 to 35 days at 4 ˚C, 7 days at 20˚C and 4 days at 25-32˚C when kept out of direct sunlight (Songserm *et al*., 2006). In addition, LPAI viruses survive longer in water than HPAI viruses because LPAI viruses excreted by waterfowls were seen to be infective for up to 207 days at 17˚C and for up to 102 days at 28˚C. These observations suggest that AI viruses could remain infective in water or moist organic materials kept at cool or freezing temperatures such as over wintering conditions for long periods of time (Songserm *et al*., 2006).

The transmission of LPAI H5 or H7 subtypes to susceptible poultry initiate a chain of infection cycles and mutational changes that may lead to the development of HPAI subtypes as means of adaptation to the new host (Webster *et al.,* 1992). The development of HPAI from LPAI is complex, dependent on conditions that exert selective pressure on circulating viruses at both host and population level that allow high rate of mutation to occur (Suarez *et al*., 2004).

Once HPAI viruses have been established in poultry, transmission and spread follow the same pathway. Live bird markets where birds are sold under crowded conditions and poor husbandry practices of poultry production play major additional roles (Webster, 2004; Aye, 2010). Airborne spread of AIVs can also occur over a short distance around heavily infected farms (Hirst *et al*., 2004). The AIVs can also be dispersed in dust particles contaminated during culling process (Capua and Alexander, 2004). The role of vectors such as rodents and flies that act as mechanical

transmitters is being investigated as the AIVs have been shown to multiply in mice without prior adaptation in addition to being isolated from blowflies in Japan (Sawabe *et al*., 2006; Swayne, 2008).

Wild birds are also known to transmit HPAI viruses. There have been several reports since 2003 in Asia and other places of some H5N1 viruses which were highly pathogenic for chickens but not for ducks (Chen *et al.*, 2005; Wakawa *et al*., 2006). Chen *et al*. (2005) reported of HPAI H5N1 outbreak among migratory wild birds at Lake Qinghai in North Western China affecting several bar-headed geese, ducks, cormorants and gulls.

2.1.7.3 Susceptible hosts of avian influenza

A wide range of avian and mammalian species are susceptible to AI. Alexander (2000) cited the range of susceptible species to include wild birds, captive pet birds, domestic poultry, swine, horses, dogs, cats, seals and humans. Among poultry, chickens and turkeys are most susceptible with fatal consequences (Capua *et al*., 2003). Most outbreaks of HPAI in Europe have been in turkeys attributable to introductions from migratory birds into most of the free range operated turkey farms (Capua *et al*., 2003). Perkins and Swayne (2003) reported that turkeys do not survive more than a few days after experimental infection with HPAI H5N1.

2.1.8 Avian influenza in Nigeria

Nigeria was the first country in Africa to report the outbreak of the Asian lineage HPAI H5N1 on February 8, 2006 (Adene *et al*., 2006; WHO, 2006). The disease spread rapidly to 25 States and the Federal Capital Territory (FCT) during which about 440,000 poultry were culled within the first two months of the outbreak (AICP, 2008). In spite of the immediate constitution of an Integrated National Avian and

Pandemic Influenza Response Plan committee by the Federal Government, the HPAI H5N1 infections continued to surge with two peaks of the outbreaks in February, 2006 and February, 2007 (AICP, 2008). The disease was later reported in two farms in Kano and Katsina States in 2008 but was quickly brought under control without further spread and report up till date (AICP, 2008).

The constituted committee on the avian and pandemic influenza response plan had three strategic objectives. These objectives were: specific response and virus containment; virus control and prevention as well as pandemic preparedness and planning (AICP, 2007). In order to achieve the set objectives, the committee was divided into four components namely: animal health; human health; social mobilization and communication as well as project management and coordination components (AICP, 2007).

The animal health component undertook a number of measures such as increasing and beefing up animal disease surveillance points from 170 to 5,000 with adequate trained personnel; prompt outbreak monitoring and response with total stamping out of confirmed cases (AICP, 2007). The first AI active surveillance in Nigeria was carried out in March, 2007 while the next surveillance was carried out in November, 2007; all involving the entire States (AICP, 2008).

The surveillance was coordinated by the Food and Agricultural Organization with the Federal Livestock Department and Pest Control Services (FAO/FLDPCS), funded by the European Union (EU) and the United State Agency for International Development (USAID) (AICP, 2007). The waves of the HPAI outbreaks from 2006 to 2008 affected 3,037 farms and led to the culling of about 1.3 million of the estimated 160

million poultry with about eight hundred and ten million naira (N810,000,000.00) paid as compensation by the Government (AICP, 2008; ILRI, 2011).

A review of the HPAI H5N1 outbreaks in Nigeria pointed towards several sources as risk of introductions as the isolates showed three divergent relationships (Brown, 2006; Ducatez *et al*., 2006). Phylogenetic analyses showed that the isolates belonged to clade 2.2 but clustered around three sublineages represented by A (EMA1) for isolates from South-west Nigeria and Niger; B (EMA2) for other isolates from South-west Nigeria, Egypt and Djibouti; and C (EMA3) for isolates from northern Nigeria, Burkina-Faso, Sudan and Cote d'Ivoire (Ducatez *et al.,* 2006). Monne *et al*. (2007) identified the presence of reassortant H5N1 virus in poultry in Nigeria as a result of the co-circulation of these three sublineages of the EMA clade 2.2.

Although trade in poultry and poultry products were initially implicated as the sources of introduction of the HPAI H5N1 to Nigeria, this route became doubtful when viewed against the facts that the subclade 2.2 is not widely spread or established in places of high poultry density that were involved with Nigeria in poultry trade (Ducatez *et al*., 2006). The presence of the same isolates from Nigeria in Egypt and other parts of Africa led credence to the vital role of migratory wild birds in the epidemiology of AI because these areas lie within the same migratory wild bird flyways (Ducatez *et al.*, 2006). The outbreaks of HPAI H5N1 in Nigeria and other parts of Africa as well as India coincided with the over-wintering season of migratory wild birds along the East Africa-East Asia flyway (WHO, 2006; Ducatez *et al*., 2007).

2.1.9 Avian influenza in Africa

After Nigeria reported the first outbreak of HPAI H5N1 on February 8, 2006; Egypt, Niger and Cameroon reported outbreaks much later in February, 2006 heralding the

establishment of the infection on the African continent (Cattoli *et al*., 2009). A total of eleven (11) African countries experienced outbreaks, namely Nigeria, Egypt, Niger, Cameroon, Sudan, Djibouti, Burkina-Faso, Cote d'Ivoire, Ghana, Togo, and Benin (Ducatez *et al.*, 2007).

The activities of the virus were not limited only to poultry but fatal cases were also recorded in humans. By March, 2006; Egypt reported its first human case and subsequently continued to confirm cases in humans with several deaths. Nigeria and Djibouti have each recorded one human death (Cattoli *et al*., 2009). Till date, Egypt continues to experience HPAI H5N1 outbreaks in both poultry and humans with a record of 168 human infections and 60 deaths (PHAC, 2012).

Molecular analyses of the HPAI H5N1 isolates in 2006 from the affected African countries showed that three sublineages; A, B, and C of the EMA clade 2.2 were circulating in the continent (Ducatez *et al.*, 2007). The circulation of different sublineages at the same time meant different routes of HPAI H5N1 introductions to Africa (Brown, 2006; Ducatez *et al*., 2006). The EMA clade 2.2 viruses were the same viruses isolated in the Qinghai Lake HPAI H5N1 outbreak in 2005 among migratory wild birds (Salzberg *et al*., 2007; Cattoli *et al*., 2009). The viruses probably might have been carried from Southeast Asia to Europe through Central Russia to the Middle East and Africa by migratory birds (Ducatez *et al*., 2007; Cattoli *et al*., 2009).

Cattoli *et al*. (2009) reported that all the Egyptian isolates analyzed showed a monophylogenetic cluster around other HPAI H5N1 viruses from Gaza and Israel. Phylogenetic relationship was seen among the isolates from Burkina-Faso, Cote d'Ivoire, Ghana and Cameroon while the isolates from Nigeria, Niger and Togo

showed similarities in their phylogenetic topology (Ducatez *et al.*, 2007; Cattoli *et al*., 2009).

Ducatez *et al*. (2007) and Cattoli *et al*. (2009) reported the clustering of the HPAI H5N1 strains from Nigeria in the three sublineages. The co-circulation of viruses of different sublineages creates opportunities for the emergence of reassortant variant. Salzberg *et al*. (2007) reported of the first reassortant HPAI H5N1 strain, A/ck/Nigeria/1047-62/2007 virus in 2006. In addition, a reassortant variant different from the first reassortant virus has been described (Monne *et al*., 2008). The HA and NA genes of A/Nigeria/6e/2007 virus that led to the death of a woman in Lagos, Nigeria in 2007 were analyzed and found to be identical to the HA and NA genes of reassortant virus detected in poultry in 2007 (Cattoli *et al*., 2009).

2.1.10 Avian influenza in wild birds

The full range of AI subtypes is found in wild birds. The first isolation of AI virus from a free living bird was in South Africa from a Tern (*Sterna hirundo*) in 1961 (Becker, 1966). The virus isolated was classified as A/tern/South Africa/61 HPAI H5N3. This was a pointer to the fact that wild birds could harbour AI viruses without showing clinical disease (Stallknecht and Brown, 2008). Generally, isolations of AI viruses have been made from over 100 species of wild birds in 12 orders but most consistent isolations have been from species in 2 orders – the Anseriformes and the Charadriiformes that share aquatic habitat (Stallknecht and Brown, 2008).

Alexander (2007) Stated four reasons why wild birds were implicated in playing a role in the epidemiology of AI. These reasons he gave were higher prevalence of AI infections in poultry units along wild bird migratory flyways, a higher level of infection in poultry kept under free range compared to those reared under closed

environments, similar virus subtypes infecting poultry and wild birds in an area of outbreak and seasonality in LPAI or HPAI outbreaks tallying with migratory wild birds patterns of migration.

Previously, wild birds were thought to be dead end host of HPAI H5N1 because isolations of AI viruses were made only from dead wild birds within a flight range of infected poultry farms (Alexander, 2007). In 2005, the outbreak of HPAI H5N1 among migratory wild birds in Qinghai Lake, Western China with no nearby poultry farms had changed the earlier view (Chen *et al*., 2005). Over 1,500 birds which were mainly bar-headed geese (*Anser indicus*) died in the outbreak (Chen *et al*., 2005).

There are strong evidences to support the speculation that wild birds are involved in the spread of HPAI viruses. In July 2005, HPAI H5N1 outbreak occurred in Central Russia spreading to Siberia with dead migratory birds found in the areas after the outbreak in Qinghai Lake, Western China (WHO, 2006). This was shortly followed on August 2, 2005 with the detection of HPAI H5N1 among poultry in Kazakhstan, then further spread to Mongolia on August 12, 2005 with isolation of H5N1 from dead wild birds at Erhel Lake (WHO, 2006). The spread of HPAI H5N1 continued to Turkey, Romania in October 2005, Ukraine and Croatia in December, 2005 (WHO, 2006). The FAO (2005) predicted that HPAI H5N1 would spread from Siberia to the Caspian and the Black sea areas along migratory wild birds' routes which actually occurred as predicted. It was probably migratory wild birds that spread the Asian lineage HPAI H5N1 to India, Nigeria, Egypt, Niger and other areas in early 2006 as these areas lie within the East Africa/East Asia flyway (WHO, 2006).

The Qinghai Lake and Erhel Lake HPAI H5N1 outbreaks led to more intensified AI viruses' surveillance in wild birds worldwide resulting in the expansion of our

knowledge on the role of wild birds in AI epidemiology (Kilpatrick *et al*., 2006). Wild bird surveillance activities were also carried out in Africa at the onset of HPAI H5N1 in the continent. Gaidet *et al*. (2007) reported the detection of LPAI in a variety of species from major wetlands in northern, western and eastern Africa indicating that the tropical environment of Africa is also suitable for the maintenance and spread of AI viruses. It is now obvious that wild birds are not just asymptomatic carriers of LPAI viruses but are also reservoirs of HPAI and are able to effectively spread the viruses over long distances on their flyways (FAO, 2005; Kilpatrick *et al*., 2006).

2.1.11 Diagnosis and detection of avian influenza

The usefulness of any diagnostic test is in its accuracy and the ease with which it can be rapidly applied either in the field or in the laboratory to obtain result for early control (Swayne, 2008). There are no pathognomonic signs for either HPAI or LPAI in birds (Abdu, 2007; Suarez, 2008). The incidence of HPAI in most cases may be peracute resulting in death without clinical signs while LPAI in the absence of exacerbating diseases causes only mild respiratory diseases or reduction in egg production in laying birds (Easterday *et al*., 1997). Confirmatory diagnosis is by isolation of the virus or identification and characterization of fragments of its genomes as well as serological detection of AI specific antibodies (OIE, 2009). Several diagnostic tests for the detection and characterization of AI viruses are discussed below with their varying sensitivity, specificity, cost and speed of applications:

2.1.11.1 Virus isolation

This is the gold standard for the confirmation/detection of AI virus in an index case before further characterization of the AI virus subtype (OIE, 2009). According to OIE (2009), the method involves the inoculation of the supernatant of a suspected clinical

sample after centrifugation at 1000 ***g*** into the allantoic sac of a specific pathogen free (SPF) or specific antibody negative (SAN) embryonated chicken egg of 9-11 day-old incubation. At least 5 SPF or SAN eggs are used per sample; the eggs are then incubated at 37˚C for 4-7 days of incubation. Eggs containing dead or dying embryos as they arise and all eggs remaining at the end of the incubation period should be chilled at 4˚C and the allantoic fluid tested for haemagglutinating (HA) activity. Detection of HA activity in a bacterial free amnio-allantoic fluid indicates the likelihood of the presence of an AI virus or a paramyxovirus. Fluid that gives a negative reaction should be further passaged into at least one batch of eggs.

The presence of AI virus can be confirmed in an allantoic fluid with HA activity by agar gel immunodiffusion (AGID) test, ELISA and RT-PCR targeting the nucleoproteins or matrix protein (OIE, 2009). Characterization of AI subtypes is done using subtype specific haemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests for the range of H1-H16 and N1-N9 subtypes. Although virus isolation is the gold standard for confirmatory diagnosis, it is costly, time consuming taking up to 2-4 weeks before result can be obtained and requires specialized laboratory with high biosafety (P3).

2.1.11.2 Serological tests

Serological tests either detect antibodies against any influenza A or antibodies against specific subtype surface proteins. Three serological tests are widely used and are discussed below:

Agar gel immunodiffusion (AGID) Test: The OIE (2005) considered this as the gold standard for serological screening of poultry flocks for avian influenza viruses. The AGID test is able to measure antibodies response to both the nucleoproteins and

matrix antigens of AI viruses (OIE,2009). The test has been widely and routinely used to detect specific antibodies in chicken and turkey flocks as an indication of infection, but AGID tests are less reliable at detecting antibodies following infection with influenza A viruses in other avian species because most avian species do not produce precipitating antibodies (OIE, 2008).

According to OIE (2009) the AGID test is carried out using gels made from 1% (w/v) agarose or purified agar and 8% (w/v) NaCl in 0.1M phosphate buffered saline (PBS) at pH 7.2, poured out to a thickness of 2-3 mm in petri dishes. Wells of about 5 mm in diameter and 2-5mm apart are cut in the agar with a template well cutter. The pattern of wells in the agar should place each suspect serum adjacent to a known positive serum and antigen so as to give a continuous precipitin line between the known positive, the suspect serum and the antigen. About 50 ul of each reagent should be added to each well and precipitin lines can be detected after 24-48 hrs depending on the concentration of the antigen and antibody. A positive result is recorded when the precipitin line between the known positive control well is continuous with the line between the antigen and test serum. Cross lines are negative, interpreted to be due to the test serum lacking identity with the antibodies in the positive control well. The test is simple, less expensive and highly specific but has low sensitivity compared to other serological tests and cannot be used in species that do not produce precipitating antibodies such as waterfowl.

Haemagglutination inhibition test: This is a standard reference method used by the OIE to detect antibodies to the haemagglutinin antigen of AI viruses. The test is based on the ability of antibodies present in test serum to inhibit the AI antigen haemagglutinating activity of chicken RBCs as a result of their coupling in antigen-

antibody reaction. The test is subtype specific and requires antigen for each of the 16 haemagglutinin subtypes. Positive test serum indicates either previous exposure to virus of the same subtype or protective antibodies derived from vaccine of the same subtype. The test is cost effective, rapid turnaround time (2-3 hours) and provides quantitative indication of virus titre.

To carry out the haemagglutination inhibition (HI) test, the haemagglutination test is first carried out to determine the 4HAU antigen to be used for the HI test. The reagents needed for these tests are isotonic PBS (0.01M) pH 7.0-7.2, and RBCs taken from at least three SPF or SAN chickens pooled in an equal volume of Alsever's solution (OIE, 2009). The RBCs should be washed three times in PBS by centrifugation at 1000 ***g*** for 5 minutes before use as a 1% (v/v) suspension of pack cell. Positive and negative control antigens and antisera should be run with each test as appropriate (OIE, 2009).

According to OIE (2009), the procedures for performing the HI test using a V-bottom microtitre plastic plates with a final volume of 75 ul is as follows:

i) Dispense 25 ul of PBS into each well of a plastic V-bottomed microtitre plate.

ii) Place 25 ul of serum into the first well of the plate.

iii) Make twofold dilutions of 25 ul volumes of the serum across the plate except the control wells.

iv) Add 25 ul of the 4 HAU antigens to each well and leave for a minimum of 30 minutes at room temperature (i.e. about 20°C) or 60 minutes at 4°C.

v) Add 25 ul of 1% (v/v) chicken RBCs to each well and mix gently, allow the RBCs to settle for about 40 minutes at room temperature, i.e. about 20°C, or

for 60 minutes at 4°C if ambient temperatures are high, by which time control RBCs should have formed a distinct button.

vi) The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 25 ul RBCs and 5 ul PBS only) should be considered to show inhibition.

vii) The validity of results should be assessed against a negative control serum, which should not give a titre >1/4 (>2^2 or >$2\log_2$ when expressed as the reciprocal), and a positive control serum for which the titre should be within one dilution of the known titre.

HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 (2^4 or 4 $\log_2$ when expressed as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titre is 1/8 (2^3 or $3\log_2$) or more. The meaning of a minimum positive titre should not be misinterpreted; it does not imply, for example, that immunised birds with that titre will be protected against challenge or that birds with lower titres will be susceptible to challenge (OIE, 2009).

Chicken sera rarely give nonspecific positive reactions in HI test and any pretreatment of the sera is unnecessary. Sera from species other than chickens may sometimes cause agglutination of chicken RBCs. Therefore, each serum should first be tested for this idiosyncrasy and, if present, it should be inhibited by adsorption of the serum with chicken RBCs. This is done by adding 25 ul of packed chicken RBCs to each 5 ul of antisera, mixing gently and leaving for at least 30 minutes; the RBCs are then

pelleted by centrifugation at 800***g*** for 2–5 minutes and the adsorbed sera are decanted. Alternatively, RBCs of the avian species under investigation can be used (OIE, 2009).

Enzyme linked immunosorbent assay: Several commercial enzyme linked immunosorbent assay:(ELISA) kits that detect antibodies to AI matrix or nucleoprotein are available (OIE, 2009). Indirect and competitive ELISA formats have been developed and are being used to detect AI specific antibodies in serum, plasma and egg yolk from chickens, turkeys and other species. ELISA has high sensitivity but low specificity leading to false positive results hence, ELISA positive tests are often confirmed with AGID test (Spackman *et al.,* 2008). The main advantage of ELISA is that it is faster than AGID, it is amenable to high throughput testing and interpretation of results is less subjective as in AGID (OIE, 2009).

2.1.11.3 Molecular techniques

Advances in science have made possible the use of molecular methods for the detection of viral nucleic acid to show the presence of AIVs and identification of HA and NA subtypes in clinical sample (Fourchier et al., 2004; OIE, 2009). Molecular methods offer numerous advantages for AIVs detection such as high sensitivity and specificity, scalability, the use of any sample type with proper sample processing, and minimization of contact with infectious materials because the virus is inactivated early during sample processing (Spackman *et al*., 2008).

The molecular methods that are available include conventional reverse transcription polymerase chain reaction (RT-PCR), real time reverse transcription polymerase chain reaction (rRT-PCR), nucleic acid sequence based amplification (NASBA) and loop mediated isothermal amplification (LAMP) (Spackman *et al*., 2008). Although NASBA and LAMP are sensitive, the tests have not been widely used.

Reverse transcription polymerase chain reaction: The RT-PCR is based on the direct detection of viral genomic riboxynucleic acid (RNA) against a template. The principle requires that the viral RNA in the clinical sample be extracted and converted to a complementary deoxyribonucleic acid (cDNA) by the enzyme, reverse transcriptase which is then amplified through several polymerase chain reactions (PCR). The use of RT-PCR for the detection of AIVs requires nucleoprotein or matrix-specific conserved primers while the detection of H5 and H7 subtypes requires H5- or H7-specific primers (Alexander, 2008).

2.1.12 Prevention and control of avian influenza

There is no single effective prevention and control strategy for AI (Cardona, 2007; Suarez, 2008). The effectiveness of any applied strategy is affected by the pathotype of the AIVs, presence of wild birds, the structure of the poultry industry, movement of birds in legal and illegal live bird trade as well as government policies regarding import and export of poultry products (Capua and Morangon, 2006; Swayne, 2008). Strategies for prevention and control of AI are geared towards achieving two main objectives of preventing the introduction of the AIVs into poultry populations from the reservoirs when absent and reducing the economic impacts of the disease or total eradication when present (Swayne, 2008).

The strategies are often used in combinations to achieve the two main objectives of prevention and control of AI. According to Swayne (2008), the strategies employed include biosecurity, surveillance, culling of infected birds, vaccination to reduce host susceptibility to infection and education.

2.1.12.1 Biosecurity

This encompasses management practices to reduce the likelihood of AIVs exposure in a naïve poultry population or once present, to reduce spread to other areas hence important in preventing disease incursion (Capua and Morangon, 2006). Biosecurity can be applied at both the national and farm levels to achieve the objectives of prevention and control.

At the farm level, biosecurity focuses on movement restriction, preventing exposure to wild birds, use of disinfection and cleaning to reduce contamination, rearing of poultry in compartments and using the practice of all-in all-out system of poultry production (Swayne, 2008). Biosecurity at the national level may include regulations restricting importation of live poultry and products into a country, restriction of bird movement within a country and the restructuring of poultry marketing and production systems (Cardona, 2007; Halifa, 2008; Pagani *et al.*, 2008).

The success of biosecurity in prevention and control of AI is dependent largely on the poultry production systems and country (Swayne, 2008). In Nigeria, the production system is mainly extensive with rural poultry predominating over commercial poultry (Adene and Oguntade, 2006). This has often made the institution of biosecurity difficult due to the prevailing husbandry practices (Ameji *et al*., 2012).

2.1.12.2 Surveillance

This refers to a continuous searchlight to detect the AIVs in the reservoir or susceptible hosts. Early and successful control of AI requires a functional surveillance system. Surveillance helps in certifying a country free of AI as well as in identifying an infected zone or compartment (Dufour *et al.*, 2001; Swayne, 2008). In an infected country, zone and compartment (CZC), the objectives of surveillance are to describe

spatial and temporal distribution of disease so as to institute control measures, assessment of control programmes and monitoring of antigenic drift.

The conduct of surveillance strategies for AI differs from country to country but generally involves activities that monitor wild birds and poultry populations either by passive or active surveillance approaches (Swayne, 2008). Wild bird surveillance is used as an early warning system to identify the probable introduction of AIVs into poultry populations (Nettles *et al.*, 1985; Burns *et al.*, 2010). Passive surveillance has been the method employed to identify LPAI viruses as the cause of respiratory disease or drop in egg production and HPAI viruses as the cause of high mortality (Easterday *et al*., 1997).

2.1.12.3 Culling of infected birds

Elimination of the source of AI viruses in CZC is important for controlling an outbreak and eradicating the disease. In an infected flock, the high risk materials such as birds, eggs and manure should be eliminated (Swayne, 2008). For HPAI, culling means humane depopulation and disposal of carcasses, eggs and manure in an environmentally friendly manner such as composting, incineration, rendering or landfill burial (Swayne, 2008).

However, for LPAI stamping out and disposal methods are less commonly used but alternatively, controlled marketing of birds 2-3 weeks after recovery from infection and washing of eggs before being marketed are used (Swayne and Halvorson, 2008). In LPAI infection, virus shedding occurs during the first 2 weeks after initial infection and by the 4^{th} week, virus can no longer be detected. Hence, antibody positive flocks have a low risk of transmission if maintained under strict biosecurity (Swayne, 2008).

Culling of infected birds through stamping out and disposal will only be successful if compensations are paid by regulatory authorities in a timely and adequate manner. Compensation is used as an incentive to encourage early disease reporting and to ensure the compliance of flock owners with the culling programme (Swayne, 2008; Abdu, 2010). A successful compensation programme to enhance avian influenza control must have a clear definition of who is to be compensated, the losses to be compensated, a clear case definition of AI and prompt reporting, an organized structure of payment, agreed compensation cut-off and availability of fund (Abdu, 2010). Successful stamping out and compensation programme had been used in Nigeria in the wake of the HPAI H5N1 outbreaks in 2006 (AICP, 2008; ILRI, 2011).

2.1.12.4 Vaccination in avian influenza infection

Vaccination is used to reduce host susceptibility to infection in order to limit or prevent disease spread (Swayne *et al*., 2001). It has been shown that vaccination can increase resistance to field challenge, reduce shedding of virus as well as the rate of disease transmission (Capua and Morangon, 2003). The use of vaccination alone may severely affect the effectiveness of AI prevention and control because vaccinated birds continue to shed the virus (Swayne *et al.,* 2001). It may be difficult to declare AI disease freedom due to the continuous viral replication in the host leading to silent spread (Swayne and Halvorson, 2008).

The use of vaccination becomes effective when combined with other components of AI control strategy such as elimination of naturally infected flock by differentiating vaccinated birds from naturally infected birds (DIVA) principle, biosecurity and education (Capua and Morangon, 2003). The use of sentinel birds placed among

vaccinated birds and tested serologically is the widely used DIVA method in most countries that have adopted vaccination in AI control (Capua and Morangon, 2003).

Production of an immune response that provides protection against the disease and prevention of infection is the targeted objective of any vaccination programme (Swayne *et al*., 2001). Several types of AI vaccines of protective value in use fall into the following categories: inactivated whole avian influenza virus vaccines, in-vitro expressed haemagglutinin vaccines, and in-vivo expressed haemagglutinin vaccines (Swayne *et al*., 2001).

2.1.12.5 Public enlightenment and awareness campaign

Education of poultry stakeholders and the general public on AIVs introduction, spread and the preventive measures to adopt is very important in prevention and control strategy (Swayne, 2008). The avoidance of risky behaviors and actions greatly reduces the spread of AIVs by controlling formite and aerosol dispersion of virus hence, preventing AIVs movement onto the farm and between farms (Swayne, 2008).

Education through public enlightenment campaigns was a component of the strategies that ensured the success of the eradication of the HPAI H5N1 outbreaks in Nigeria (AICP, 2008).

2.1.12.6 Inactivation and decontamination of avian influenza viruses

Inactivation and decontamination are methods used by regulatory authority, poultry farmers and personnel involved in AI disease prevention, control and eradication to get rid of the AIVs from a contaminated environment (Meroz and Samberg, 1995). Inactivation and decontamination of AIVs is achieved mainly through the processes of cleaning and disinfection (Songserm *et al.*, 2006).

Cleaning: A thorough cleaning and removal of all organic materials from the environment should precede disinfection so that pathogens come in direct contact with the disinfectants (Bohm, 1998). The presence of organic substances reduces the efficacy of disinfectants and renders some inactive (Meroz and Samberg, 1995). Effective cleaning reduces pathogens in the environment below infectious levels. Cleaning is usually done with water, soaps and detergents before disinfection with disinfectants (Block, 2001).

Disinfection by physical method: This method of disinfection employs various natural or artificial physical means to reduce the load of pathogens in the environment. The method when applied often gives variable results depending on the presence of organic materials, high temperatures and contact time (Meroz and Samberg, 1995). The physical means often used are ultra violet light, dry heat and steam heat.

Disinfection by chemical method: Most poultry farmers use chemical disinfectants for routine cleaning and disinfection procedures (Mauldin, 1984). In the USA, disinfectants are registered by the Environmental Protection Agency (EPA) which has so far registered over 100 disinfectants to be used against AIVs on hard, non porous, inanimate surfaces (EPA, 2006).

The major classes of chemicals used in AIVs disinfection include soap and detergents; inorganic compounds such as iodine; peroxygen compounds; ethanol; hypochlorites; phenols; saponated cresols; quaternary ammonium compounds; aldehydes; acids and bases (Bohm, 1998; EPA, 2006).

2.1.13 Public health importance of avian influenza

Avian influenza is a zoonotic disease that spreads from animals to humans (Brankston *et al.*, 2007). All human influenza pandemics have been caused by viruses that contain viral genetic components from AI viruses (Suarez, 2008).

At present, HPAI H5N1 viruses are panzootic in poultry and pose an ominous threat to global public health due to their ability to directly infect humans often, with fatal consequences (Peiris, 2007). At the moment, these viruses are still inefficiently transmitted from infected birds to humans but there is fear of a likely danger of HPAI H5N1 viruses gaining the ability to transmit and spread among humans in an efficient manner to cause the next influenza pandemic (Suarez, 2008). The grave concern about the HPAI H5N1 human epidemics is the unusually high case-fatality rate of about 60% among infected humans since 2003 and the devastating impacts that may occur if these viruses gain the ability to efficiently transmit amongs humans (Sims and Brown, 2008).

Human influenza is a highly contagious acute respiratory disease (ARD) caused by infection with human influenza A, B or C viruses (Call *et al*., 2005). Influenza infection is often self limiting but complications may result in substantial morbidity and mortality worldwide. Human influenza viruses cause an annual toll of 300,000-500,000 deaths worldwide and in the 1957-1958 pandemic years the death toll was approximately 1 million while an estimated 40-50 million deaths occurred during the 1918-1919 devastating pandemic (Beveridge, 1991).

The public health impact of influenza is mainly due to influenza A and B virus infections (Beveridge, 1991; Subbarao and Katz, 2000). Among the subtypes of influenza A, only subtypes H1N1, H1N2 and H3N2 viruses are currently circulating

in humans (Peiris, 2007). Influenza viruses circulating in humans are evolving continuously and in an unpredictive manner due to antigenic drift in which random point mutations in the HA gene results in amino acid changes in the HA surface protein during replication (Capua and Alexander, 2004).

Antigenic drift is the cause of seasonal influenza epidemics of unpredictable and variable severity that occurs during the winter months of October-April and May-September in the northern and southern hemispheres respectively (Suarez, 2008). Influenza occurrence is poorly described in developing countries however, in the tropics, human influenza generally occurs all year round but with increases during the cold months and rainy periods (Brankston *et al*., 2007).

The attack rates of influenza are usually highest in toddlers, pre-school and young school-aged children leading to high school absenteeism and high rates of outpatient visits to hospitals (O'Brien *et al.,* 2004). Clinical manifestations of human influenza are variable depending on age, underlying chronic disease and associated complications (Call *et al.,* 2005). A common feature in most cases is what is termed the influenza-like illness (ILI) characterized by fever, malaise and upper respiratory disease seen as oculonasal discharge, sneezing, coughing, rhinitis, etc (Call *et al.*, 2005). Older persons from the age of 65 with cardiopulmonary disease, and infants have the highest rates of hospitalization due to complications. Although deaths have occurred in previously healthy children, the elderly have the highest rates of mortality (Call *et al*., 2005).

Human-to-human transmission is via viral particles in droplets expelled through coughing and sneezing by infected person (Brankston *et al.,* 2007). After infection the incubation period is 2 days with a range of 1 to 4 days (O'Brien *et al.,* 2004). Virus

shedding in infected person begins a day prior to onset of illness and continues 4 to 5 days after occurrence of symptoms (Brankston *et al.*, 2007). So far, from 2003 -2014, over 668 persons had been infected worldwide since the HPAI H5N1 epidemic started from Southeast Asia with 393 (60%) case fatality (WHO, 2014; PHAC, 2014). These fatal cases included the one case of a woman that died of bird flu in Lagos in 2007 and up to 63 deaths in Egypt (PHAC, 2014, WHO, 2014).

2.2 Historical Perspective of Newcastle Disease

Newcastle disease (ND) was first reported in Newcastle upon Tyne in 1926 (Jordan, 1990). It was initially referred to as fowl plague as a result of the high virulence associated with the disease and the perceived thoughts that it was the same as avian influenza (Swayne, 2008). There was a general disagreement in accepting ND as a different disease even after Doyle had described it as another disease and this lasted until 1955 when the viral cause of fowl plague became known as avian influenza virus (Kaleta and Catherine, 2008).

Newcastle disease has a worldwide distribution and had caused three major panzootics in poultry in the past (Miller and West, 1972). The first was at the emergence of the disease in fowls in the mid 1920s around Europe and Far East spreading slowly throughout the world Miller and West, 1972). The second panzootic was in the late 1960s among fowls in the Middle East which spread much faster than the first as a result of international trade in pet birds to reach all continents and most countries by 1973 (Jordan, 1990). The third panzootic was associated with neurotropic and enteric form in the pigeons around the Middle East that spread rapidly among racing, show, meat and feral pigeons with concomitant infections of other poultry in some countries. Control measures employed within these periods with

success was a compulsory slaughter policy until 1963 when it was replaced by vaccination policy (Miller and West, 1972).

Newcastle disease is the most devastating disease of rural chickens in Nigeria and Africa hence, it is the most researched disease in rural chickens in a bid to reduce the disease impacts on poultry health and productivity (Adene, 1996; Yongolo, 1996).

2.2.1 Aetiology of Newcastle disease

Newcastle disease is caused by Paramyxovirus-1 (PMV-1) which is an enveloped single stranded non-segmented RNA virus belonging to the genus, *Avulavirus* and family, *Paramyxoviridae* (Abdu, 2007). Viruses of the *Paramyxoviridae* family are pleomorphic, assuming filamentous or spherical forms with a size of 100-500 nm in diameter (Jordan, 1990). The viral envelope has two surface projections made up of two glycoproteins of variable lengths. The longest surface projection is made of a single glycoprotein with both haemagglutinin and neuraminidase activities while the shorter surface projection is the F glycoprotein associated with fusion of the virus with host cell membrane (Jordan, 1990).

2.2.2 Pathotypes of Newcastle disease

Newcastle disease was first described in 1926 and when the virus was later isolated, it was thought to be the only member of the genus, *Paramyxovirus* (Jordan, 1990). Several isolations have been made from other avian species of members of the *Paramyxovirus* that are distinct from ND virus which led to their grouping into serotypes ranging from PMV-1 to 9 (Jordan, 1990).

Newcastle disease virus has different strains with ability to cause disease of variable signs and severity even in the same host (Jordan, 1990). Based on the disease effects produced in chickens, ND viruses have been grouped into five pathotypes which are:

i. Viscerotropic velogenic ND virus strains that cause highly virulent form of ND with haemorrhagic lesions in the GIT.

ii. Neuerotropic velogenic ND virus strains that cause high mortality with respiratory and nervous signs.

iii. Mesogenic ND virus strains that cause respiratory and sometimes nervous signs with low mortality.

iv. Lentogenic ND virus strains that cause mild or inapparent respiratory infection.

v. Asymptomatic ND virus strains that cause inapparent enteric infection (Jordan, 1990).

2.2.3 Transmission of Newcastle disease

The ND virus is shed in faeces and aerosols from infected birds (Abdu, 2007). People play a major role in the spread of ND virus between farms usually by movement of live birds, fomites, personnel and poultry products from infected birds and premises to susceptible birds and premises (Jordan, 1990). Wild birds and other wildlife undoubtedly contribute to the spread of the virus during epizootics either by infection or mechanical transfer (Sa'idu *et al*., 2004; Abdu, 2007; Swayne, 2008).

Birds shed virus during incubation, clinical disease and for variable time during convalescence. The virus may be present in eggs laid during clinical disease and in every part of carcass during acute virulent infection (Cynthia and Scott, 2008). The ND virus can persist in improperly cleaned, infected premises and dead birds for several weeks or years if held frozen (Jordan, 1990).

There is seasonality in the occurrence of ND which is more around the cold months between October and March where increase in wind speed during the harmattan enhances its transmission (Sa'idu *et al*., 1994).

2.2.4 Hosts of Newcastle disease

The ND virus affects over 200 species of birds either naturally or experimentally (Jordan, 1990). The virus strains have been shown to affect all the major species of domestic poultry although some species such as ducks and geese tend to show inapparent infections even with the most virulent strains of ND virus for chickens (Nwanta *et al.*, 2006; Abdu, 2007).

The ND virus affects all ages of birds but the young are more susceptible with variable clinical signs depending on the strains and pathotypes involved as well as the type of birds (Nwanta *et al.*, 2006). Onset of clinical signs is sudden and rapid after an incubation period of 2–12 days. There is sudden death in per acute cases, marked dropped in egg production in laying hens, eggs may be mishapened or soft shelled, somnolence, inappetance, prostration, greenish diarrhoea, dyspnoea, sneezing, oedema of head and nervous signs may occur with mortality of up to 100% (Miller and West, 1972; Abdu, 2007).

There are no pathognomonic lesions for any form of ND (Jordan, 1990). Remarkable lesions are usually seen only with very virulent ND in which there are severe congestion of skeletal muscles, serous exudates in nasal passages, thickened air sacs, haemorrhages and necrosis of caecal tonsils, haemorrhages and necrosis of the intestinal wall as well as haemorrhagic ovarian follicles with ruptured yolk in abdominal cavity in laying hens (Abdu, 2007).

2.2.5 Public health importance of Newcastle disease

Newcastle disease is zoonotic though, the zoonotic risk is minimal (Cynthia and Scott, 2008). Virulent or live vaccine strains of ND virus can produce conjunctivitis in humans when exposed to large doses of the virus (Miller and West, 1972). Prior to the

wide spread adoption of vaccination to control ND, conjunctivitis due to ND virus infection occurred in personnel eviscerating poultry meat in processing plant but the disease has not been reported in poultry farmers or consumers of poultry products (Miller and West, 1972; Cynthia and Scott, 2008).

2.2.6 Diagnosis of Newcastle disease

The gold standard and definitive methods of diagnosis are isolation and identification of the virus after demonstration of haemagglutinating antibodies in haemagglutination inhibition test. Diagnosis using ELISA kits is available for rapid field use to screen for ND virus (Jordan, 1990).

2.2.7 Prevention and control of Newcastle disease

Strict biosecurity measures and voluntary vaccination are strategic prevention and control policies adopted to control ND (Miller and West, 1972; Jordan, 1990). Live vaccines made from the lentogenic and mesogenic strains are used to protect birds against ND field virus challenge (Boven *et al.,* 2008). Killed vaccines or oil–adjuvanted inactivated vaccines are also available but they are only used after priming with the lentogenic strains (Jordan, 1990).

Vaccination alone is not enough to control ND under field conditions (Abdu, 2007). Hence, it has to be coupled with strict biosecurity measures to achieve effective control (Nwanta *et al.,* 2008). Good management practice such as adequate housing and stocking to avoid overcrowding, good ventilation and proper cleaning and disinfection of poultry premises and equipment help to keep out infection and its spread (Jordan, 1990; Abdu, 2007).

The development of the ND thermostable vaccine (NDV4) raised the hope for the effective control of ND among rural poultry in the tropics (FAO, 1998). Most African

countries adopted the trial use of the vaccine which later failed due to lack of an appropriate food carrier for the vaccine (FAO, 1998). Rural poultry farmers on the other hand utilize risk reduction measures such as sales or slaughter of birds during the cold months to reduce flock size and stocking density as well as movement control to avoid contact with infective materials or carrier birds in the prevention and control of ND (Nwanta *et al*., 2006; Nwanta *et al.,* 2008).

2.3 Wild Bird – Poultry Interactions

Wild bird – poultry interactions refer to the communication of wild birds and poultry either directly or indirectly. Direct communication enables close contact of wild bird and poultry while indirect communication is via contact with contaminated environments by faeces, secretions and other body parts that may lead to the sharing of pathogens (Hoye *et al.,* 2010). Wild bird species can transmit infectious agents either by active shedding or by mechanical transfer of the agents in water droplets or feces containing AIVs and other pathogens. Wild bird species that enter poultry houses could carry AIVs directly into the poultry flock or could contaminate environments from where AIVs could be carried into poultry houses by farm workers, equipment, pets, rodent or insects (Hoye *et al.,* 2010).

The outbreaks of HPAI (H5N1) first started in Hong Kong SAR in 1997, persisted in Mainland China and the Asian subregion up to 2003 and by the second half of 2005, the HPAI (H5N1) virus appeared in Russia and Romania (Chen *et al.,* 2006; Gilbert *et al.* 2006). The disease became panzootic in 2006 around the Black Sea, the Mediterranean, Western Europe and Africa (Si *et al.* 2009). The tempo-spatial occurrence and spread of HPAI (H5N1) along major intercontinental migratory bird flyways supported the suggestions that wild birds' migration and trade in domestic

poultry could be responsible for the global dispersion of the HPAI H5N1 virus (Brown, 2006; Kilpatrick *et al.,* 2006).

Two major pathways of HPAI (H5N1) disease spread had been put forward and are currently been investigated (Si et al., 2010). The first pathway is linked with trade in poultry and products from infected areas while the second is linked with the movement of migratory wild birds. Hence, knowledge of wild bird – poultry interactions and human activities are keys to the complete understanding of the mechanisms of introduction and spread of HPAI (H5N1) and other avian pathogens from infected regions to areas that were hitherto free of the agents (Sabir *et al.,* 2010).

There are several factors that encourage wild bird-poultry interactions, the understanding of which will help in identifying high risk environments, setting priorities for preventions and developing preventive measures against future outbreaks (Si *et al.,* 2010). Some of the environmental factors known to influence wild bird-poultry interaction include: surface water availability, topography, climate, crop farming system), the distance to roads, poultry density, human population density, LBMs among others (Gilbert *et al*., 2012; Si *et al.,* 2010). In addition, biosecurity and poultry husbandry practices play important role as the confounding factors that bring poultry to close activity space with wild birds (FAO, 2008).

2.4 Biosecurity and Husbandry Practices in poultry Production

Biosecurity refers to all the management practices aimed at excluding or reducing the potential for the transmission and spread of diseases to animals, humans or an area initially free from the disease causing agents (Halifa, 2008). It is a term coined from two words: Bio – life, and Security – protection, with the two main objectives of biosecurity being bio-exclusion and bio-containment (AICP, 2008; USAID, 2009).

Either of the two objectives of biosecurity has three components consisting of isolation, containment and sanitation.

Biosecurity is of much importance in poultry production in so much that the FAO based the classification of poultry production systems on the levels of biosecurity (Adene & Oguntade, 2006). Strict biosecurity measures in addition to vaccinations are strategic prevention and control policies adopted to control some contagious poultry diseases as vaccinations alone are not enough to control them under field conditions (Abdu, 2007). Based on the level of biosecurity, the FAO classified the poultry production systems into generally into the following:

Sector 1: Industrial integrated system with high level of biosecurity and birds or products are marketed commercially (e.g. farms that are part of an integrated broiler production enterprise with clearly defined and implemented standard operating procedures for biosecurity).

Sector 2: Commercial poultry production system with moderate to high biosecurity and birds or products is usually marketed commercially (e.g. farms with birds kept indoors continuously; strictly preventing contact with other poultry or wildlife).

Sector 3: Commercial poultry production system with low to minimal biosecurity and birds/products entering live bird markets (e.g. a caged layer farm with birds in open sheds; a farm with poultry spending time outside the shed; a farm producing chickens and waterfowl).

Sector 4: Rural or backyard production with minimal biosecurity and birds or products are consumed locally.

However, there are two distinct poultry production systems in Nigeria, as in most developing countries of Africa and Asia. The two poultry production systems are the commercial and the rural poultry differing in scale, stock, husbandry and productivity (Adene and Oguntade, 2006; Abdu, 2010). The commercial production system is industrial in its scale, husbandry and productivity with large, dense and uniform stocks of exotic or modern improved poultry. The rural poultry production refers to the small scale extensive method of poultry farming with small flock size, mixed and multiage species maintained under free range usually with little or no input in housing and veterinary care (Sonaiya, 1990; FAO, 1998). The rural poultry comprises of indigenous domestic fowls (chickens, guinea fowls, ducks, turkeys, pigeons, geese, ostriches, quails and peacocks) (Adene and Oguntade, 2006).

Traditionally, based on husbandry or management practices, poultry production is grouped into intensive and extensive management systems (Pagani *et al*., 2008)). The main management criteria used in these groupings are feeding, housing and biosecurity. Under the intensive management system, feeding, housing and other management requirements are adequately provided while feeding and housing are rarely provided under the extensive system (Adene & Oguntade, 2006). It is well known that poultry production under extensive management system do not receive proper nutrition, suffer from effects of harsh weather, and are exposed to various diseases than the intensive system of management (Sonaiya, 1990).

Good husbandry practices such as adequate feeding, housing and stocking to avoid overcrowding, good ventilation, proper disposal of wastes, cleaning and disinfection of poultry premises help to keep out infections and their spread (Jordan, 1990). Hence, efforts should be geared towards the improvement the rural poultry

management as they remain a valuable part of the rural economy, source of family protein and could negatively limit commercial poultry production by acting to spread diseases (Sonaiya, 1990; Abdu, 2010).

Generally, current and comprehensive information on the poultry production sector in Nigeria is lacking (Adene & Oguntade, 2006). Similarly, in Kogi State, commercial poultry production is low with majority of poultry being rural poultry that are kept under extensive system of management (Adene & Oguntade, 2006). In spite of the fact that HPAI H5N1 was not reported in the State during the HPAI (H5N1) outbreaks in Nigeria, the low level of poultry production has continued long after the socio-economic impacts of the disease that led to drastic shortfall in poultry production in most parts of the country had improved (Ameji *et al.*, 2011).

CHAPTER THREE

SERO-PREVALENCE OF AVIAN INFLUENZA AND NEWCASTLE DISEASE IN POULTRY

Abstract

The negative impacts of avian influenza (AI) and Newcastle disease (ND) on poultry production in Nigeria are enormous. Commercial poultry production in Kogi State is low even though the State had not reported any outbreak of HPAI (H5N1), AI and ND are attributted to be the major causes. A survey was carried out to determine the seroprevalence of AI and ND in poultry from twelve Local Government Areas (LGAs) in Kogi State. A total of 779 sera were analyzed for AI (H5) antibodies by haemagglutination inhibition (HI) test which gave an overall seroprevalence of 4.2% with mean antibody titre of 8.42 ± 0.254$\log_2$. The seroprevalence of AI (H5) among the LGAs was highest in Kabba/Bunu LGA with 27.3% (mean antibody titre of 8.78 ± 0.572 $\log_2$) while it was zero in Ajaokuta and Okene LGAs. The seroprevalence of AI (H5) in the three sampling units was highest for backyard farms with 42.4% (mean antibody titre of 8.64 ± 0.401 $\log_2$) and lowest for rural poultry with 27.3% (mean antibody titre of 7.56 ± 0.503 $\log_2$). Using ELISA, 452 of the poultry sera were analyzed for AI nucleoprotein (NP) antibodies which gave an overall seroprevalence of 30.8% (mean antibody titre of 16.588 ± 0.882 EU). The seroprevalence of AI (NP) in the surveyed LGAs was highest for Mopamuro with 41.8% (mean antibody titre of 21.009 ± 3.250 EU) and lowest for Adavi with 18.6% (mean antibody titre of 10.610 ± 2.959 EU); among the species it was highest in chicken with 31.6% (mean antibody titre of 16.427 ± 0.888 EU) and zero in duck while among the sampling units, rural poultry was highest with 35.0% (mean antibody titre of 18.987 ± 1.921 EU) and lowest in backyard poultry farm with 26.5% (mean antibody titre of 14.480 ± 1.167 EU). Similarly, 779 poultry sera were analyzed by HI test for ND antibodies which

gave an overall seroprevalence of 75.9% with mean antibody titre of 6.77 ± 0.141 log_2. The seroprevalence of ND by HI test among the surveyed LGAs was highest for Lokoja with 100.0% (mean antibody titre of 9.59 ± 0.159 log_2) and lowest for Ofu with 42.9% (mean antibody titre of 3.41 ± 0.489 log_2). Among species the seroprevalence of ND using HI was highest in chicken with 75.9% (mean antibody titre of 6.80 ± 0.15 log_2) and lowest in ducks with 72.7% (mean antibody titre of 5.36 ± 0.99 log_2), while among the sampling units, it was highest in backyard poultry farms with 76.0% (mean antibody titre of 6.75 ± 0.224 log_2) and lowest in rural poultry with 75.6% (mean antibody titre of 6.71 ± 0.252 log_2). Using ELISA, 367 of the poultry sera were analyzed for ND antibodies with an overall seroprevalence of 68.1% and mean antibody titre of 34.487 ± 1.444 EU. The seroprevalence of ND using ELISA in the surveyed LGAs was highest in Ankpa with 84.6% (mean antibody titre of 48.665 ± 5.175 EU) and lowest in Ogori-Mangogo with 46.7% (mean antibody titre of 26.400 ± 5.075 EU). The ND seroprevalence using ELISA among poultry species was highest in turkey with 76.2% (mean antibody titre of 27.848 ± 3.824 EU), while among the sampling units, it was highest in backyard poultry farms with 75.3% (mean antibody titre of 40.702 ± 2.399 EU) and lowest in rural poultry with 62.4% (mean antibody titre of 29.399 ± 2.625 EU).The presence of AI (H5) antibodies in poultry sera in the surveyed area indicated a previous exposure to the AI viruses which calls for further virological investigation and subtype characterization. The presence of ND antibodies in backyard poultry might be due to vaccination of flocks but that of rural poultry might be due to natural infection of susceptible poultry as flocks from this sampling unit are not routinely vaccinated. Poultry from rural households and live bird markets (LBMs) are sources of ND infection to backyard and commercial poultry, hence, should be prevented from having contact.

3.1 Introduction

Avian influenza (AI) and Newcastle disease (ND) both have low and high pathogenic pathotypes based on the ability to cause dease or death in the host (Alexander, 1987; Jordan, 1990). The highly pathogenic pathotype of either AI or ND can cause morbidity and mortality of up to 100% and had independently resulted in several panzootic and pandemic in the past (Miller and West, 1972; Swayne, 2008). Mixed infection of the low pathogenic pathotypes can occur with severe cliincal manifestations leading to misdiagnosis (Easterday *et al*., 1997; Wakawa *et al*., 2009). Durosinlorun *et al*. (2008) reported that in spite of the high number of people that had heard about HPAI, they could not recognize the disease probably due to confusion with ND or because the radio was the medium through which they had heard of the disease.

Newcastle disease is endemic in Africa and outbreaks have been reported in some countries including Nigeria in domestic and semi – domestic poultry (Ezeokoli *et al*., 1984; Sa'idu *et al*., 2004). Oladele *et al*. (1996) had reported the presence of ND antibodies in pigeons and other semi – domestic species of poultry in Zaria.

Before the maiden outbreak of HPAI H5N1 in Nigeria on 8th February 2006, there had been reports of the detection of AI antibodies of H1N1 and H5N1 subtypes in poultry and pigs (Adeniji *et al*. 1993; Owoade *et al*. 2002). The AI infection once established in a region is always difficult to eradicate, especially for countries that undertake vaccination of poultry as control policy such as Egypt and China (FAO, 2011; Wakawa *et al*., 2012). Vakuru *et al*. (2012) carried out surveillance in wild birds from 8 States across Nigeria and identified species which could potentially act as bridge for spill over infection from wild bird reservoirs to poultry due to the reoccurrence of

HPAI in two Nigerian States in 2008 after a long absence. Presently, as at May, 2015, there are reports of HPAI outbreaks in poultry farms and LBMs in about 18 States of Nigeria, speculated to be from Wild birds (Vetline, 2015).

Continuous surveillance is key to effective control for AI especially in countries that had adopted eradication policy as well as for ND so as to be able to evaluate vaccination options, vaccinal strains against field strains and development of new vaccines. Presently, there is no Government funded surveillance for AI in Kogi State and the institutionalized disease reporting system is weak with no feedback disease control policy needed for improved poultry production (KGSADP, 2009). In an earlier study, the presence of AI, ND and GD antibodies were detected by agar gel precipitin test (AGPT) in chickens from six LGAs of Kogi State (Ameji, 2010). The present study was designed to determine the seroprevalence of AI and ND in poultry from twelve LGAs in Kogi State, Nigeria.

3.2 Statement of the Research Problem

Commercial poultry production is low in Kogi State even though there had not been any report of HPAI outbreak in the State, AI is attributted to be one of the causes because the State is surrounded by about eight States including the Federal Capital Territory (FCT) where HPAI outbreaks were reported. Avian influenza viruses are known to spread along high way networks by travelers and vehicles (Gilbert *et al*., 2008).

The prevalent endemic strains of NDV in Nigeria are known to be highly virulent held in circulation by unvaccinated rural poultry and wild birds (Adu *et al.,* 1985). There have been several reports of ND antibodies in unvaccinated rural poultry in Nigeria (Ezeokoli *et al*., 1984; Abdu *et al.,* 1992; Ameji, 2011). Commercial poultry

production in Kogi State is still low and the negative effects of ND may be a contributing factor.

Presently, as at May, 2015, there are reports of HPAI outbreaks in poultry farms and LBMs in about 18 States of Nigeria, speculated to be from Wild birds (Vetline, 2015).

3.3 Justification of the Study

The presence of AI antibodies will provide evidence as to whether poultry in Kogi State were previously exposed to the AIVs which might be the cause of the commercial poultry production in Kogi State.

The study will help to update the knowledge on the status of AI and ND in poultry in Kogi State which will be useful for the design of prevention and control programmes against the diseases.

The study will help in identifying the threat of scavenging rural and unvaccinated poultry in the maintenance and spread of ND to commercial poultry.

3.4 Aim of the Study

The aim of the study was to determine the seroprevalence of AI and ND in poultry in Kogi State, Nigeria.

3.5 Objectives of the Study

The objectives were to determine:

1. The sero-prevalence of AI in poultry in Kogi State.
2. The sero-prevalence of ND in poultry in Kogi State.

3.6 Research Questions

1. Are there evidence of AI in poultry in Kogi State, Nigeria?

2. Are there evidence of ND in poultry in Kogi State, Nigeria?

3.7 Materials and Methods

3.7.1 Definitions

For the purpose of this research, the folowing terms are defined and applied:

3.7.1.1 Rural poultry

Rural poultry also known as village, local or native poultry refer to the scavenging indigenous domestic fowls that have no regular health programme, roam freely for food and shelter may or may not be provided. The flock size is small, between 1-50 birds reared under traditional management (extensive system) with zero to minimal biosecurity level (FAO sector 4) (Abdu, 2010). Birds of mixed species are kept but the predominant species of rural poultry is the indigenous domestic fowl (*Gallus domestica*) often referred to as the rural chicken (FAO, 1998).

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3.7.1.2 Backyard poultry

Backyard poultry refer to improved commercial or exotic domestic fowls which are kept in household's backyard under improved management systems. The biosecurity level in backyard farms is minimal to low (FAO sector 3) with flock sizes ranging from 50-999 birds and sometimes up to 2,000 birds (Abdu, 2010). The predominant species in this class is the improved domestic fowl referred to as exotic chickens (FAO, 1998). This scale of production represents the intermediate level between free-range non-commercial and medium scale commercial poultry production systems in Nigeria (Abdu, 2010).

3.7.1.3 Live bird market

Live bird market refers to the market where live poultry of all species are sold and taken away to homes or slaughtered in the market and processed for meat (FAO, 1998).

3.7.2 Study area

The study area is Kogi State which lies between Latitude 6°44' - 7°36'N and Longitude 7°49' - 8°27'E situated at a height of about 789 km above sea level and covering a land area of 29,833 Km^2 (Figure 3.1). The State is bordered by FCT and Niger State on the north, Benue and Nasarawa States on the east, Ekiti and Kwara States on the west, Edo, Anambra and Enugu States on the south.

The vegetation of Kogi State is guinea savannah on the north and a belt of rain forest on the southern fringe with rivers Niger and Benue passing through the State, which later converged at a point to form a confluence. The annual rainfall ranges from 1100 mm – 1250 mm starting from April to October (KGSADP, 2009).

Kogi State has a total of twenty-one (21) Local Government Areas (LGAs). The human population of the State is 2,099,046 with the major economic activities of the people being farming, fishing and trading (KGSADP, 2009). The poultry population is estimated to be 3,685,211 with 91.5% being rural or backyard poultry and the rest being commercial poultry (Adene and Oguntade, 2006).

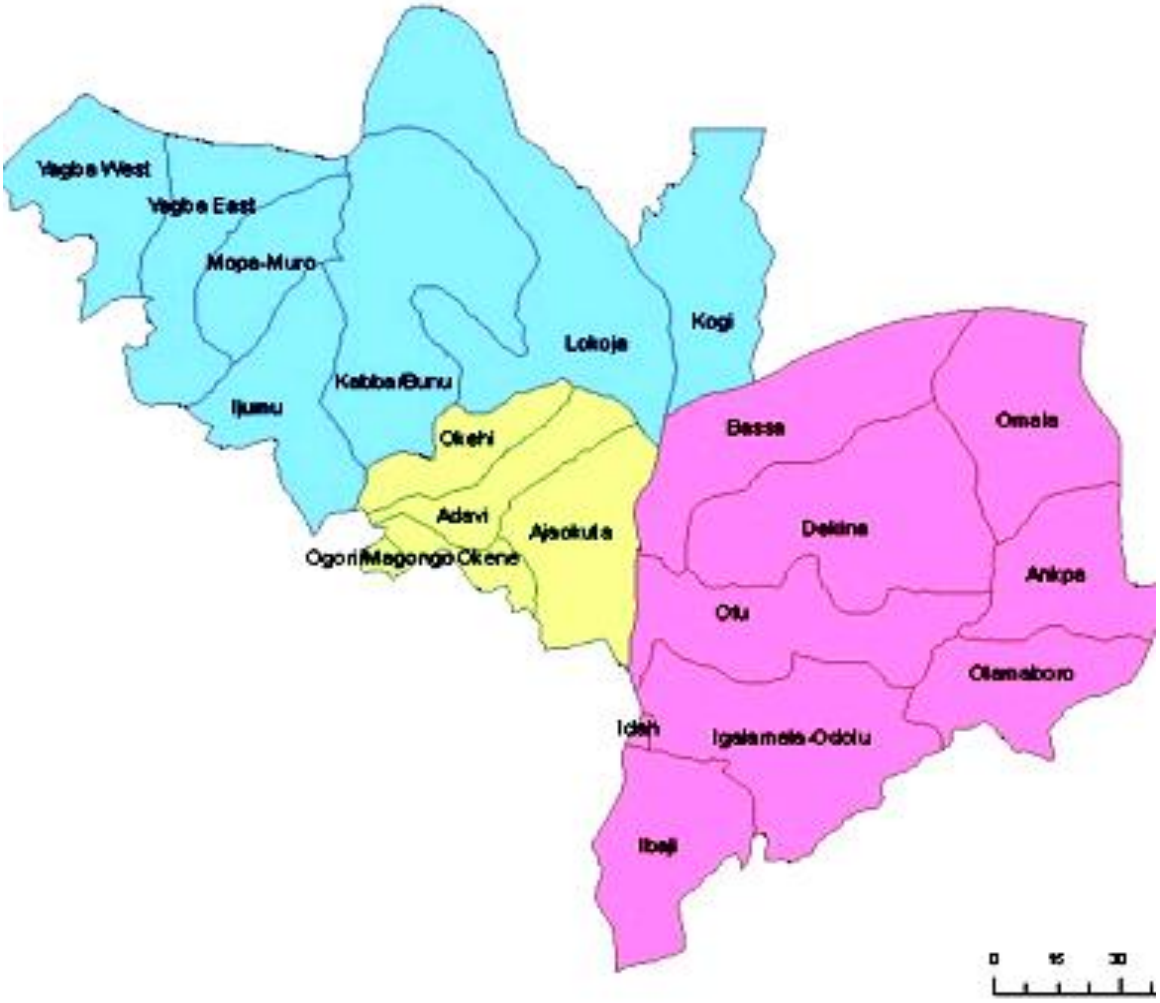


Figure 3.1: Map of Kogi State showing the 21 Local Government Areas grouped into western (blue area), central (yellow area) and eastern (pink area) senatorial districts. (Source: www.ncocusa.com, accessed September 14th, 2014; 3pm)

3.7.3 Sample size

3.7.3.1 Sample size for avian influenza survey in rural poultry

The sample size for the study was determined using the formula by Cohen (1988):

$N = Z^2Pq/L^2$

Where N = Number of samples to be taken from a population

Z = Z-score (for a two-tailed test at 95% confidence interval) = 1.96

P = Prevalence of AI = 18.7% (Ameji, 2010)

q = 1-P = 1 – 0.187 = 0.813

L = a measure of the confidence interval = allowable error (5%) = 0.05

$$N = \frac{1.96^2 \text{ X } 0.187 \text{ X } 0.813}{0.05^2} = 233.6 = 234$$

A) Sample size for AI survey in poultry from the 3 sampling units = 234 x 3 = **702**

3.7.3.2 Sample size for Newcastle disease survey in rural poultry

Sample size for ND survey in poultry (Prevalence of ND= 13.3% Ameji, 2010) = 177.

B) Sample size for ND survey in poultry from the 3 sampling units = 177 x 3 = **531**

3.7.4 Sampling method for poultry from backyard farms, live bird markets and rural households

Multistage, simple random and convenient sampling methods were used. The survey was carried out in the three senatorial districts involving 12 LGAs out of the 21 LGAs of the State. In each senatorial district, 4 LGAs were selected using AI studies risk map of areas with concentrated poultry population, wetlands, road (high way) networks, high population of people and live bird markets from a list of the LGAs in the senatorial district.

In each of the 4 LGAs selected, 5 wards were chosen at random from a list of between 10 – 13 wards in each LGA and a village was selected at random and visited per ward from a list of villages for sampling of at least six rural poultry in a household chosen at random. Also in each of the 4 LGAs, two poultry farms were selected at random from a list of registered poultry farms in the area for sampling of at least fifteen backyard poultry at random per farm. Equally, in each of the 4 LGAs, daily or weekly LBM was selected at random from a list of LBMs in the area where at least six poultry was sampled from 5 poultry sellers chosen at random from the poultry sellers in the LBM.

3.7.5 Collection of blood

The blood sample was collected from a bird after proper restraint to make the area over the wing vein visible and with the use of a 21 gauge needle and a 5 ml syringe, 2 ml of blood was withdrawn from the wing vein. The blood collected was allowed to stand for two hours for clotting to occur and the serum decanted into a bijou bottle, stored under ice then transported to the laboratory within 48 hours and kept at -20 °C until used.

A total of 779 poultry sera were collected from poultry during the survey from June, 2012 to February, 2013.

3.7.6 Detection of avian influenza antibodies

3.7.6.1 Haemagglutination inhibition test

Antigen and antisera: An inactivated AI (H5) antigen (Batch 20060212) produced from China and H5N2 antiserum (Batch 1/10) produced by OIE/FAO reference laboratory for AI and ND, delle Venezie, Italy were used.

Preparation of chicken red blood cells: Two millilitres of blood were collected from 5 unvaccinated day-old chicks and pooled in an equal volume of Alsever's solution. The RBCs were then washed three times in PBS (pH7.2) by centrifugation at 800 ***g*** for 5 minutes (OIE, 2009). One per cent RBC suspension (v/v) was made by adding 40 ul of washed RBC to 40 ml of PBS.

Determination of avian influenza antigen titre: Haemagglutination (HA) test was carried out according to the OIE (2009) protocol to determine the AI (H5) antigen titre that was used for the HI test. Twenty five microlitre of PBS was dispensed into each well of the micro titre plates on two rows of the plate up to the 24^{th} well. Twenty five microlitre of the antigen suspension was placed in the first well. Using a 25 ul micropipette, two-fold dilutions of the antigen was made across the wells of the plate. Twenty five microlitres of 1% RBC was dispensed into each well and mixed by tapping the plate gently then allowing the RBCs to settle for about 40 minutes at room temperature. The HA was determined by tilting the plate to observe the presence or absence of tear-shaped streaming of the RBCs. The antigen titre to the highest dilution that gave complete HA (no streaming of RBCs), was the end point. This end point represented 1 HA unit (1HAU) and it was observed to be 1:8192 (the 14^{th} well), calculated accurately from initial range of dilutions (Appendix I). However, 8HAU (1:1024) was taken as the dilution of antigen that was used.

The haemagglutination inhibition test: The HI test was carried out as described by OIE (2009). A 25 ul of PBS was dispensed into each well of a plastic V-bottom microtitre plates. A 25 ul of test serum was dispensed into the first well of the plate. Two-fold dilutions of the 25 ul serum were made across the plate except the control wells. A 25 ul of 8 HAU of the antigen suspension was added to each well and left for

30 minutes at room temperature. A 25 ul of chicken RBCs was added to each well, gently mixed and allowed to settle for 40 minutes at room temperature. The positive HI titres were the highest dilutions of the test sera that cause complete inhibition of RBCs agglutinations of the 8 HAU antigen. This was made easy as positive wells were compared with positive control which stream at the same rate. The results were expressed in $\log_2$.

3.7.6.2 Detection of avian influenza antibodies by enzyme link immunosorbent assay

The AI ELISA antibody test kit used was manufactured by Affinitech Ltd, Bentonville, AR 72712, USA. The reagents supplied included antigen coated microtitre plates, sample diluent, wash solution, conjugate (IgG alkaline phosphate), substrate (p-Nitrophenyl phosphate), stop solution (3.0 M NaOH), positive and negative controls which were prepared and used according to manufacturer's instructions.

The reagents were warmed to room temperature, mixed by inverting and swirling according to manufacturer's instructions before use. To each microtitre plate, a 100 ul of the negative and positive controls was dispensed to duplicate wells. The test sample was diluted with sample diluent in the ratio of 1:400 and 100 ul of the diluted test sample was dispensed into each microtitre well. The plate was allowed to stand for 30 minutes at room temperature.

Thereafter, the plate was emptied and washed by dispensing 300 ul of the wash solution per well. The washing was done three times with excess water tapped out on paper towel after which 100 ul of the conjugate was dispensed per well and allowed to stand at room temperature for 30 minutes. The plate was again washed with 300 ul of wash solution three times, excess water was drained with paper towel and 100 ul of

substrate was dispensed into each well then, allowed to stand for 30 minutes at room temperature. However, the plate was not emptied after the substrate was added of its contents but 100 ul of stop solution was dispensed per well and read using dual wavelength microtitre plate ELISA reader with 405 nm primary filter and 630 nm reference filter blanked on air.

Following the manufacturer's instructions, the following were ensured for validity of test results:

1. Positive and negative controls were included in each test run per plate.
2. The average O.D of positive control minus average O.D of negative control was more than 0.7 O.D.
3. The average O.D of the negative control was less than 0.4 O.D.

The reactivity of each test sample was calculated by subtracting the average optical density (O.D) of negative control from the O.D of test sample divided by the difference between the average O.D of positive control and average O.D of negative control as follows:

$$\frac{\text{O.D of test sample} - \text{Average O.D of negative control}}{\text{Av. O.D of positive control} - \text{Av. O.D of negative control}} = \text{sample to positive ratio}$$

Sample to positive ratio (Sp) X 100 = ELISA units (EU)

ELISA Units less than 15 EUs = Negative for antibodies to AIVs

ELISA Units greater than 15 EUs = Positive for antibodies to AIVs

3.7.7 Detection of Newcastle disease antibodies

3.7.7.1 Haemagglutination inhibition test

Antigen and antisera: The antigen used was La Sota strain of Newcastle disease virus (NDV) and antiserum obtained from NVRI, Vom, Nigeria.

Preparation of chicken red blood cells: Two millilitres of blood was collected from 5 unvaccinated day-old chicks and pooled in an equal volume of Alsever's solution. The RBCs were washed three times in PBS (pH7.2) by centrifugation at 800 ***g*** for 5 minutes (OIE, 2009). One per cent RBC suspension (v/v) was made by adding 40 ul of washed RBC to 40 ml of PBS.

*Determination of Newcastle disease antigen titre**:*** Haemagglutination (HA) test was carried out according to the OIE (2009) protocol to determine the antigen titre to be used for the HI tests. Twenty five microlitre of PBS was dispensed into each well of the microtitre plates. Twenty five microlitre of the antigen suspension was placed in the first well. Using a 25 ul micropipette, two-fold dilutions of the antigen was made across the plates. A 25 ul of 1% RBC was dispensed into each well and mixed by tapping the plates gently then allowing the RBCs to settle for about 40 minutes at room temperature. The HA was determined by tilting the plate to observe the presence or absence of tear-shaped streaming of the RBCs. The antigen titre was the highest dilution that gave complete HA (no streaming of RBCs). This end point represented 1 HA unit (1HAU) and it was observed to be 1:1024 (the 11^{th} well), calculated accurately from initial range of dilutions. The antigen was diluted to 4 HAU in PBS and used for the HI test.

The Newcastle disease haemagglutination inhibition test: The ND HI test was carried out as described by OIE (2009). A 25 ul of PBS was dispensed into each well of a plastic V-bottom microtitre plates. 25 ul of test serum was dispensed into the first well of the plate. Two-fold dilutions of the 25 ul serum were made across the plate except the control wells. A 25 ul of 4 HAU of the antigen suspension was added to each well and left for 30 minutes at room temperature. A 25 ul of chicken RBCs was added to

each well, gently mixed and allowed to settle for 40 minutes at room temperature. The positive HI titre was the highest dilutions of the test sera that cause complete inhibition of RBCs agglutinations of the 4 HAU antigens. This was made easy as positive wells were compared with positive control which streamed at the same rate. The results were expressed in $\log_2$.

3.7.7.2 Detection of Newcastle disease antibodies by enzyme link immunosorbent assay

The ND ELISA antibody test kit used was manufactured by Affinitech Ltd, Bentonville, AR 72712, USA. The reagents supplied included antigen coated microtitre plates, sample diluent, wash solution, conjugate (IgG alkaline phosphatase), substrate (p-Nitrophenyl phosphate), stop solution (3.0 M NaOH), positive and negative controls which were prepared and used according to manufacturer's instructions.

The reagents were warmed to room temperature, mixed by inverting and swirling according to manufacturer's instructions before use. To each microtitre plate, a 100 ul of the negative and positive controls was dispensed to duplicate wells. The test sample was diluted with sample diluent in the ratio of 1:400 and 100 ul of the diluted test sample was dispensed into each microtitre well. The plate was allowed to stand for 30 minutes at room temperature.

Thereafter, the plate was emptied and washed by dispensing 300 ul of the wash solution per well. The washing was done three times with excess water tapped out on paper towel after which 100 ul of the conjugate was dispensed per well and allowed to stand at room temperature for 30 minutes. The plate was again washed with 300 ul of wash solution three times, excess water was drained with paper towel and 100 ul of substrate was dispensed into each well then, allowed to stand for 30 minutes at room

temperature. However, the plate was not emptied after the substrate was added of its contents but 100 ul of stop solution was dispensed per well and read using dual wavelength microtitre plate ELISA reader with 405 nm primary filter and 630 nm reference filter blanked on air.

Following the manufacturer's instructions, the following were ensured for validity of test results:

1. Positive and negative controls were included in each test run per plate.
2. The average O.D of positive control minus average O.D of negative control was greater than 0.7 O.D.
3. The average O.D of the negative control was less than 0.4 O.D.

The reactivity of each test sample was calculated by subtracting the average optical density (O.D) of negative control from the O.D of test sample divided by the difference between the average O.D of positive control and average O.D of negative control as follows:

$$\frac{\text{O.D of test sample} - \text{Average O.D of negative control}}{\text{Av. O.D of positive control} - \text{Av. O.D of negative control}} = \text{sample/positive ratio}$$

Sample to positive ratio (Sp) X 100 = ELISA Units (EU)

ELISA Units less than 15 EUs = Negative for antibodies to NDV

ELISA Units greater than 15 EUs = Positive for antibodies to NDV

3.8 Results

3.8.1 Avian influenza antibodies detection by haemagglutination inhibition test and enzyme link immunosorbent assay

Thirty three (4.2%) of the poultry sera were positive for AI (H5) antibodies and none of the poultry species other than chicken had AI (H5) antibodies by HI test (Table 3.1). The seroprevalence of AI (H5) in the 12 LGAs surveyed were 27.3% for

Kabba/Bunu; 15.2% for Ijumu, Mopamuro and Ofu; 6.1% for for Dekina, Lokoja, and Ogori – Mangogo; 3.0% for Ankpa, Adavi and Olamaboro while Ajaokuta and Okene had zero (Table 3.2). The seroprevalence of AI (H5) in the different sampling units was 42.4% for backyard poultry, 30.3% for poultry from LBMs and 27.3% for rural poultry (Table 3.3). The overall AI (H5) antibody mean titre by HI test was 8.42 ± 0.254 with 95.8% of the sera having antibody titre $\leq 3\log_2$ and 3.7% having antibody titre $\geq 7\log_2$ (Table 3.4). The calculated χ^2 values for the group seroprevalence on the basis of LGAs was found to be significant but was not significant on the basis of sampling units.

One hundred and thirty nine (30.8%) poultry sera were positive for AI (NP) antibodies by ELISA with seroprevalence for chicken being 31.6%, 26.9% for turkey and 0.0% for ducks (Table 3.5). The seroprevalence of AI in the surveyed 12 LGAs were 41.8% for Mopamuro; 40.0% for Olamaboro; 39.0% for Ijumu; 33.3% for Kabba/Bunu; 32.6% for Ofu; 28.6% for Dekina and Ogori – Mangogo; 26.9% for Lokoja; 26.7% for Okene; 20.0% each for Ajaokuta and Ankpa while Adavi had 18.6% respectively (Table 3.5).

The overall ELISA mean antibody titre (Mean EU±SEM) is 16.588±0.882 EU while the seroprevalence of AI (NP) for sampling units were 26.5% for backyard farms, 32.9% for LBMs and 35.0% for rural poultry (Table 3.6).

3.8.2 Newcastle disease antibodies by haemagglutination inhibition test and enzyme link immunosorbent assay

Five hundred and ninety one (75.9%) of the poultry sera were positive for ND antibodies with species seroprevalence being 76.0% for chicken, 75.8% for turkey, 72.7% for duck and overall HI test mean antibody titre of 6.77 ± 0.141 (Table 3.7).

Table 3.1: Seroprevalence of avian influenza (H5) antibodies using haemagglutination inhibition test in different poultry species surveyed in Kogi State.

Poultry species	No. tested	No. positive	Mean antibody titre ± SEM log_2
Chicken	734	33(4.5)	8.42 ± 0.254
Duck	12	0	-
Turkey	33	0	-
Overall	779	33(4.2)	8.42 ± 0.254

Table 3.2: Seroprevalence of avian influenza (H5) antibodies in poultry using haemagglutination inhibition test in twelve Local Government Areas of Kogi State.

Local Government Area	No. tested	No. positive (%)	Antibody titre ≤ 3 $\log_2$ (%)	Antibody titre ≥ 7 $\log_2$ (%)	Mean antibody ± SEM $\log_2$
Adavi	70	1 (3.0)	69 (98.6)	1 (3.0)	10.00 ± 0.00
Ajaokuta	48	0 (0.0)	48 (100.0)	0 (0.0)	-
Ankpa	42	1 (3.0)	41 (97.6)	1 (3.0)	10.00 ± 0.00
Dekina	62	2 (6.1)	60 (96.8)	2 (6.1)	8.00 ± 1.000
Ijumu	78	5 (15.2)	73 (93.6)	3 (3.8)	7.60 ± 0.927
Kabba/Bunu	73	9 (27.3)	64 (87.7)	8 (11.0)	8.78 ± 0.572
Lokoja	80	2 (6.1)	78 (97.5)	2 (2.5)	8.50 ± 0.500
Mopamuro	63	5 (15.2)	58 (92.1)	5 (7.9)	8.60 ± 0.400
Ofu	60	5 (15.2)	53 (91.6)	5 (8.3)	8.20 ± 0.374
Ogori-Mangogo	60	2 (6.1)	57 (93.8)	2 (6.1)	8.00 ± 2.000
Okene	61	0 (0.0)	61 (100.0)	0 (0.0)	-
Olamaboro	82	1 (3.0)	79 (96.3)	1 (3.0)	8.00 ± 0.000
Overall	779	33 (4.2)	734 (94.2)	30 (3.9)	8.42 ± 0.254

$\chi^2 = 23.81$ $p = 0.014$

Table 3.3: Seroprevalence of avian influenza (H5) antibodies in poultry using haemagglutination inhibition test in the surveyed sampling units in Kogi State, Nigeria.

Sampling unit	No. tested	No. positive (%)	Mean antibody titre ± SEM log_2
Backyard farm	313	14 (42.4)	8.64 ± 0.401
Live bird market	232	10 (30.3)	8.90 ± 0.348
Rural poultry	234	9 (27.3)	7.56 ± 0.503
Overall	779	33 (4.2)	8.42 ± 0.254

Table 3.4: Seroprevalence of avian influenza antibodies in different poultry species surveyed using enzyme link immunosorbent assay in Kogi State.

Species	No. tested	No. positive (%)	Mean antibody titre ± SEM EU
Chicken	418	132 (31.6)	16.427± 0.888
Duck	8	0 (0.0)	-
Turkey	26	7 (26.9)	22.081 ± 5.490
Overall	452	139 (30.8)	16.588 ± 0.882

Table 3.5: Seroprevalence of avian influenza antibodies in poultry using enzyme link immunosorbent assay in the surveyed twelve Local Government Areas of Kogi State.

Local Government Area	No. tested	No. positive (%)	Mean antibody ± SEM EU
Adavi	43	8 (18.6)	17.477 ± 3.281
Ajaokuta	25	5 (20.0)	12.752 ± 2.318
Ankpa	20	4 (20.0)	10.610 ± 2.959
Dekina	35	10 (28.6)	15.471 ± 3.081
Ijumu	41	16 (39.0)	18.476 ± 3.227
Kabba/Bunu	30	10 (33.3)	14.810 ± 2.824
Lokoja	52	14 (26.9)	16.348 ± 2.820
Mopamuro	55	23 (41.8)	21.009 ± 3.250
Ofu	46	15 (32.6)	18.057 ± 2.992
Ogori-Mangogo	35	10 (28.6)	12.503 ± 1.554
Okene	30	8 (26.7)	15.520 ± 2.754
Olamaboro	40	16 (40.0)	18.310 ± 2.970
Overall	452	139 (30.9)	16.588 ± 0.882

Table 3.6: Seroprevalence of avian influenza antibodies in poultry using enzyme link immunosorbent assay in the surveyed sampling units from Kogi State.

Sampling unit	No. tested	No. positive (%)	Mean antibody titre ± SEM EU
Backyard farm	189	50 (26.5)	14.480 ± 1.167
Live bird market	143	47 (32.9)	17.360 ± 1.662
Rural poultry	120	42 (35.0)	18.987 ± 1.921
Overall	452	139 (30.9)	16.588 ± 0.882

Table 3.7: Seroprevalence of Newcastle disease in different poultry species using haemagglutination inhibition test in Kogi State.

Poultry species	No. tested	No. positive	Mean antibody titre ± SEM log_2
Chicken	734	558 (76.0)	1.96 ± 0.008
Duck	11	8 (72.7)	-
Turkey	33	25 (75.8)	-
Overall	779	592 (75.9)	1.96 ± 0.007

The seroprevalence of ND by HI test in the 12 LGAs surveyed were 100.0% for Lokoja; 95.1% for Mopamuro; 94.5% for Kabba/Bunu; 88.3% for Olamaboro; 88.1% for Ajaokuta; 80.8% for Ijumu; 75.0% for Ankpa; 66.1% for Dekina; 60.0% for Okene; 57.1% for Adavi; 50.8% for Ogori – Mangogo and 42.9% for Ofu, respectively (Table 3.8). The seroprevalence of ND in the different sampling units was 76.0% for backyard poultry, 75.9% for poultry from live bird market and 75.6% for rural poultry (Table 3.9). The overall HI test NDV antibody mean titre was 6.77 ± 0.141 with 24.3% of the sera having antibody titre $\leq 3\log_2$ and 55.3% sera having antibody titre $\geq 7\log_2$ (Table 3.8). The calculated χ^2 values for the group specific mean antibody titres on the bases of LGAs and sampling units were found to be significant.

Two hundred and fifty (68.1%) poultry sera were positive for ND antibodies with seroprevalence for chickens being 69.0%, 72.6%for turkeys and zero for ducks (Table 3.10). The seroprevalence of ND by ELISA in the 12 LGAs were 84.6% for Ankpa; 84.2% for Kabba/Bunu; 77.8% for Mopamuro; 76.9% for Adavi; 75.0% for Ajaokuta; 68.3% for Lokoja; 67.9% for Okene; 66.7% for Olamaboro; 65.5 for Ijumu 59.1% for Dekina; 50.0% for Ofu and 46.7% for Ogori – Mangogo respectively (Table 3.11). The ELISA overall mean ND antibody titre is 34.487 ± 1.444 EU while the seroprevalence for sampling units were 75.3% for backyard farms, 63.4% for live bird markets and 62.4% for rural poultry (Table 3.12). The odd of reactors to the two tests were calculated and was significant in Ijumu, Kabba/Bunu, Lokoja and Olamaboro LGAs (Table 3.13).

Table 3.8: Seroprevalence of Newcastle disease in poultry using haemagglutination inhibition test in the twelve Local Government Areas of Kogi State.

Local Government Area	No. tested	No. positive (%)	Antibody titre ≤ 3 $\log_2$ (%)	Antibody titre ≥ 7 $\log_2$ (%)	Mean antibody titre ± SEM $\log_2$
Adavi	70	40 (57.1)	30 (42.9)	22 (31.4)	4.50 ± 0.425
Ajaokuta	42	37 (88.1)	5 (11.9)	33 (78.6)	8.17 ± 0.528
Ankpa	48	36 (75.0)	12 (25.0)	36 (75.0)	7.54 ± 0.617
Dekina	62	41 (66.1)	21 (33.9)	37 (59.7)	6.34 ± 0.583
Ijumu	78	63 (80.8)	15 (19.2)	55 (70.5)	6.87 ± 0.393
Kabba/Bunu	73	69 (94.5)	4 (5.5)	54 (74.0)	8.29 ± 0.304
Lokoja	80	80 (100.0)	0 (0.0)	74 (92.5)	9.59 ± 0.159
Mopamuro	82	78 (95.1)	4 (4.9)	71 (86.6)	8.95 ± 0.254
Ofu	63	27 (42.9)	37 (58.7)	18 (28.6)	3.41 ± 0.489
Ogori-Mangogo	61	31 (50.8)	30 (49.2)	23 (37.7)	3.75 ± 0.453
Okene	60	36 (60.0)	24 (40.0)	23 (38.3)	4.48 ± 0.473
Olamaboro	60	53 (88.3)	7 (11.7)	52 (86.7)	8.47 ± 0.415
Overall	779	591 (75.9)	189 (24.3)	431 (55.3)	6.77 ± 0.141

Table 3.9: Seroprevalence of Newcastle disease antibodies in poultry using haemagglutination inhibition test in the surveyed sampling units in Kogi State.

Sampling unit	No. tested	No. positive (%)	Mean antibody titre ± SEM log_2
Backyard farm	313	238 (76.0)	6.75 ± 0.224
Live bird market	232	176 (75.9)	6.87 ± 0.261
Rural poultry	234	177 (75.6)	6.71 ± 0.252
Overall	779	591 (75.9)	6.77 ± 0.141

Table 3.10: Seroprevalence of Newcastle disease in different poultry species using enzyme link immunosorbent assay in Kogi State.

Species	No. tested	No. positive (%)	Mean antibody titre ± SEM EU
Chicken	339	234 (69.0)	35.533 ± 1.524
Duck	7	0 (0.0)	3.757 ± 1.801
Turkey	21	16 (76.2)	27.848 ± 3.824
Overall	367	250 (68.1)	34.487 ± 1.444

χ^2 =15.72 p=0.002

Table 3.11: Seroprevalence of Newcastle disease antibodies in poultry using enzyme link immunosorbent assay in the surveyed twelve Local Government Areas of Kogi State.

Local Government Area	No. tested	No. positive (%)	Mean antibody titre ± SEM EU
Adavi	39	30 (76.9)	37.697 ± 4.450
Ajaokuta	16	12 (75.0)	37.475 ± 6.727
Ankpa	26	22 (84.6)	48.665 ± 5.175
Dekina	22	13 (59.1)	24.936 ± 4.438
Ijumu	29	19 (65.5)	38.766 ± 5.715
Kabba/Bunu	19	16 (84.2)	40.379 ± 6.883
Lokoja	41	28 (68.3)	34.634 ± 4.485
Mopamuro	45	35 (77.8)	31.189 ± 3.385
Ofu	36	18 (50.0)	23.647 ± 3.790
Ogori-Mangogo	30	14 (46.7)	26.400 ± 5.072
Okene	28	19 (67.9)	45.757 ± 6.819
Olamaboro	36	24 (66.7)	31.494 ± 3.979
Overall	367	250 (68.1)	34.487 ± 1.444

χ^2 =21.950 p=0.025

Table 3.12: Seroprevalence of Newcastle disease antibodies in poultry using enzyme link immunosorbent assay in the surveyed sampling units.

Sampling unit	No. tested	No. positive (%)	Mean antibody titre ± SEM EU
Backyard farm	154	116 (75.3)	40.702 ± 2.399
Live bird market	112	71 (63.4)	30.531 ± 2.282
Rural poultry	101	63 (62.4)	29.399 ± 2.625
Overall	367	250 (68.1)	34.487 ± 1.444

χ^2 =6.368 p=0.041

Table 3.13: The odd of reactors of poultry sera to Newcastle disease antibodies using enzyme link immunosorbent assay and haemagglutination test in the surveyed twelve Local Government Areas of Kogi State.

Local government area	No. positive by ELISA (%)	No. positive by HI Test (%)	Odd Ratio (95% CI)	p – value
Adavi	28 (76.9)	40 (57.1)	0.476 (0.201-1.29)	0.089
Ajaokuta	12 (75.0)	37 (88.1)	2.467 (0.569-10.698)	0.218
Ankpa	22 (84.6)	36 (75.0)	0.545 (0.156-1.903)	0.337
Dekina	13 (59.1)	41 (66.1)	1.352 (0.498-3.672)	0.554
Ijumu	17 (65.5)	63 (80.8)	2.718 (1.057-6.989)	0.034*
Kabba/Bunu	15 (84.2)	69 (94.5)	4.600 (1.032-20.496)	0.032*
Lokoja	28 (68.3)	80 (100.0)	0.700 (0.571-0.857)	0.001*
Ofu	17 (50.0)	27 (42.9)	0.750 (0.325-1.732)	0.500
Ogori-Mangogo	12 (46.7)	31 (50.8)	1.638 (0.679-3.944)	0.271
Okene	18 (67.9)	36 (60.0)	0.750 (0.289-1.944)	0.553
Olamaboro	24 (66.7)	53 (88.3)	3.786 (1.325-10.813)	0.010*

* = p-value ≤0.05 (significant level) CI = 95% Confidence interval

3.9 Discussion

The overall seroprevalence for AI (H5) of 4.2 % detected by HI test in the surveyed 12 LGAs of Kogi State from this study was lower than the seroprevalence of 22.4 % reported earlier in 2010 in chickens from the same study area (Ameji *et al.,* 2011). The difference may be attributed to the diagnostic test used and time variation would have played a role as antibodies are known to decay over a period of time in the absence of active infection. The test employed in the previous survey was agar gel immunodiffusion test (AGID) that targeted the nucleoprotein or matrix protein of AIVs and not a subtype specific test like HI test (OIE, 2009). The seroprevalence of AI (H5) in the present study is also lower than that of Wakawa *et al.* (2012) in HPAI (H5N1) affected and non affected commercial farms in Kano State, Nigeria.

However, using ELISA, a seroprevalence of 30.8 % for AI was detected in poultry sera from the surveyed 12 LGAs of the State. This was mainly due to the high sensitivity of ELISA to detect all avian influenza virus subtypes targeting the nucleoprotein or matrix protein compared to the high specificity of HI test in detecting only a particular haemagglutinin subtype. The seroprevalence of AI (NP) obtained by the ELISA fairly agreed with the previous seroprevalence of 22.4% using AGID test in sera of poultry obtained from the surveyed area (Ameji *et al*., 2011).

None of the poultry species apart from chickens had detectable AI antibodies by HI test in the present study similar to the previous study in 2010 in the same study area (Ameji, 2010). This consistent trend is a pointer to a different incriminating source of infection other than the duck or any other poultry species in spreading AI infection (Alexander, 2007). It was speculated that some breeder farmers were vaccinating their chickens against AI at the wake of HPAI H5N1 outbreak in Nigeria in 2006 and were

encouraging commercial and backyard farmers that obtain chicks from them to vaccinate their birds as well as was reported by Wakawa *et al*. (2012). If the sharp practice was true, it would curtail outbreak but would not stop AI viral replication with the AIVs becoming endemic in poultry population and antibody response.

On the other hand, using ELISA, an AI (NP) seroprevalence of 26.9% was detected in turkey indicating the circulation of AIVs of other subtypes than H5 in turkey population in the sampled area. Turkeys and chickens are known to be highly susceptible to HPAI of either H5 or H7 subtypes as reported by Swayne (2008). The circulation of LPAI for long in these species of poultry is a serious risk of an inherent outbreak due to mutation of AIVs. Similarly, there was no freedom of AI infection in the surveyed LGAs as antibodies were detected by ELISA in all the LGAs at higher levels than the seroprevalence by HI test due to the ability of ELISA to detect antibodies to all subtypes of AIVs. However, the seroprevalence of AI by HI test in Ijumu, Kabba/Bunu and Ofu LGAs were higher than that by ELISA probably due to the type and age of poultry sampled in these LGAs which were more of rural poultry, cockerels, layers and older birds than in other areas.

The sampling units' seroprevalence of AI had no significant difference from one another because the same types of chickens were found in all the sampling units. During the survey, cockerels, layers and broilers were seen in the live bird markets as well as some rural poultry. However, the seroprevalence of AI in the LGAs by HI test were significantly different from one another. Kabba/Bunu had the highest seroprevalence by HI test followed by Ijumu and Mopamuro which were contiguous areas with same live bird markets, culture and road network to the south west from where they source day old chicks and biologicals which is similar to the reports of

Capua and Morangon (2004) that AI viruses spread along highway road network and high poultry density area.

Incidentally, there were no AI (H5) antibodies detected by HI test in Ajaokuta and Okene LGAs relative to the number of poultry sera and types of chicken surveyed in these areas. The poultry surveyed in Ajaokuta and Okene LGAs were mainly rural poultry (native breed) which are kept for long period of up to 4 years which might result in loss of antibody titres beyond detectable level if they were earlier exposed to the AIVs. This may reinforce the speculation that the presence of AI (H5) antibodies in poultry might be due to illegal vaccination of backyard/commercial poultry by some farmers in the State. This finding might not mean freedom from disease or AI infection as the sampled poultry were apparently healthy as Komar and Olsen (2008) reported that it was statistically more likely to finding HPAIVs (H5 and H7) in sick or dead poultry than apparently healthy birds.

The survey showed high overall seroprevalence of ND in contrast to a previous study in 2010 involving half of the study area (Ameji, 2010). A greater number of the poultry sera, 55.3% had antibody titre $\geq 7 \log_2$. This is an indication of recent infection or vaccination as well as the protective status of the poultry population in the study area. This was a great improvement over the previous study and more should be done about the vaccination of the poultry flocks to sustain this growth. However, Boven *et al*. (2008) reported that for adequate herd immunity against NDV, 85% of poultry flock needed to be vaccinated hence, much effort is required to prevent and control ND outbreaks in the LGAs where the seroprevalence of ND antibodies in backyard poultry is below this stated value.

Variable seroprevalence of ND in rural chickens have been reported in the past but the infective strains were not characterized. The seroprevalence of ND for rural poultry detected in this study is similar to that reported by Ezeokoli *et al.* (1984) in Zaria but higher than that of Orjaka *et al.* (1999) in south eastern Nigeria and Musa *et al.* (2009) in Plateau State. However, the seroprevalence of ND in the present study was slightly lower than that reported by Sa'idu *et al.* (2004) in Zaria. The difference may be due to ecological variability of NDV activity, time of sampling and epidemiology as well as difference in sampling methods. The high seroprevalence of ND in the various sampling units surveyed was an indication of natural exposure with seroconversion and the danger posed by free range rural poultry as well as poultry from LBMs to backyard poultry production.

The detection of high seroprevalence of ND in chickens and turkeys compare to ducks agrees with the report that galliformes were more susceptible to ND than the anseriformes and other birds which might not show clinical signs (Echeonwu *et al.*, 1993; Sa'idu *et al.,* 2004). In most rural communities in Nigeria, mixed poultry species are often kept together which makes cross transmission of ND and other pathogens possible. The high seroprevalence of ND coupled with the high HI test mean antibody titre of between 5.0 – 6.80 $\log_2$ across the poultry species indicated recent infection or vaccination and the protective status of flocks in the face of an outbreak if the ND challenge strain is not different from the circulating NDV strain to which antibodies were produced.

Lokoja LGA had the highest seroprevalence of ND probably due to improved vaccination of backyard/commercial poultry flocks in the area. Lokoja is an urban centre and the headquarters of Kogi State with enlightened poultry farmers that can

assess veterinary and extension services. The ND HI test mean antibody titre range from 4.50 – 9.59 log_2 for all the LGAs indicating recent infection or vaccination and protection of the flocks in the face of outbreaks apart from Ofu and Ogori/Mangogo LGAs where mean HI test mean antibody titre range from 3.41 – 3.75 log_2 below the protective titre of 4 log_2. Hence, the poultry flocks in Ofu and Ogori/Mangogo LGAs are at risk of ND infection and outbreak.

Other workers have reported good relativity and measure of association between ELISA and HI test (Brown *et al*., 1999; Ricardo *et al*., 2000). The percentage of samples that were positive to both ELISA and HI test was 67.8%, this gave an idea of the true seroprevalence of ND in poultry sera. The high variability observed between the ND ELISA and ND HI test in the present study might be due to difference ELISA techniques or the coated ND antigen on the ELISA plates could be less immunogenic resulting in low reactions with antibodies in test sera by ELISA thereby giving false negative. The choice of a high cut-off value for positive sample by ELISA as predetermined from a controlled experiment by different manufacturers might affect the sensitivity and specificity of the ELISA.

The odds of positive reactors to ND by ELISA in Ijumu, Kabba/Bunu and Olamaboro are more than that by HI test probably due to age of the birds sampled and stage of infection with NDV. Most of the poultry sampled from these LGAs were rural poultry that would have been kept by rural poultry farmers over a long time.

From this study it was concluded that the poultry had been exposed to AIVs and ND was endemic in Kogi State, Nigeria. It is recommended that continuous surveillance for AIVs should be undertaken; vaccination of backyard poultry against ND should be stepped up and as well, instituted in rural poultry.

CHAPTER FOUR

SERO-PREVALENCE OF AVIAN INFLUENZA AND NEWCASTLE DISEASE IN FREE FLYING WILD BIRDS

Abstract

Wild birds are known to introduce avian influenza (AI) and Newcastle disease (ND) into poultry as well as spread them over long distance. A survey was carried out from June, 2012 – February, 2013 to determine the presence of AI and ND antibodies in wild birds from twelve Local Government Areas (LGAs) in Kogi State. Sera of 50 free flying wild birds from 8 families were analyzed by HI test for AI (H5) antibodies but the results were negative. However, 44 sera were analyzed using ELISA with an overall AI nucleoprotein (NP) seroprevalence of 4.5% and mean titre of 6.61 ± 0.78 EU in wild birds. The seroprevalence of AI (NP) was 28.6% for Lokoja LGA, while the seroprevalence was zero in the other LGAs. The seroprevalence of AI (NP) in wild birds' species was 100.0% in black Stork, while the seroprevalence in the other wild bird species surveyed was zero. The sera of 48 species of free flying wild birds were analyzed for ND antibodies by HI test with an overall seroprevalence of 29.2% and mean antibody titre of 2.69 ± 0.51 $\log_2$ while the seroprevalence was highest in Ankpa, Ofu and Olamaboro LGAs each with 50.0% and zero in Adavi, Mopamuro and Okene LGAs. Swallow had the highest ND seroprevalence of 50.0% by HI test while laughing dove had the lowest seroprevalence of 23.1% with 18.9% of the wild birds having antibody titre $\geq 7\log_2$. Using ELISA, the overall seroprevalence of ND in free flying wild birds was 27.1% with mean antibody titre of 11.06 ± 1.28 EU. The seroprevalence of ND using ELISA in wild birds was highest in Ankpa LGA with 42.9% and zero in Mopamuro and Okene LGAs while among wild bird species, it was highest in black kite with 66.7%. There were no AI (H5) antibodies in wild birds which was an indication that wild birds had not been exposed to AI viruses of this

subtype in the study area. The presence of AI (NP) antibodies in wild birds was an indication of natural exposure of wild birds to the AI viruses other than H5 subtype in the study area. It is recommended that effort should be made to avoid contact of poultry and black Stork so as not to expose poultry to infection of low pathogenic avian influenza viruses. The presence of ND antibodies in wild birds was an indication of the presence of wild type ND in wild birds in Kogi State. It is recommended that vaccination of rural poultry against ND should be encouraged, backyard poultry should be screened from wild birds and scavenging rural poultry.

4.1 Introduction

Avian influenza viruses (AIVs) have been isolated from over 100 species of wild birds, most of which are wild aquatic birds such gulls, terns and shorebirds or waterfowls such as ducks, geese and swans (Swayne, 2008). Most of the AIVs found in wild birds are low pathogenic avian influenza (LPAI) and do not cause disease in the reservoir hosts. Wild birds often serve as means of introduction and spread of both AI and Newcastle disease (ND) viruses to susceptible hosts over long distances (Abdu, 2007; Swayne and Halvorson, 2008). Avian influenza viruses have been isolated from free-flying wild birds as evident in the isolation of HPAI (H5N1) viruses from apparently healthy Tree Sparrows (*Passer montanus*) in China in 2003 and 2004 (Kelly *et al.*, 2008). Also, large-scale outbreak of HPAI occurred in a zoological institution in Cambodia that affected a variety of captive wild bird species including eagles and owls (Desvaux *et al.*, 2009).

Avian influenza and ND are not limited to birds alone but they affect humans and other mammals with fatal consequences (Suarez, 2008). Hence, AI and ND are

reportable due to the high economic losses and zoonotic effects associated with them affecting negatively international poultry trade (Dufour, 2001).

Wild birds are known to be liable for the wide intercontinental spread of AI, ND and other pathogens (Jourdain *et al.*, 2007). In most parts of Africa and Nigeria, ND is endemic often causing outbreak in backyard and commercial poultry (Ezeokoli *et al.*, 1984; Adene, 1996). Although, ND is mostly pathogenic for the domestic chickens, turkeys and guinea fowls, other poultry and wild birds may not show clinical disease but do develop antibodies to the virus (Jordan, 1990; Mohammed *et al*., 1998; Abdu, 2007).

There was a report of the detection of AIVs (H5N2) by means of molecular tests in free-living and apparently healthy White-faced Whistling-duck (*Dendrocygna viduata*) and Spur-winged Goose (*Plectropterus gambensis*) in Nigeria (Gaidet *et al.*, 2008; Chantal *et al.*, 2011). These findings suggested that land-based wild birds might also be a major reservoir of AIVs and other avian pathogens. This fact make them a threat to commercial poultry made possible by their free interactions with wild and domestic populations of waterfowl and poultry making them important bridge in the transmission of AIVs from aquatic birds to poultry and vice versa (Vakuru *et al.*, 2012).

There are increasing evidence incriminating wild birds in the spread of AI on the African continent (Ducatez *et al.*, 2007). Nigeria and other parts of Africa where HPAI H5N1 has been reported are traversed by 3 major migratory bird flyways, namely the East Africa/West Asia, East Atlantic and Black sea/Mediterranean flyways (Williams *et al.*, 2008).

The present study was designed to determine the seroprevalence of AI and ND in captive and free flying wild birds on poultry farms, rural households, live bird markets and wetlands from twelve LGAs of Kogi State, Nigeria.

4.2 Statement of the Research Problem

Kogi State is traversed by two major rivers, Benue and Niger that pass through the State and converged at a point to form a confluence with abundant wetlands in Kogi, Lokoja, Bassa, Ibaji, Ajaokuta and Idah LGAs that may serve as resting points for resident and migratory wild birds (KGSADP, 2009). These may provide good grounds for direct and indirect interactions of large population of rural poultry and wild birds where pathogens are shared in their competition for food and water.

Presently, there are reports of HPAI outbreaks in poultry farms and LBMs in over 18 States of Nigeria, speculated to be from Wild birds (Vetline, 2015).

4.3 Justification of the Study

A surveillance study involving wild birds associated with poultry will shed more light on their roles in AI and ND epidemiology in Kogi State.

Outbreak and spread of ND had been reported in the UK via feed contaminated by faeces of pigeons (Alexander, 2001).

To the best of my knowledge there has not been any report of the presence of antibodies to AI and ND in wild birds in Kogi State.

4.4 Aim of the Study

The aim of the study was to determine the seroprevalence of AI and ND in wild birds in Kogi State, Nigeria.

4.5 Objectives of the Study

The objectives were to determine:

1. The sero-prevalence of AI in wild birds in Kogi State.
2. The sero-prevalence of ND in wild birds in Kogi State.

4.6 Research Questions

1. Are there antibodies against AI in wild birds in Kogi State?
2. Are there antibodies against ND in wild birds in Kogi State?

4.7 Materials and Methods

4.7.1 Study area

The study area is as described in section 3.7.2

4.7.2 Sample size for wild birds

Data on population size of wild bird species and prevalence of AI/ND in wild birds are lacking in Kogi State, a problem adjudged to be a major challenge for designing disease surveys in wild birds in most countries (Wilking *et al.,* 2009). Hence, the sample size was not predetermined but was thought reasonable to limit the sample size to available number of wild birds captured as done by other workers (Vakuru *et al*., 2012; Assam, 2014).

4.7.3 Sampling method for wild birds

Wild birds kept in households and those sold in the LBMs were sampled. Also, free flying wild birds seen around poultry farms and wetlands were caught using hands, rubber glue and mist net, photographed for identification and samples collected. In each of the 12 LGAs, thirty-two wild birds were targeted to be trapped/captured using mist net and locally made glue trap. However, only 50 blood samples were obtained

from wild birds in all due to limited number of wild birds caught as well as little volume of blood in some of the wild birds.

4.7.3.1 Identification of wild birds surveyed

The wild birds that were sampled were alive and apparently healthy. The wild birds were trapped with mist nets and glue traps with cautions taken to avoid injuries to them and they were identified according to the methods of Borrow and Demey (2008).

4.7.4 Collection of blood sample

The collection of blood was as in *section 3.7.5*

4.7.5 Detection of avian influenza antibodies

4.7.5.1 Haemagglutination inhibition test

The detecion of AI antibodies by HI test was as in ***section 3.7.6.1***

Sera of 50 species from 8 families of free flying wild birds were analyzed for antibodies to AI by HI test.

4.7.5.2 Avian influenza antibodies by enzyme link immunosorbent assay

The detection of AI antibodies by ELISA was as in ***section 3.7.7.2***

Using ELISA, 44 sera of the wild bird species were analyzed.

4.7.6 Detection of Newcastle disease antibodies

4.7.6.1 Newcastle disease antibodies by haemagglutination inhibition

The detection of ND antibodies by HI was as in ***section 3.7.6.1***

A total of 48 sera of wild bird species were analyzed for antibodies to ND by HI test.

4.7.6.2 Newcastle disease antibodies by enzyme link immunosorbent assay

The detection of ND antibodies by ELISA was as in ***section 3.7.6.2***

4.8 Results

The families, species and migratory status of wild birds surveyed for the detection of AI and ND antibodies are as shown (Table 4.1).

4.8.1 Avian influenza antibodies detection by haemagglutination test and enzyme link immunosorbent assay

None of the sera from the 50 species of wild birds in any of the 9 LGAs where wild birds were caught and sampled was positive for AI (H5) antibodies (Tables 4.2 and 4.3).

Two (4.5%) of these sera were positive for AI antibodies, the calculated χ^2 values were found to be significant across species and LGAs (Table 4.4 and 4.5). The group seroprevalence of AI was 100.0% for Lokoja LGA while the seroprevalence was 0.0% for the other LGAs (Table 4.4). Equally, species seroprevalence of AI was 100.0% for black stork while the other species had 0.0% seroprevalence (Table 4.5).

4.8.2 Newcastle disease antibodies detection by haemagglutination test and enzyme link immunosorbent assay

Fourteen (29.2%) of the sera were positive for antibodies to ND with overall mean antibody titre of 2.69±0.51 and the seroprevalence in the LGAs were 50.0% for Ankpa, Ofu and Olamaboro; 33.3% for Ajaokuta; 22.2% for Lokoja; 20.0% for Dekina while Adavi, Mopamuro and Okene LGAs had 0.0% respectively (Table 4.5). Based on species, the seroprevalence of ND by HI test were 50.0% for swallow, 33.3% for swifts and 23.1% for dove while the other species had 0.0% respectively (Table 4.5). Thirty-four (70.9%) of the wild bird species had antibody titre ≤ 3 $\log_2$ while 9 (18.9%) had antibody titre ≥ 7 $\log_2$ (Tables 4.4). Thirteen (27.1%) of the sera were positive for antibodies to ND with overall mean antibody titre of 11.06±1.28 (Table 4.6).

Table 4.1: The families, species and migratory status of the wild birds that were surveyed for avian influenza and Newcastle disease viruses in Kogi State.

S/No	Common Name	Family	Species	Migratory status
1	White rumped swift[2]	Apodidae	*Apus caffer*	Intercontinental
2	Little swift[2]	Apodidae	*Apus affinis*	Continental
3	Ethopian swallow[2]	Hirundinidae	*Hirundo aethiopica*	Intercontinental
4	Barn swallow[2]	Hirundinidae	*Hirundo rustica*	Intercontinental
5	Laughing dove[2]	Columbidae	*Streptopelia senegalensis*	Resident
6	Rose ringed parakeet	Psittacidae	*Psittacula krameri*	Resident
7	Melodious warbler	Sylviidae	*Hippolais polyglotta*	Resident
8	Sooty fly catcher	Muscicipidae	*Muscicapa infuscata*	Resident
9	Black stork[1]	Ciconiidae	*Ciconia nigra*	Intercontinental
10	Black kite[2]	Accipitridae	*Milvus migrans*	Continental

[1] Species of wild birds with AI antibodies

[2] Species of wild birds with ND antibodies

Table 4.2: Seroprevalence of avian influenza antibodies in wild bird using enzyme link immunosorbent assay in the surveyed Local Government Areas of Kogi State.

Local Government Area	No. tested	No. positive (%)	Mean antibody titre ± SEM EU
Adavi	5	0 (0.0)	-
Ajaokuta	3	0 (0.0)	-
Ankpa	10	0 (0.0)	-
Dekina	5	0 (0.0)	-
Lokoja	7	2 (28.6)	9.83 ± 3.88
Mopamuro	2	0 (0.0)	-
Ofu	2	0 (0.0)	-
Okene	5	0 (0.0)	-
Olamaboro	5	0 (0.0)	-
Total	44	2 (4.5)	6.61 ± 0.78

Table 4.3: Seroprevalence of avian influenza antibodies using enzyme link immunosorbent assay in different wild bird species surveyed in Kogi State.

Species	No. tested	No. positive (%)	Mean antibody titre ± SEM EU
Black kite	3	0 (0.0)	-
Dove	13	0 (0.0)	-
Fly catcher	2	0 (0.0)	-
Parakeet	3	0 (0.0)	-
Black stork	2	2 (100.0)	24.50 ± 2.40
Swallow	15	0 (0.0)	-
Swift	4	0 (0.0)	-
Warbler	2	0 (0.0)	-
Total	44	2 (4.5)	6.61 ± 0.78

Table 4.4: Seroprevalence of Newcastle disease antibodies using haemagglutination test in wild birds from the surveyed Local Government Areas of Kogi State.

Local Government Area	No. tested	No. positive (%)	Antibody titre $\leq 3\log_2$	Antibody titre $\geq 7\log_2$	Mean antibody titre ± SEM $\log_2$
Adavi	4	0 (0.0)	4 (100.0)	0 (0.0)	-
Ajaokuta	3	1 (33.3)	2 (66.7)	0 (0.0)	2.00 ± 2.00
Ankpa	6	3 (50.0)	3 (50.0)	2 (33.3)	3.17 ± 1.52
Dekina	5	1 (20.0)	4 (80.0)	0 (0.0)	1.60 ± 1.03
Lokoja	9	2 (22.2)	7 (77.8)	3 (33.3)	2.78 ± 1.34
Mopamuro	2	0 (0.0)	2 (100.0)	0 (0.0)	-
Ofu	6	3 (50.0)	3 (50.0)	2 (33.3)	4.33 ± 1.87
Okene	5	0 (0.0)	5 (100.0)	0 (0.0)	-
Olamaboro	8	4 (50.0)	4 (50.0)	3 (37.5)	4.63 ± 1.41
Overall	48	14 (29.2)	34 (70.9)	9 (18.9)	2.69 ± 0.51

Table 4.5**:** Seroprevalence of Newcastle disease antibodies using haemagglutination test in different wild bird species from Kogi State.

Poultry species	No. tested	No. positive (%)	Antibody titre ≤ 3 $\log_2$ (%)	Antibody titre ≥ 7 $\log_2$ (%)	Mean antibody titre ± SEM
Black kite	3	0 (0.0)	3 (100.0)	0 (0.0)	-
Dove	13	3 (23.1)	10 (76.9)	1 (7.7)	2.31 ± 0.79
Fly catcher	1	0 (0.0)	1 (100.0)	0 (0.0)	-
Parakeet	2	0 (0.0)	2 (100.0)	0 (0.0)	-
Stork	2	0 (0.0)	2 (100.0)	0 (0.0)	-
Swallow	18	9 (50.0)	9 (50.0)	8 (44.4)	4.44 ± 1.02
Swift	6	2 (33.3)	4 (66.7)	0 (0.0)	2.67 ± 0.96
Warbler	3	0 (0.0)	3 (100.0)	0 (0.0)	-
Overall	48	14 (29.2)	34 (70.9)	9 (18.9)	2.69 ± 0.51

Table 4.6: Seroprevalence of Newcastle disease antibodies in wild birds using enzyme link immunosorbent assay from the surveyed Local Government Areas of Kogi State.

Local Government Area	No. tested	No. positive (%)	Mean antibody titre ± SEM
Adavi	3	1 (33.3)	11.77 ± 6.96
Ajaokuta	3	1 (33.3)	15.53 ± 3.39
Ankpa	7	3 (42.9)	15.79 ± 3.35
Dekina	6	2 (33.3)	14.38 ± 2.62
Lokoja	9	3 (33.3)	9.08 ± 3.34
Mopamuro	2	0 (0.0)	-
Ofu	6	1 (16.7)	6.78 ± 3.82
Okene	5	0 (0.0)	-
Olamaboro	7	2 (28.6)	12.24 ± 3.94
Overall	48	13 (27.1)	11.06 ± 1.28

On the basis of LGAs, the seroprevalence of ND by ELISA were 42.9% for Ankpa; 33.3% for Adavi, Ajaokuta, Dekina and Lokoja; 28.6% for Olamaboro and 16.7% for Ofu while Mopamuro and Okene LGAs had 0.0% respectively (Table 4.6). Based on species the seroprevalence of ND by ELISA were 66.7% for black kite, 38.9% for swallow, 15.4% for laughing dove and 33.3% for swifts while the rest species had zero (Table 4.7).

The ND HI test and ELISA were compared to see the number of samples that were positive and negative to both tests. Four (8.3%) sera were positive and 25 (52.1%) sera were negative with both tests (Table 4.8). The odd of reactors to the two tests were calculated but were not significant across the LGAs (Table 4.9).

Table 4.7**:** Seroprevalence of Newcastle disease antibodies in sera of wild bird species using enzyme link immunosorbent assay from Kogi State.

Wild bird species	No. tested	No. positive (%)	Mean antibody titre ± SEM
Black kite	3	2 (66.7)	16.57 ± 2.68
Dove	13	2 (15.4)	9.88 ± 2.06
Fly catcher	2	0 (0.0)	-
Parakeet	1	0 (0.0)	-
Stork	2	0 (0.0)	-
Swallow	18	7 (38.9)	13.39 ± 2.46
Swift	6	2 (33.3)	11.90 ± 4.02
Warbler	3	0 (0.0)	-
Overall	48	13 (27.1)	11.06 ± 1.28

Table 4.8: Distribution of positive and negative sera to Newcastle disease antibodies by enzyme link immunosorbent assay and haemagglutination inhibition test in the wild birds surveyed in Kogi State, Nigeria.

	ND HI test		
ND ELISA	Positive	Negative	Total (%)
Positive	4	9	13 (27.1)
Negative	10	25	35 (72.9)
Overall (%)	14 (29.1)	34 ((70.8)	48 (100.0)

Table 4.9: The odd of reactors of wild bird sera to Newcastle disease antibodies by enzyme link immunosorbent assay and haemagglutination tests in the surveyed Local Government Areas of Kogi State.

Local Government Area	ELISA positive	HI test positive	OR (95%CI)	p
Ajaokuta	1 (7.7)	1 (7.2)	1.00 (0.034-29.807)	1.000
Ankpa	3 (23.1)	3 (21.4)	1.33 (0.149-11.929)	0.797
Dekina	2 (15.4)	1 (7.2)	0.50 (0.031-7.794)	0.621
Lokoja	3 (23.1)	2 (14.3)	0.57 (0.070-4.644)	0.599
Ofu	1 (7.7)	3 (21.4)	5.00 (0.344-72.767)	0.221
Olamaboro	2 (15.4)	4 (28.6)	2.500 (0.164-9.538	0.398

OR = Odd ratio CI = Confidence interval

4.9 Discussion

The absence of detectable AI (H5) antibodies by HI test in the sera of all the wild birds surveyed indicated that wild birds in Kogi State had not been exposed to AIVs (H5) subtype and the absence of the infection in the wild birds investigated. It could as well be that infected birds would have died if they were infected with HPAI thereby missing the true status of infection because the survey involved only apparently healthy birds. Antibodies are known to decay over time. The absence of antibodies in sera of birds tested by HI test might indicate absence of AI (H5) subtype infection in wild birds and circulation in poultry population.

However using ELISA, AI (NP) antibodies were detected in black stork species of wild bird. This was an indication of the presence of AIVs infection of other subtypes than H5 subtype in this species. Black storks and other members of the ciconiiformes are medium to large wading birds found around wetlands and shallow waters in which AIVs have been isolated (FAO, 2007). Black stork and members of the ciconiiformes have ability to live in aquatic and terrestrial habitats. This ability of dual habitation makes black stork a potential threat as source of infection and to play the role of spreading infection from aquatic wild birds to terrestrial poultry and vice versa (FAO, 2007).

Lokoja LGA was the only area where AI (NP) antibodies were detected in wild birds. In survey for a new or an emerging disease in an area initially thought to be free, it is an “all or none” situation where a single detection indicates the presence of the disease or infection (Joannis *et al.,* 2008). Lokoja is traversed by two major rivers, Benue and Niger that converged at a point within the city to form a confluence. These two rivers run downward from some States such as Kebbi, Niger, Kwara, Benue,

Taraba and Nasarwa where HPAI (H5N1) outbreaks were earlier reported, the water provides good habitat for the black storks that were positive for AI (NP) antibodies as well as other water birds. The AI (NP) antibodies found in black stork species of wild birds poses danger to free range poultry as well as in contact humans in the area. The AIVs have been known to survive in water for a long period especially under freezing and cold condition. Hence, excreted AIVs from black stork and other wild birds often survive in water and may be carried away by water current over long distance to remain infective to susceptible poultry (Stallknecht *et al*., 1990; FAO, 2007).

The detection of antibodies to ND in free flying wild birds in this study confirms reports of the prevalence in nature of the highly virulent NDV strains among free range poultry, apparently healthy ducks and other wild birds (Ibu *et al.,* 2009; Saidu *et al.,* 2004). The overall ND seroprevalence of 29.2% determined in this study is higher than that reported by Schelling *et al.* (1999) who determined a seroprevalence of ND in wild birds using tissue fluids from dead birds. Detectable antibody levels are higher during active infection than in convalescent and morbid birds.

Most of the LGAs where wild birds were sampled showed the presence of ND antibodies in wild birds except in Mopamuro and Okene LGAs where antibodies were not detected probably due to the organization of the poultry industry and ecological structures of these areas. In the two LGAs where ND antibodies were not detected, the backyard poultry is more organized to avoid contact with free range poultry and wild birds. However, other LGAs had rural poultry on free range that interact freely with wild birds during scavenging for food around dump sites, farms and open fields. The live bird markets in these LGAs also may create avenues of interaction and contamination of the environment because there were no market stalls or bird cages in

most of these areas but the birds were being sold in open spaces under trees that provided shades for the birds and their sellers.

The association of ND infected but apparently healthy free flying wild birds with free range and commercial poultry may be responsible for vaccine failure often seen in the field. Maminiaina *et al.* (2010) reported the existence of divergence of up to 15 – 25% between the NDV strains (genotype VII) that are endemic in Africa, Asia and Europe; wild type NDV strains in comparison to the La Sota vaccine strain (genotype II) resulting in variable immunological responses.

On the basis of species, swift, swallow and laughing dove had detectable levels of ND antibodies. The source of the infection is not known but ND antibodies have been detected elsewhere in these species (Schelling *et al.*, 1999; Ibu *et al.*, 2009), which are found in association with rural and commercial poultry. The occurrence of ND antibodies in these species was an indication of their susceptibility to ND above other species as a result of their close interactions with vaccinated poultry and or other wild birds that might be carriers of NDV. Laughing dove, swallow and swift are insects and seed eaters which are likely to pick NDV from poultry manure which are often disposed indiscriminatingly in refuse dump. Airborne spread of NDV within short distance is also established hence, these species of wild birds could acquire NDV from infected or vaccinated flocks as a result of their close association via air dispersal during the cold, dry months when the wind speed is high.

Schelling *et al.* (1999) reported an ND seroprevalence of 10.2% in Switzerland, while Dimitrov *et al.* (2008) reported a seroprevalence of 14.8% among wild birds in Bulgarian zoos. In Nigeria, Sa'idu *et al*. (2004) reported high ND seroprevalence of between 4.0% - 52.0% in semi domestic and wild birds which included anseriformes,

columbiformes and struthioformes, while Ibu *et al.* (2009) reported antigen prevalence of 7.0% among wild and captive birds that included passeriformes, falconiformes and strigiformes. The detection of ND antibodies in some of the species of wild birds surveyed was an indication of the susceptibility of these species to ND. There is need to determine the pathotype of the strains of the NDV in wild birds.

The ELISA however, detected ND antibodies in black kite, a species in which HI test failed to detect antibodies. Black kite is a diurnal raptor that eats other birds which may contract the NDV when it feeds on NDV infected birds. There have been reports of the isolation of NDV from raptors, such as eagles, owls, hawks, buzzards and vultures elsewhere (Schelling *et al.,* 1999; Ibu *et al.,* 2009). Black kite (*Milvus migrans)* is a continental migrant that may play vital role in the introduction and spread of ND.

The absence of ND antibodies in fly catcher, parakeet, black stork and warbler either by HI test or ELISA showed the non susceptibility of these species of wild birds to NDV and may be of lesser threat to backyard and commercial poultry. However, psittacine and most passerine birds are susceptible to ND (Alexander, 2001) and the absence of the antibodies in these species may be relative to the few number of birds sampled which might not have represented the true status of the population.

From this study, there were no AI (H5) antibodies but there were AI (NP) antibodies of other subtypes than H5 subtype in wild birds indicating exposure of wild birds to low pathogenic AIVs. It is recommended that poultry should be screened from having contact with black stork, the species that had AI (NP) antibodies. The presence of ND antibodies in wild birds was an indication of wild type ND in Kogi State. It is

recommended that vaccination of rural poultry should be encouraged, backyard poultry should be screened from wild birds and scavenging rural poultry

CHAPTER FIVE

SURVEY FOR AVIAN INFLUENZA VIRUSES IN POULTRY AND FREE FLYING WILD BIRDS

Abstract

The global spread of HPAI (H5N1) from Asia was blamed on movement of migratory wild birds as well as trade in live poultry and poultry products across continents from infected regions. A survey was carried out between June, 2012 to February, 2013 to detect the presence of avian influenza (AI) viruses in poultry from rural households, backyard farms and live bird markets as well as free flying birds from twelve Local Government Areas (LGAs) of Kogi State, Nigeria. A total of 810 cloacal and oropharyngeal swabs were tested using conventional RT-PCR with set of primers targeting the avian influenza matrix proteins. Based on sampling units, the highest samples, 32.1% were collected from backyard poultry farms while the lowest samples, 12.3% were from wild birds. Also, based on LGAs, the highest samples, 11.1% were from Ijumu, Lokoja and Okene LGAs, while the lowest, 4.9% were from Adavi and Ajaokuta LGAs, respectively. However, on the basis of species, the highest samples, 76.5% were from chickens while the lowest, 1.2% were from swifts. All the tested samples were negative for AI viruses. The failure to detect the presence of AI viruses in the samples was an indication that the viruses were not circulating in wild birds and poultry in the study area. The limitations of the sampling method for wild birds which might not represent a random sample and possibly its effects on the study so as to make concrete conclusions were acknowledged. It is recommended that a broad based sustained surveillance for the AI viruses should be carried out to establish the free status of poultry and wild birds from the activities of AIVs in Kogi State. The study will serve as reference for future surveillance of AI viruses in the study area as there was no previous study done in the area for these species surveyed.

5.1 Introduction

Poultry and wild birds are important in the transmission of AI and other diseases within the avifauna as well as to humans. Hence, it is necessary to know the status of these diseases and their levels of threats to commercial poultry production as well as the precautions to take when coming in contact with or handling birds (Jacquie *et al.*, 2011).

Continous surveillance is necessary for the prevention and control of AI by providing early warning of disease incidence as well as identifying possible hosts and modes of disease transmission. Hence, surveillance helps to inform timely intervention and a tool for evaluating the success of prevention and control measures (Swayne 2008). Surveillance also enables early detection of AIVs in formerly unknown reservoir hosts and environment that may serve as sources of introduction of the disease into new regions and may provide access to updated data for subtype's classification of the viruses.

Ameji *et al*. (2011) reported the detection of antibodies to AI by agar gel precipitin test (AGPT) in chickens from six LGAs of Kogi State. The presence of AI antibodies in chickens in Kogi State which did not report HPAI (H5N1) outbreak, indicated exposure to AIVs requiring further investigations.

5.2 Statement of the Research Problem

The global spread of HPAI (H5N1) from Asia was blamed on the movement of migratory wild birds as well as trade in live poultry and poultry products across continents from infected regions. The maiden introduction of HPAI (H5N1) into Nigeria in 2006 and later to other parts of Africa was explained on these two routes (Brown, 2006; Ducatez et al., 2007).

In Africa and Asia, where the structure of the poultry industry is mostly non formal, most poultry die off are not reported or investigated (Sonaiya, 1990; Komar and Olsen, 2008). This can lead to the build up of pathogen loads in the environment and in reservoir hosts with negative impacts on commercial poultry production.

5.3 Justification of the Study

The fear of the study area being a corridor for hiding of AIVs due to the presence of factors that might favor the spread of the AIVs in the study area informed the survey. Such factors include presence of wide range of wild birds around wetlands and major river basins in the study area, non structured backyard poultry farming, abundance of free range rural poultry and live bird market trades with States where HPAI H5N1 outbreaks were reported.

Due to the problem of food safety, AI negatively affects poultry trade. Knowledge of its status in commercial, rural poultry and wild birds will help in establishing disease freedom (Dufour, 2001).

The spread of AIVs and other pathogens are known to occur through several routes such as trade in live poultry and wild birds as well as migratory wild birds (Brown, 2006; Kilpatrick, *et al.*, 2006). Kogi State has a number of LBMs and it is involved in poultry trade with other States across Nigeria. In Kogi State, most people sourced birds for rearing from the LBMs, a practice which needs to be discouraged based on facts.

There has not been any virological study of AIVs in poultry and wild birds in Kogi State. Ameji (2011) reported of antibodies to AI in chickens from six LGAs in Kogi State using agar gel precipitin test (AGPT) requiring further investigations.

5.4 Aim of the Study

The aim of the study was to determine the prevalence of AIVs in poultry and wild birds in Kogi State, Nigeria.

5.5 Objectives of the Study

The objectives were to undertake:

1. The detection of AI viruses in poultry in Kogi State by RT – PCR.
2. The detection of AI viruses in Wild birds in Kogi State by RT – PCR.

5.6 Research Questions

1. Are there AI viruses in poultry in Kogi State?
2. Are there AI viruses in wild birds in Kogi State?

5.7 Materials and Methods

5.7.1 Definitions

Definitions backyard poultry, rural poultry and LBMs as in ***section 3.7.1***

5.7.2 Study area

The study area was as described in ***section 3.7.2***

5.7.3 Sample size

5.7.3.1 Sample size for avian influenza survey in poultry

The sample size for the AI study was as determined in ***section 3.7.3.1***

5.7.3.2 Sample size for avian influenza survey in wild birds

Sample size for wild birds was as determined in ***section 4.7.2.2***

5.7.4 Sampling method for poultry from rural, backyard, live bird market and wild birds

The sampling method used was as described in ***section 3.7.4***

5.7.5 Cloacal and oropharyngeal swabs sampling

A total of 810 swabs made up of 710 oropharyngeal swabs from poultry and 100 cloacal swabs from wild birds were collected and analyzed for AI viruses. These were the same birds whose sera collected and analyzed for AI antibodies in previous sections.

The oropharyngeal swab was collected after proper restraint of a bird by an assistant, the beak was held open with the left hand of the researcher and the right hand used to place sterile cotton swab at the base of the tongue, gently rotated and taken out. The swab was then placed in a cryo-vial of viral transport medium containing 10,000 units/ml penicillin, 10 mg/ml streptomycin, 25 μg/ml gentamycin, 5000 units /ml mycostatin and 1% bovine serum albumin in phosphate buffered saline (PBS) (OIE, 2009).

Similarly, the cloacal swab was collected after proper restraint by an assistant, the researcher used the left hand to lift the tail feather up as well as to stabilize the cloaca and with the right hand, placed the sterile cotton swab into the cloaca, rotated, taken out and placed in a cryo-vial of viral transport medium of the same constituents as Stated above. Care was taken to avoid massive faeces (OIE, 2009). The swab samples were maintained at 4 °C in ice pack upon collection and stored in liquid nitrogen at -70 °C within 4-6 hours until they were processed.

5.7.6 Detection of avian influenza viruses in poultry and wild birds

A total of 810 swabs consisting of 710 (87.7%) oropharyngeal and 100 (12.3%) cloacal swab samples were collected from poultry and wild birds during the field survey from June, 2012 to February, 2013.

5.7.6.1 Preparation of samples

The swab sample tubes were decontaminated by spraying with virkon^{R} and dried with tissue paper. The samples were then pooled based on species and sampling unit into sterile tubes. Ten samples were pooled into one in sterile tubes and labeled (Killian, 2008; Wakawa, 2012).

5.7.6.2 Extraction of Nucleic Acid

The ribonucleic acid (RNA) was extracted from each of the pooled test samples using QIAamp Viral RNA mini kit (QIAGEN GmbH, Germany) by spin protocol according to the manufacturer's instructions. A 560 ul of buffer AVL and carrier RNA, 140 ul of test sample was added and mixed by pulse vortexing for 15 seconds. The mixture was incubated at room temperature (15 – 25°C) for 10 minutes to lyse the virus and release the nucleic acid. The mixture was short spunned to remove some liquid drops from the lid of the eppendorf tube. To the sample mixture, 560 ul of absolute ethanol was added and mixed by pulse vortexing for 15 seconds. The mixture was short spunned to remove some liquid drops from the lid of the eppendorf tube. A 630 ul of the solution from step 3 was carefully added to QIAamp Mini spin column in a 2 ml collection tube without wetting the rim. The cap of the column was closed and centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp spin column was placed in a new 2 ml collection tube and the old tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened and 500 ul of buffer AW1 was added, the cap closed and centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp Mini spin column was placed in a new 2 ml collection tube, the cap carefully opened and 500 ul of buffer AW2 added and centrifuged at 20,000 x g (14,000 rpm) for 3 minutes. The QIAamp Mini spin was then placed in a new 2 ml collection and centrifuged (dry spin) at full speed for 1 minute. Elution of the viral

RNA was done by placing the QIAamp Mini spin in a clean 1.5 ml eppendorf tube, the cap was carefully opened, 60 ul of buffer AVE was added and centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp Mini spin column was discarded and the eluted RNA was stored at -20°C until used.

5.7.6.3 Preparation of Polymerase Chain Reaction Master Mix

The master mix for the 25 µl final volume PCR was prepared from stock of reagents as shown (Appendix II). The AIVs forward and reverse primers targeting the M-gene of 250 bp size with the following sequences: M52C = 5′-CTT CTA ACC GAG GTC GAA AGG-3′ and M253R = 5′-AGG GCA TTT TGG ACA AAG/T CGT CTA - 3′ (Fourchier *et al.,* 2000) were used.

5.7.6.4 Reverse Transcription- Polymerase Chain Reaction

The conventional one step RT – PCR was used to amplify sample in final reaction mixture of 25 µl containing nuclease free H_2O, 10x PCR Buffer, 50 mM $MgCl_2$, 10 mM deoxynucleoside triphosphate (dNTP), 20 mM dithiothreitol (DTT), 10 µM each of oligonucleotide (M52C and M253R – forward and reverse primers), 20 U of reverse transcriptase, 5 U of ribonuclease inhibitor, 5 U of Ampli – Taq DNA polymerase (all reagents by Applied Biosystems,USA) and 5 µl of RNA extract. Thermocycling was done in GeneAmp^R PCR System 9700, Applied Biosystems (USA) with the following cycling conditions: activation at 40°C for 20 min and initial denaturation at 95°C for 5 min (stages I & II once), denaturation at 94°C for 1 min, annealing at 55°C for 1 min, elongation at 72°C for 1 min (stage III 40 times), final elongation at 72°C for 10 min and hold at 4°C until used (stage IV once).

5.7.6.5 Preparation of tris-borate ethylene diamine tetra-acetic acid buffer

The TBE 1x buffer that was used in the preparation of 1.5 % agarose gel for electrophoresis was prepared from TBE 10x stock solution. A 1000 ml solution of TBE 1x was prepared by adding 100 ml of TBE 10x to 900 ml of distilled water.

5.7.6.6 Preparation of 1.5 % 100 ml gel

A chemical balance was used to weigh 1.5 g of agarose and added to 100 ml of TBE 1x then mixed by swirling. The solution was heated at maximum heat for 10 min in a microwave for the agarose to dissolve and allowed to cool to about 45°C. Before casting the gel, 5 µl of ethidium bromide was added as dye and mixed by shaking the Erlinmeyer flask.

5.7.6.7 Agarose gel electrophoresis of the amplicons

The amplicons were mixed with a loading dye (blue/green, Promega^{R}) on a parafilm paper in the ratio of 2:8. Thereafter, the gel was placed in the electrophoresis tank, submerged by the TBE buffer and loaded in the wells of the gel. One Kb plus DNA ladder (Fermentas^{R}) was used as band marker with each lane of the wells having positive and negative controls. The electrophoresis voltage regulator was set at 120 V for 35 min after which the bands were read in a Gel Doc XR+ with Image LabSoftware (BIO – RAD Molecular Imager^{R}, USA) viewed on a computer monitor.

5.8 Results

The total of 810 swabs analyzed by RT – PCR for AI viruses, based on sampling units, the highest number of samples, 32.1% was collected from backyard poultry farms, while the lowest, 12.3%, was from wild birds (Figure 5.1). On the basis of species, the highest samples, 76.5%, were from chickens while the lowest, 1.2%, were from swifts (Figure 5.2).

All the birds from the three poultry sampling units and wild birds in the survey areas were negative for avian influenza viruses (Plates I and II). There was no presence of cDNA bands corresponding to a base pair of 250 bp specific for the gene generated by the pairs of primers used in the amplification of the RNA extract of test samples.

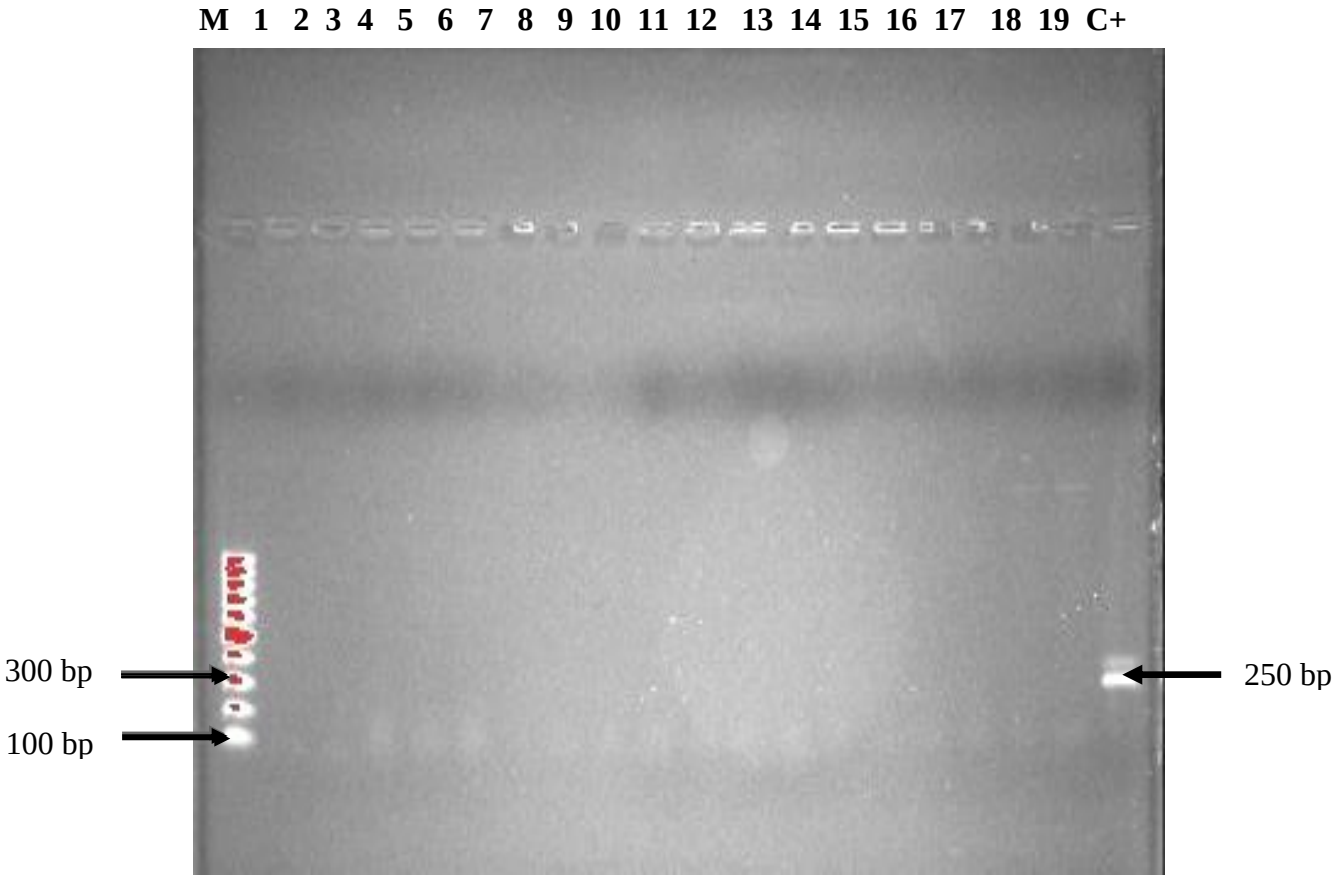


Plate I: Avian influenza virus RT-PCR results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 1 – 18, **M** = molecular weight marker, **C+** = positive control).

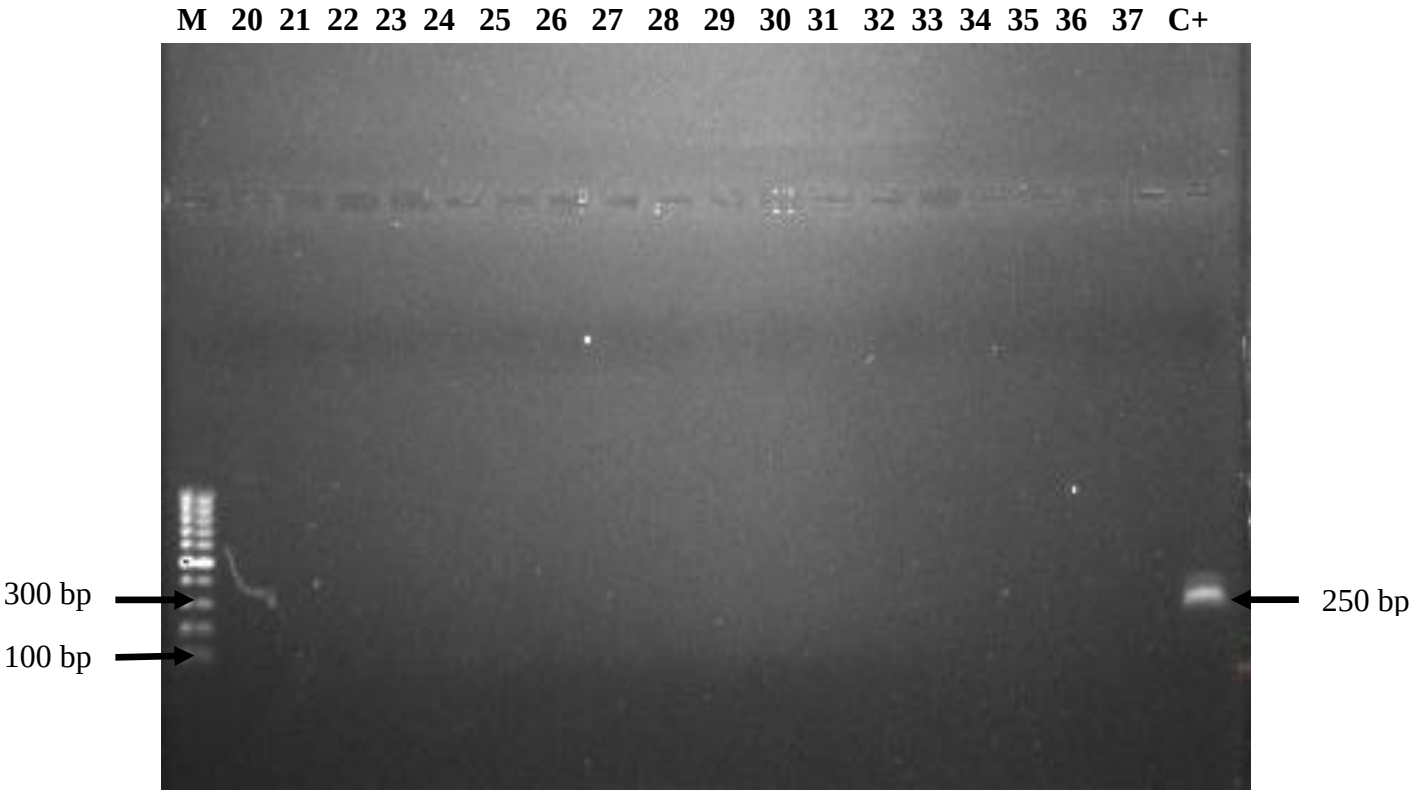


Plate II: Avian influenza virus RT-PCR results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 20-37, **M** = molecular weight marker, **C+** = positive control).

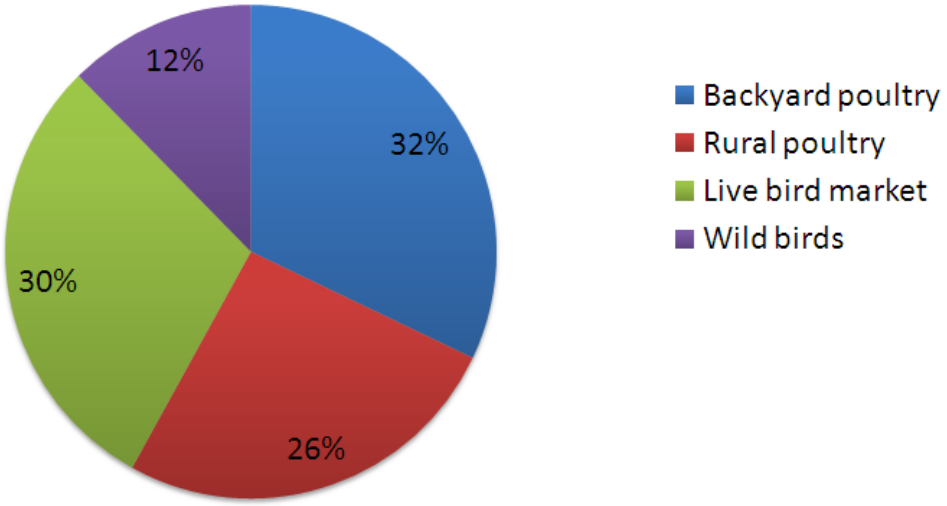


Figure 5.1: Distribution of cloacal and oropharyngeal swabs collected from the various sampling units in the twelve Local Government Areas of Kogi State, Nigeria.

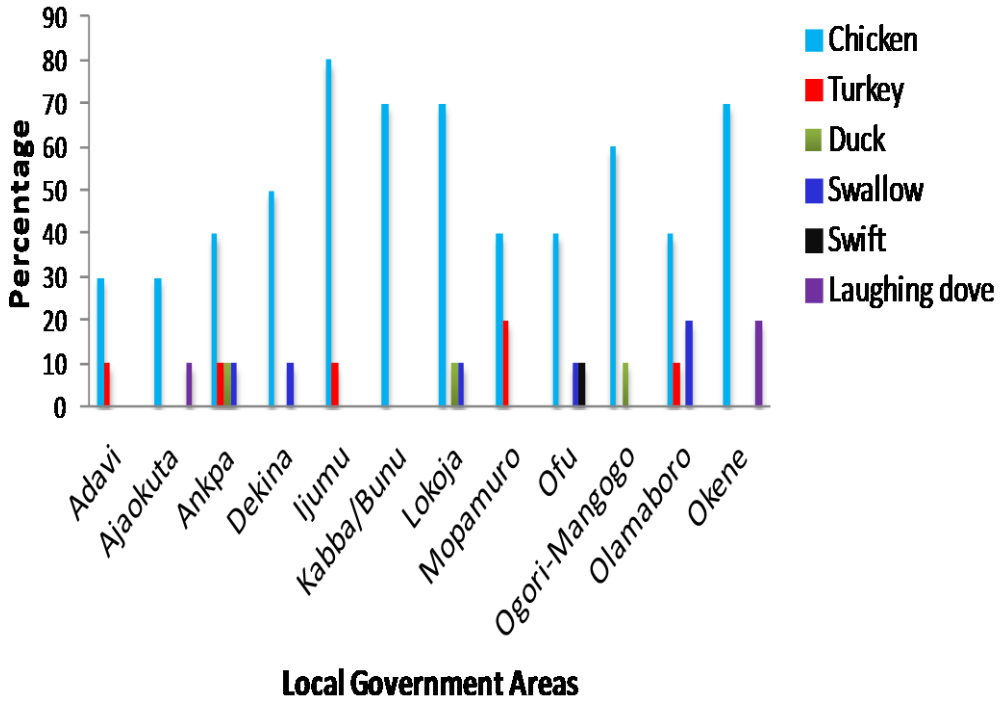


Figure 5.2: Distribution of cloacal and oropharyngeal swabs collected from the various species of poultry and wild birds in the surveyed twelve Local Government Areas of Kogi State, Nigeria.

5.9 Discussion

Conventional RT – PCR is highly sensitive and can detect viruses with titre as low as 3 EID50 (fifty per cent egg infective dose) in clinical samples (Joannis *et al.,* 2008). The failure to detect any positive band of AIVs in the RT – PCR test results from the cloacal and oropharyngeal swabs even when AI antibodies were detected in these birds was an indication that the AIVs were not being shed in poultry and wild birds in the study area. The threshold of infection in the population may be below the level that could be detected from the sampled population. The sampled population might have been small and would not have represented the population characteristics. This might be true, especially in relation to the number of wild birds captured and the sampling method used which might not represent random sample.

There might be limitations in the RT – PCR test used in terms of sensitivity and specificity of detecting AIVs which were reported to be 88.2% and 99.5%, respectively (OIE, 2008). Starick *et al.* (2005) and OIE, (2008) suggested repeating the conventional RT – PCR several times on the samples, using rRT – PCR or nested PCR as ways to overcome the limitations of conventional RT – PCR.

However, Fouchier *et al.* (2000) reported that a negative RT – PCR result did not imply the absence of AIVs in the samples. However, the absence of reported cases of HPAI in the study area during the outbreak of 2006 in Nigeria coupled with the failure to detect AIVs in the samples tested by RT- PCR in this study might indicate that the viruses were not circulating in poultry and wild birds in the area.

The result of the present study is similar to the work of other workers in other parts of Nigeria that failed to detect AIVs in poultry and wild birds. For example, Vakuru *et al.* (2012) surveyed wild birds in live poultry markets, LBMs and around poultry

farms from eight States where HPAI outbreaks were reported across Nigeria but failed to detect AIVs in 403 cloacal swabs by isolation. Also, Wakawa *et al.* (2012) surveyed 33 affected and 25 non-affected poultry farms in Kano State but could not detect the AI viruses by RT-PCR. In addition, Assam (2014) surveyed wild birds from live poultry markets and LBMs in Kaduna State but could not detect AI viruses by RT-PCR. It has been shown that wild birds other than the Anseriformes and Charadriiformes are usually less susceptible to AIVs and when infected, aften excrete less viruses (Stallknecht and Shane, 1988).

The present study could not detect the AIVs in the samples tested by RT – PCR which indicated the freedom status of poultry and wild birds from the AIVs in the study area. Even though AI antibodies were detected in the same birds, the absence of AI viruses showed that the exposure might not be recent or likely due to vaccination of commercial poultry with inactivated vaccines. Wakawa *et al*. (2012) in a study indicated the presence of AI (H5) antibodies in commercial flocks to be as a result of illegal vaccination of commercial poultry by some famers in Kano State, Nigeria. The consequence of vaccination against AI is enormous especially for a country like Nigeria that has adopted the stamping out policy for eradication of AI. Vaccination does not stop repication in the host and shedding of the AIVs which may lead to an endemic situation. It has also been established that vaccination provides the backbone for reassortant AIVs and makes eradication difficult with severe disease impacts (Capua and Morangon, 2003). As well, vaccination creates a problem for surveillance and effective monitoring of AI, evaluation of situation reports and control policy formulation (Capua and Alexander, 2004; Cardona, 2007).

The failure to detect the presence of AI viruses in the cloacal and oropharyngeal swabs was an indication that the viruses were not circulating in wild birds and poultry in the study area. The limitations of the sampling method for wild birds which might not represent a random sample and possibly its effects on the study so as to make concrete conclusions were acknowledged. It is recommended that a broad based sustained surveillance for the AI viruses should be carried out to establish the free status of poultry and wild birds from the activities of AIVs in Kogi State. The study will serve as reference for future surveillance of AI viruses in the study area as there was no previous study done in the area for these species surveyed.

CHAPTER SIX

SURVEY FOR NEWCASTLE DISEASE VIRUSES IN POULTRY AND FREE FLYING WILD BIRDS

Abstract

Newcastle disease outbreak even in the face of effective vaccination is a common problem in Nigeria. A survey was carried out between June, 2012 to February, 2013 to detect the presence of Newcastle disease viruses in poultry from rural households, backyard farms and live bird markets (LBMs) as well as in free flying wild birds in twelve Local Government Areas (LGAs) of Kogi State, Nigeria. A total of 810 cloacal and oropharyngeal swabs were tested using conventional RT-PCR with set of primers targeting the avian paramyxovirus-1 (APMV-1) matrix protein with 60.5% being positive for the viruses. Backyard poultry accounted for 32.1% of the total samples with 57.7% being positive for ND viruses, poultry from live bird market accounted for 29.6% of the samples with 62.5% positive, rural poultry accounted for 25.9% with 61.9% being positive while wild birds accounted for 12.3% with 60.0% being positive for ND viruses. Dekina had the highest APMV-1 prevalence of 100% followed by Ofu with 83.3%; 77.8% for Ijumu, 71.4% each for Ankpamand Olamaboro; 66.7% for Mopamuro; 57.1% for Ogori-Mangogo; 50.0% each for Adavi and Ajaokuta; 44.4% for Okene; 42.9% for Kabba/Bunu, while Lokoja had 22.2%, respectively. On the other hand, Chicken accounted for 76.5 % of samples with 75.5% being positive, while Turkey accounted for 7.4% with 8.2% being positive, swallow accounted for 7.4 % with 10.2% being positive, Duck accounted for 3.7% with 4.1% being positive, swifts accounted for 1.2% with 2.0% being positive, while Laughing dove accounted for 3.7% with none being positive for ND viruses. Groups were compared with each other using χ^2 and the relative risk of infection between groups were calculated as odd ratio. The χ^2 values of backyard poultry sampling unit against

other sampling units (OR = 0.70, 95% CI = 0.28 – 1.76), LGAs without wild bird captured against LGAs with wild birds captured (OR = 0.99, 95% CI = 0.40 – 2.46) and poultry species against wild bird species (OR = 0.98, 95% CI = 0.25 – 3.77) were not significant but that of contiguous areas A against contiguous areas B were significant (χ^2 = 6.59, p≤ 0.01, OR = 4.10 at 95% CI = 1.34 – 12.28). The study revealed the circulation of APMV-1 in poultry and wild birds in Kogi State. There is need for further sequencing of the F-protein or isolation and characterization of isolates to determine the pathotypes of the APMV-1. Control activities apart from vaccination should involve adequate screening of barn swallow and swifts from roosting in poultry houses.

6.1 Introduction

Three Newcastle disease (ND) panzootic were recorded in the past and one of such was caused by feral pigeon fecal contamination of poultry feed materials (Alexander, 2001). The presence of wild type Newcastle disease virus (NDV) has for long remained a threat to rural and commercial poultry production. Wild birds particularly waterfowls (order Anseriformes), tend to carry these viruses asymptomatically as reservoirs (Alexander, 2001). Most of the viruses found in wild birds are lentogenic however, virulent Avian paramyxovirus-1 (APMV-1) has become established in some cormorant populations (*Phalacrocorax* spp; order Pelecaniformes) causing disease in juvenile birds (Seal *et al.,* 1998).

Susceptibility to ND varies widely among poultry and pet birds. Members of the order Phasianiformes (gallinaceous birds) particularly chickens, are highly susceptible to disease (Alexander, 2001). Turkeys are less likely to develop severe symptoms and the susceptibility of game birds (pheasants, partridges, quail and guinea fowl) varies

with the species. Ducks and geese usually have inapparent infections but some clinical cases have also been described occasionally in ducks (Alexander, 2001). Outbreaks have been reported in ostriches (order Struthioniformes) while pigeons (order Columbiformes) are susceptible to the disease with APMV-1 strains maintained in pigeon populations having some antigenic differences from other NDV isolates and are sometimes called pigeon paramyxovirus type 1 (Alexander, 2001). Susceptibility to disease varies widely in psittacine birds (order Psittaciformes); cockatiels and parrots often die or develop neurological signs but some species tend to carry velogenic strains subclinically (Seal *et al.*, 1998).

Some wild birds found in zoos also become ill with penguins (order Sphenisciformes) highly susceptible to Newcastle disease and often die acutely (Alexander, 2001). Severe disease has been reported in some raptors (order Falconiformes) including a bearded vulture (*Gypaetus barbatus*), a captive white-tailed sea eagle (*Haliaeetus albicilla)* and a wild osprey (*Pandion haliaetus*) while other raptors tend to be resistant to clinical disease. Clinical disease has also been reported in gulls (order Charadriiformes), owls (order Strigiformes), pelicans (order Pelecaniformes) and a Northern gannet, *Morus bassanus* (order Pelecaniformes) while susceptibility varies among passerine birds (order Passeriformes) with some species excreting virus subclinically and others developing severe clinical signs (Seal *et al.*, 1998). Sporadic deaths have also been reported in Corvidae such as crows and ravens (Alexander, 2001).

The epidemiology of APMV-1 is incompletely understood; however, wild birds, particularly waterfowls may be the reservoir hosts for lentogenic viruses (Alexander, 2001). This virus could become more virulent after becoming established in poultry.

Some outbreaks were apparently caused by velogenic strains that emerged from local, low pathogenic isolates (Alexander, 2001). Acquisition of virulence has also been reported in experimentally infected birds. Psittacine birds have introduced APMV-1 to poultry flocks in some outbreaks (Alexander, 2001).

In Africa, ND is a major constraint against the development of both commercial and village poultry production (Ezeokoli *et al.,* 1984; Adene, 1996). In Nigeria, ND is endemic with the occurrence of outbreaks even after vaccination (Sai'du *et al.,* 2004). This study was designed to determine the molecular epidemiology of ND in poultry and free flying wild birds in Kogi State, Nigeria so as to plan and adopt appropriate control measures against the impact of the disease.

6.2 Statement of the Research Problem

Newcastle disease is a primary disease of birds. Some avian species become ill, while others carry this virus asymptomatically. Infections are known to occur in humans such as veterinarians and laboratory personnel who handle the virus but have not been reported in other species of mammals. The transmission of NDV occurs through aerosol, newly introduced birds, buying or giving away sick birds, exposure to fecal and other excretions from infected birds and contact with contaminated feed, water, equipment and clothing (Abdu, 2007; Nwanta *et al.,* 2008). The disease causes high economic losses due to high mortality, morbidity, stress, decreased egg production and hatchability (Alexander, 2000; Sa'idu *et al.,* 2004). Vaccination has been reported as a safeguard against endemic ND but outbreaks still occur in most instances after vaccination (Orajaka *et al*., 1999; Sa'idu *et al.*, 2004).

6.3 Justification of the Study

Outbreaks of ND are known to occur even after vaccination (Sa'idu *et al*., 2004; Nwanta *et al*., 2008). The detection of wild type or field NDV strains will help in evaluating the efficacy of current vaccines as well as the development of new vaccines.

The detection of ND viruses in wild birds will help in a change of approach of the present prevention and control policy which rely entirely on vaccination.

Ameji *et al*. (2011) reported of antibodies to ND in chickens from six LGAs of Kogi State in a survey which warranted further virological investigations.

6.4 Aim of the Study

The aim of the study was to determine the prevalence of NDV in poultry and wild birds in Kogi State, Nigeria.

6.5 Objectives of the Study

The objectives were to undertake:

1. The detection of ND viruses in poultry in Kogi State by RT – PCR.
2. The detection of ND viruses in Wild birds in Kogi State by RT – PCR.

6.6 Research Questions

1. Are there ND viruses in poultry in Kogi State?
2. Are there ND viruses wild birds in Kogi State?

6.7 Materials and Methods

6.7.1 Definitions

Definitions of backyard poultry, rural poultry and LBMs are as in ***section 3.7.1***

6.7.2 Study area

The study area is as described in ***section 3.7.2.***

6.7.3 Sample size

6.7.3.1 Sample size for Newcastle disease survey in poultry

Sample size for ND survey in poultry was as determined in ***section 3.7.3.1***

6.7.3.2 Sample size for Newcastle disease survey in wild birds

Sample size for wild birds was as determined in ***section 4.7.2.2***

6.7.4 Sampling method for poultry from rural, backyard, live bird market and wild birds

The sampling method was as described in ***section 3.7.4.***

6.7.5 Cloacal and oropharyngeal swabs sampling

A total of 810 swabs made up of 710 oropharyngeal swabs from poultry and 100 cloacal swabs from wild birds were collected and analyzed during the survey.

The oropharyngeal swab was collected after proper restraint of a bird by an assistant, the beak was held open with the left hand of the researcher and the right hand used to place sterile cotton swab at the base of the tongue, gently rotated and taken out. The swab was then placed in a cryo-vial of viral transport medium containing 10,000 units/ml penicillin, 10 mg/ml streptomycin, 25 µg/ml gentamycin, 5000 units /ml mycostatin and 1% bovine serum albumin in phosphate buffered saline (PBS) (OIE, 2009).

Similarly, the cloacal swab was collected after proper restraint by an assistant, the researcher used the left hand to lift the tail feather up as well as to stabilize the cloaca and with the right hand, placed the sterile cotton swab into the cloaca, rotated, taken out and placed in a cryo-vial of viral transport medium of the same constituents as

Stated above. Care was taken to avoid massive faeces (OIE, 2009). The swab samples were maintained at 4 °C upon collection and stored in liquid nitrogen at -70 °C within 4-6 hours until they were processed.

6.7.6 Detection of Newcastle disease viruses in poultry and wild birds

A total of 810 swabs consisting of 710 oropharyngeal and 100 cloacal swab samples were collected from poultry and wild birds during the field survey from June, 2012 to February, 2013 and analyzed for ND viruses.

6.7.6.1 Preparation of samples

The samples were prepared as described in section 5.7.6.1

6.7.6.2 Extraction of nucleic acid

The nucleic acid was extracted from the samples as described in section 5.7.5.2

6.7.6.3 Preparation of polymerase chain reaction master mix

The master mix for the 25 µl final volume PCR was prepared from stock of reagents as shown (Appendix I). The APMV-1 forward and reverse primers targeting the M-gene of 121 bp size with the following sequences: F=5′-AGTGATGTGCTCGGGACCTTC-3′ and R=5′-CCTGAGGAGAGGCATTTGCTA-3′ (Wise *et al*., 2004) were used.

6.7.6.4 Reverse transcription- polymerase chain reaction

The conventional one step RT – PCR was used to amplify sample in final reaction mixture of 25 µl containing nuclease free H_2O, 10x PCR Buffer, 50 mM $MgCl_2$, 10 mM deoxynucleoside triphosphate (dNTP), 10 µM each of oligonucleotide (APMV-1 and 121 bp – forward and reverse primers), 20 U of reverse transcriptase, 5 U of ribonuclease inhibitor, 5 U of Ampli – Taq DNA polymerase (all reagents by Applied

Biosystems, USA) and 5 µl of RNA extract. Thermocycling was done in GeneAmp^{R} PCR System 9700, Applied Biosystems (USA) with the following cycling conditions: activation at 50°C for 20 min and initial denaturation at 95°C for 15 min (stages I & II once), denaturation at 94°C for 45 sec, annealing at 60°C for 45 sec, elongation at 72°C for 45 sec (stage III 40 times), final elongation at 72°C for 5 min and hold at 4°C until used (stage IV once).

6.7.6.5 Preparation of tris- borate ethylene diamine tetra-acetic acid buffer

The TBE 1x buffer that was used in the preparation of 1.5 % agarose gel for electrophoresis was prepared from TBE 10x stock solution. A 1000 ml solution of TBE 1x was prepared by adding 100 ml of TBE 10x to 900 ml of distilled water.

6.7.6.6 Preparation of 1.5 % 100 ml gel

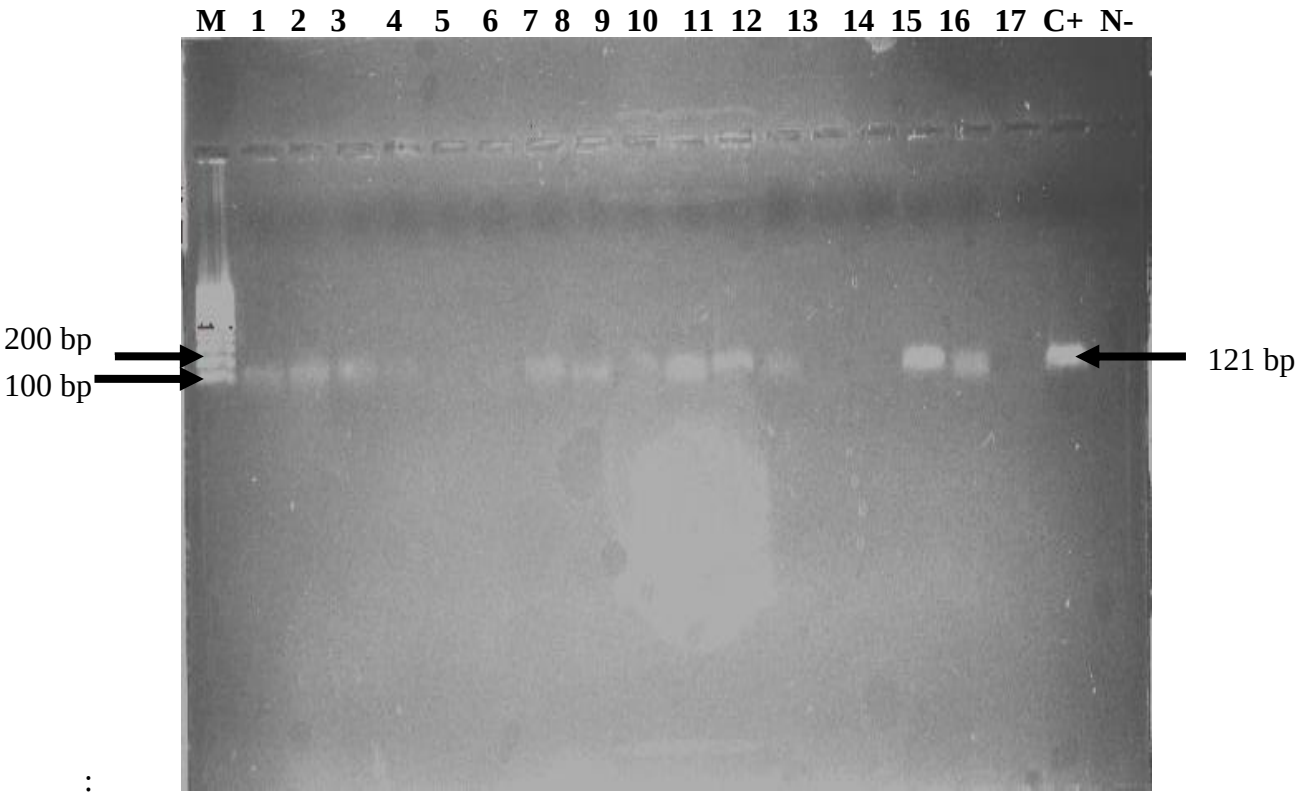
The gel was prepared as described in section 5.7.6.6

6.7.6.7 Agarose gel electrophoresis of the amplicons

The agarose gel electrophoresis of the amplicons was carried out as described in section 5.7.5.7

6.8 Results

A total of 490 (60.5%) swabs from the three poultry sampling units and wild birds in the survey areas were positive for ND viruses (Plates III - V). The positive samples had the presence of cDNA bands corresponding to the region of 121 bp specific for the gene generated by the pairs of primers used in the amplification of the RNA extract of test samples.



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Plate III: Newcastle disease virus RT-PCR results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 1 – 17 with 1, 2, 3, 7, 8, 10, 11, 12, 15 and 16 having positive cDNA bands. **M** = molecular weight marker, **C+** = positive control, **N -**= negative control).

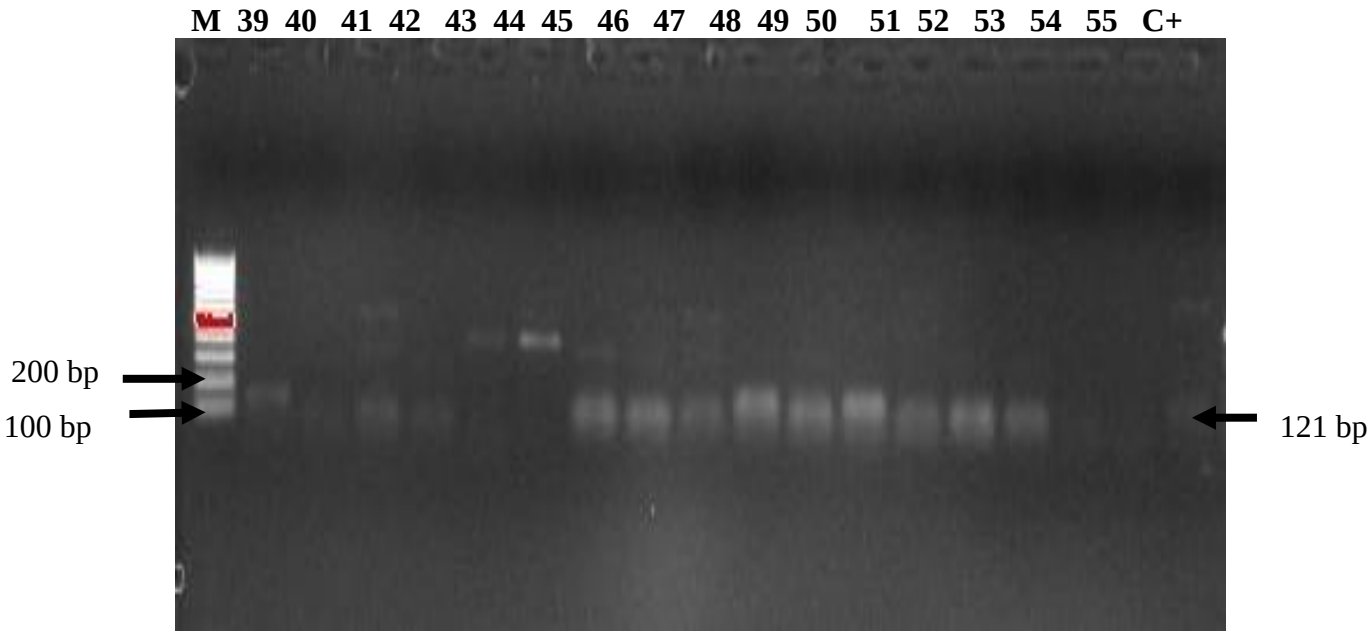


Plate IV: Newcastle disease virus RT-PCR results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 39 – 55 with 39, 41, 42, 45, 46, 47, 48, 49, 50, 51, 52 and 53 being positive for NDVs. **M** = molecular weight marker, **C+** = positive control.

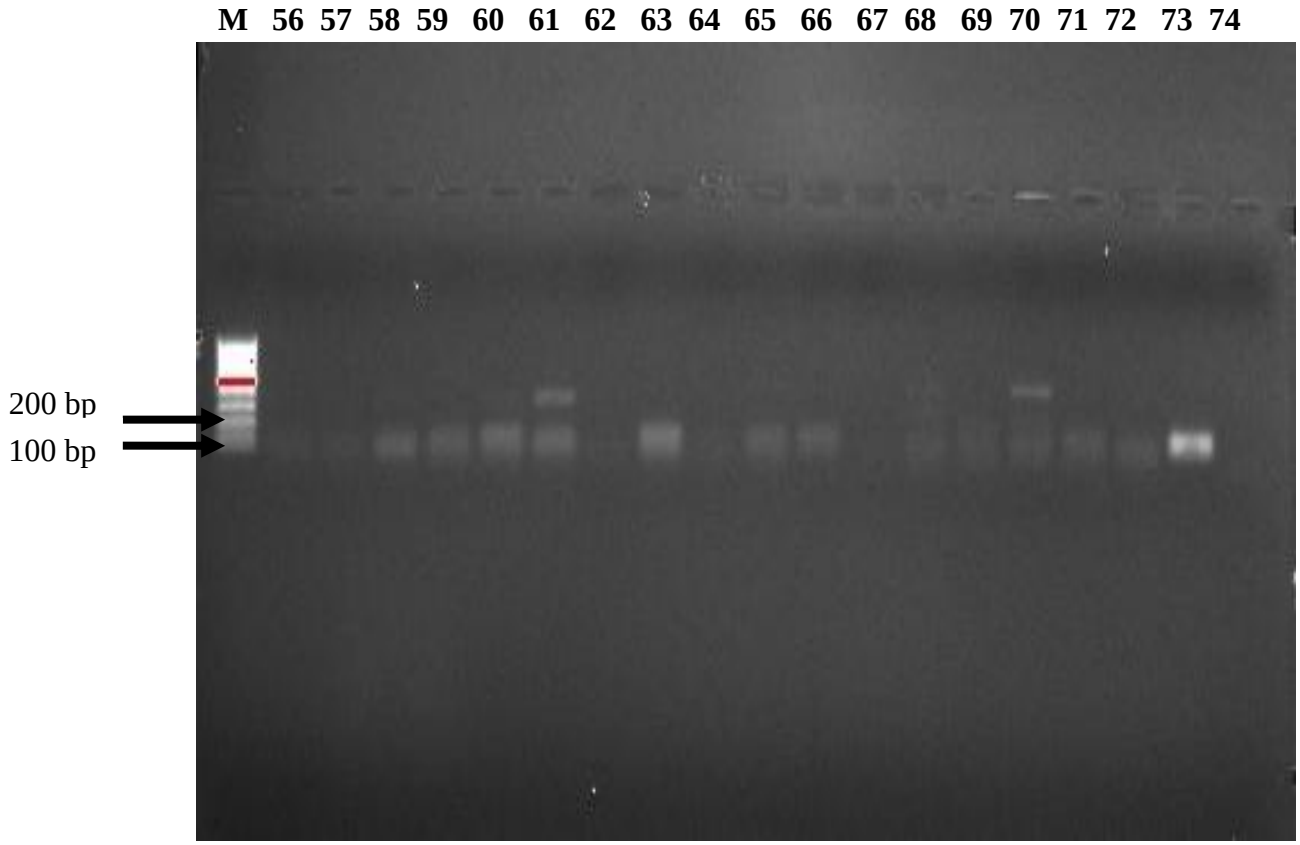


Plate V: Newcastle disease virus RT-PCR results of oropharyngeal and cloacal swabs from poultry and wild birds in the surveyed areas of Kogi State (Pooled samples 56 – 73 with 58, 59, 60, 61, 63. 65, 66,69, 70, 71, 72 and 73 being positive for NDvs, **M** = molecular weight marker).

On the basis of LGAs, the prevalence of NDV was 100.0% for Dekina; 83.3% for Ofu; 77.8% for Ijumu; 71.4 % for Ankpa and Olamaboro; 66.7% for Mopamuro; 57.1% for Ogori-Mangogo; 50.0% each for Adavi and Ajaokuta; 44.4% for Okene; 42.9% for Kabba/Bunu; and 22.2% for Lokoja (Table 6.1). However, on the basis of sampling units, the prevalence of NDV were 57.7% for backyard poultry, 61.9% for rural poultry, 62.5% for live bird market and 60.0% for wild birds (Table 6.1). Similarly, based on avian species, prevalence of NDV was zero for laughing dove, 75.5% for Chicken, 8.2% for Turkey, 4.1% for Duck, 10.2% for swallow and 2.0% for swifts respectively (Table 6.2).

The LGAs were grouped into LGAs where wild birds were surveyed and LGAs where wild birds were not surveyed and or as two contiguous zones, A and B for evaluating epidemiological characteristics. The LGAs where wild birds were surveyed, included Ankpa, Ajaokuta, Dekina, Lokoja, Ofu and Olamaboro while the LGAs where wild birds were not surveyed, included Adavi, Kabba/Bunu, Ijumu, Mopamuro and Okene. On the other hand, LGAs within contiguous zone A included Adavi, Ajaokuta, Ijumu, Kabba/Bunu, Lokoja, Mopamuro, Ogori-Mangogo and Okene, while those within contiguous zone B included Ankpa, Dekina, Ofu and Olamaboro. These groups were compared using χ^2 and relative risk of infection as odd ratio. The χ^2 values of backyard poultry sampling unit against other sampling units (OR = 0.70, 95% CI = 0.28 – 1.76), LGA where wild birds were not surveyed against LGA where wild birds were surveyed (OR = 0.99, 95% CI = 0.40 – 2.46) and poultry species against wild bird species (OR = 0.98, 95% CI = 0.25 – 3.77) were not significant but that of contiguous areas A against contiguous areas B were significant (χ^2 = 6.59, $p \leq 0.01$, OR = 4.10 at 95% CI = 1.34 – 12.28) (Table 6.3).

Table 6.1: Prevalence of Newcastle disease viruses in the surveyed sampling units and Local Government Areas of Kogi State.

Local Government Area	Positive samples to NDVs in different sampling units				
	Backyard farm (%)	Rural household (%)	Live bird market (%)	Wild bird (%)	Total (%)
Ankpa	10 (6.7)	30 (23.1)	0 (0.0)	10 (16.7)	50 (71.4)
Adavi	10 (6.7)	10 (7.7)	0 (0.0)	0 (0.0)	20 (50.0)
Ajaokuta	10 (6.7)	10 (7.7)	0 (0.0)	0 (0.0)	20 (50.0)
Dekina	10 (6.7)	10 (7.7)	30 (20.0)	10 (16.7)	60 (100.0)
Ijumu	40 (26.7)	10 (7.7)	20 (13.3)	0 (0.0)	70 (77.8)
Kabba/Bunu	20 (13.3)	0 (0.0)	10 (6.7)	0 (0.0)	30 (42.9)
Lokoja	0 (0.0)	20 (15.4)	0 (0.0)	0 (0.0)	20 (22.2)
Mopamuro	10 (6.7)	10 (7.7)	20 (13.3)	0 (0.0)	40 (66.7)
Ofu	10 (6.7)	10 (7.7)	10 (6.7)	20 (33.3)	50 (83.3)
Ogori-Mangogo	30 (20.0)	0 (0.0)	10 (6.7)	0 (0.0)	40 (57.1)
Okene	0 (0.0)	10 (7.7)	30 (20.0)	0 (0.0)	40 (44.4)
Olamaboro	0 (0.0)	10 (7.7)	20 (13.3)	20 (33.3)	50 (71.4)
Total	150 (57.7)	130 (61.9)	150 (62.5)	60 (60.0)	490 (60.5)

Table 6.2: Prevalence of Newcastle disease viruses among avian species surveyed in the various Local Government Areas of Kogi State.

Local Government Area	No. of species of poultry and wild bird positive to ND viruses						Total (%)
	Chicken (%)	Turkey (%)	Duck (%)	Swallow (%)	Swifts (%)	Dove (%)	
Adavi	20 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (50.0)
Ajaokuta	20 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (50.0)
Ankpa	20 (5.4)	10 (25.0)	10 (50.0)	10 (20.0)	0 (0.0)	0 (0.0)	50 (71.4)
Dekina	50 (13.5)	0 (0.0)	0 (0.0)	10 (20.0)	0 (0.0)	0 (0.0)	60 (100.0)
Ijumu	60 (16.2)	10 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	70 (77.8)
Kabba/Bunu	30 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	30 (42.9)
Lokoja	10 (2.7)	0 (0.0)	10 (50.0)	0(0.0)	0 (0.0)	0 (0.0)	20 (22.2)
Mopamuro	30 (8.1)	10 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	40 (66.7)
Ofu	30 (8.1)	0 (0.0)	0 (0.0)	10 (20.0)	10 (100.0)	0 (0.0)	50 (83.3)
Ogori-Mangogo	40 (10.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	40 (57.1)
Okene	40 (10.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	40 (44.4)
Olamaboro	20 (5.4)	10 (25.0)	0 (0.0)	20 (40.0)	0 (0.0)	0 (0.0)	50 (71.4)
Total	370 (75.5)	40 (8.2)	20 (4.1)	50 (10.2)	10 (2.0)	0 (0.0)	490 (60.5)

Table 6.3: The relative risk of Newcastle disease viruses infection in different categories of birds in the surveyed areas of Kogi State, Nigeria.

Category of odds	OR value	95% CI		Chi-square
		Lower	Upper	
Backyard poultry/other poultry & wild bird	0.698	0.277	1.758	χ^2 =0.583, p≤0.445
Areas without captured wild bird/Areas with captured wild bird	0.992	0.401	2.457	χ^2 =0.001, p≤0.986
Poultry species/wild bird species	0.977	0.253	3.774	χ^2 =0.021, p≤0.973
Contiguous zone A/contiguous zone B	4.050	1.336	12.281	χ^2 =6.587, p≤0.01*

OR = Odd ratio * = significant p value

6.9 Discussion

The detection of cDNA bands of avian paramyxovirus 1 (APMV-1) in this survey by RT-PCR is an indication that strains of the virus are prevalent among poultry and wild birds in the study area. The sources of these strains in the wild birds are unknown but indiscriminate immunization of exotic chickens with live lentogenic and mesogenic vaccines as well as disposal of poultry litter and carcasses in refuse dump are common (Ibu *et al*., 2009). Wild birds are taken to be the reservoirs of both avirulent and the virulent ND virus strains and may play major roles in the maintenance of the disease in domestic poultry (Echeonwu *et al.,* 1993; Alexander, 2001).

An overall NDV prevalence of 60.5% seen in this study was an indication of the high viral activity in the study area. The birds sampled were apparently healthy which showed that NDV strains in circulation were vaccine strains or lentogenic field strains with concomitant mild disease and production losses. However, it had been revealed that the highly virulent strains could evolve from virus strains of low virulence by mutation (Alexander *et al.,* 1997) lending credence to the impending danger of the co-circulation of strains of APMV-1 in wild birds and poultry as seen in the present study.

The NDV prevalence of 60.0% for wild birds found in this study is much higher than the prevalence of 7.0% determined in a previous study in North Central Nigeria (Ibu *et al.,* 2009). The wide gap in the prevalence may be due to the larger sample size and wide area of coverage in the former study compared to the present study that involved some LGAs within a State. Also, the former study used virus isolation to determine the prevalence, a method of high specificity but very low sensitivity.

Chickens had the highest NDV prevalence of 75.5% among the species of poultry sampled. This showed an infectivity of the chickens with the NDV vaccinal strains if the vaccination was recent as most of the NDV vaccines in use in backyard poultry were live vaccines. However, this high prevalence is a combination from the three sampling units which meant that the viruses might not have entirely come from vaccine strains alone. This argument is obvious considering the fact that the ND antigens prevalence in poultry from backyard farms alone was 57.7% while that of rural poultry where routine vaccination against ND is not done is 61.9%.

The low prevalence of ND in backyard poultry farms might be an indication of low vaccine takes if vacination of backyard flocks with live vaccine was recent which further meant that chickens in this cluster would not be properly protected against NDV in the event of an ND outbreak in the study area. It could also be that vaccinated and sampled poultry from backyard farms were being challenged by ND field virus or late stage of vaccination which had seroconverted. Newcastle disease is one of the viral diseases that cannot be treated but preventable through vaccination and improved biosecurity hence, the findings of this study underscore the threat of the disease to poultry production in the study area.

Poultry from LBMs had an NDV prevalence of 62.5%. The poultry of this cluster are a collection of poultry from rural households and backyard poultry farms and do not have a particular rearing or management conditions but a combination of its constituents. The LBM acts as a mixing pool for vaccinated and unvaccinated poultry where pathogens are shared with the possible danger of spread to areas where poultry are taken to after purchase from the LBMs. The implication of this finding is that there is circulation of

NDV in the LBMs with the likely outbreak of ND when naïve birds are introduced and may results in disease spread to households and farms.

The NDV prevalence of 61.9% in rural poultry where routine vaccination is not done is also high, a pointer of the endemicity of APMV-1 in the environment. The circulation of APMV-1 in rural poultry is majorly due to natural exposure because the birds are not routinely vaccinated. Rural poultry are kept on free range where they roam freely to scavenge for food and in the process may pick NDV from the environment in refuse dumps, contaminated food and water by infected, vaccinated poultry flocks and wild birds or even via aerosol. Seropositivity to ND antibodies had been detected in unvaccinated flocks in Australia, Switzerland and elsewhere where poultry are kept on free range with direct contact with wild birds and Ducks which often do not show clinical disease (Schelling *et al.,* 1999).

The prevalence of 60.0% in wild birds in the study area is also high. All the swallow and swift tested were positive to the ND viral RNA contrary to an earlier study carried out within three States of North central Nigeria that failed to detect the virus in these species of wild birds (Ibu *et al.,* 2009). Ibu *et al.* (2009) in the earlier study attempted ND viral isolation from cloacal swabs which failed to yield any positive result as it has been shown to be often difficult isolating NDV from seropositive flocks (Schelling *et al*., 1999). The 10.2% prevalence of NDV in swallow and 2.0 % in swifts is an indication of the susceptibility of these wild birds' species to infection with the virus. The detection of ND viruses in swallow and swifts species of wild birds may be related to their preference to roost or breed in old buildings, barns and around poultry houses where they may be infected via aerosol, contamination of water and feed by vaccinated poultry flock.

The absence of ND viruses in laughing dove (*Streptopelia spp*) is in agreement with the work of Ibu *et al.* (2009) which may be due largely to the fewer number of wild birds caught across the survey area. The sampling of wild birds was done through the use of glue, hand and mist net trapping to avoid harm and death of the birds which limited the available birds sampled. Free flying wild birds were not previously considered in the control of ND in Nigeria. However, the presence of the ND virus in a single species is a proof of the likely role free flying wild birds may play in the epidemiology of the disease.

The NDV prevalence in duck species was 4.1%. Ducks do not show clinical disease but are known to be carriers of the avirulent NDV which may mutate to virulent NDV strains (Alexander, 1997). The detection of ND viruses in ducks was an indication that these species were infected and were shedding the ND viruses. The risk of NDV transmission from ducks or water birds may be either through direct or indirect contact with poultry flocks such as contaminated feed and water or infection of wild bird species at common water sites and then transmission of the infection into poultry flocks. Isolations of lentogenic NDV strains are frequently reported in wild waterfowls but two velogenic NDV strains were isolated from outbreaks in Ireland, which were antigenetically closely-related to avirulent strains in waterfowls (Alexander, 2001). There was evidence that NDV with low pathogenicity may mutate and become virulent for poultry hence, ducks/waterfowls must be regarded as reservoirs also for pathogenic strains for chickens (Alexander *et al.*, 1997).

The prevalence of NDV in turkey was 8.2%, indicating the susceptibility of this species to the virus. This finding has an implication that rearing of turkeys and chickens together

will result in ND outbreak with devastating effect on Chicken production as clinical disease in turkeys is often variable.

Dekina LGA had the highest NDV prevalence probably due to the age and type of poultry sampled from this LGA. The poultry sampled in Dekina LGA were more of broilers from backyard poultry than in any of the LGAs. This is closely followed by Ofu LGA then, Ijumu; Ankpa and Olamaboro LGAs with same prevalence of NDV. These LGAs apart from Ijumu are contiguous (contiguous zone B) to one another on the eastern flank of Kogi State and the findings might be linked with poultry husbandry practice in the areas. Backyard and local poultry were often housed together and allowed a free run during the day thereby enabling the sharing of common pathogens. The LBMs in these areas also had these same types of poultry kept in the same cages for sale. All the birds sampled were apparently healthy hence, the prevalence of ND viruses indicated the infection of naïve birds with wild lentogenic or vaccinal strains of NDV. The high prevalence of ND viruses in Ankpa, Dekina, Ofu and Olamaboro LGAs above other LGAs might be due to the important role of wild birds in the maintenance and dissemination of ND infection within this contiguous zone. Wild birds were surveyed from all the LGAs in this zone with ND antibodies and almost all the cloacal swabs being positive to ND viruses.

On the other hand, Ijumu LGA had the highest prevalence of ND viruses followed by Mopamuro, Ogori-Mangogo, Okene and Kabba/Bunu LGAs while Adavi, Ajaokuta and Lokoja LGAs had the least prevalence. These LGAs were grouped as contiguous zone A on the west and central flanks of Kogi State. Airborne transmission over some distance had been reported for NDV (Alexander *et al.*, 1997). This may account for the presence

of ND viruses in non vaccinated poultry flocks in addition to the role of wild birds. The detection of relatively low prevalence of NDV in this contiguous zone might be due to sampling of older birds from vaccinated flocks which had antibodies that made them more resistant to infection with the virus. It was also indicative of the little role of wild birds in the dissemination of NDV in these areas as wild birds were surveyed in only 3 out of 8 LGAs in this contiguous zone with none of the cloacal swabs from wild birds being positive for ND viruses.

The odds of infection with NDV in the contiguous zone A were 4 times higher than that of contiguous zone B. This was due to the fact that more of the tested swabs with 52% being positive for ND viruses were from zone A. The implication of the finding is that contiguous zone A had more backyard poultry farms and LBMs as well as other factors which favor the infection of birds with NDV. The odds of infection in backyard poultry was less than that of other poultry and wild bird species probably due to the vaccination of backyard poultry against ND infection. Moreover, the odds of infection in LGAs without wild birds captured were less than that from LGAs where wild birds were captured. The high odd of infection in areas with wild birds captured under scored the role of wild birds in the epidemiology of ND as well as other factors such as naivity and age of birds sampled in this zone.

CHAPTER SEVEN

ASSESSMENT OF POULTRY – WILD BIRD INTERACTIONS BIOSECURITY AND HUSBANDRY PRACTICES IN POULTRY PRODUCTION IN KOGI STATE

Abstract

Wild birds are known to be reservoirs of most avian pathogens, especially avian influenza (AI) and Newcastle disease (ND) viruses and are involved in their spread over long distances to commercial poultry flocks. A study was conducted from June – September, 2012 to assess the presence of poultry – wild bird interactions, biosecurity and husbandr practices in poultry production in twelve Local Government Areas (LGAs) of Kogi State, Nigeria. A total of 108 respondents made up of backyard and rural poultry farmers and fowl sellers were interviewed, 77.8% of the respondents affirmed to wild birds scavenging for food around their farms, households and live bird markets, 66.7% of the respondents kept poultry on free range or under inadequate housing, 63.9% of the respondents had trees where wild birds could roost around their farms, households or live bird markets, 8.3% of the respondents had water body/fish pond around their farms or households, 85.2% of the respondents would dispose poultry litter or dead birds in refuse dumps, 50.0% of the respondents were in areas with major rivers or wetlands while 74.1% of the respondents were in areas that serve as transit routes for live birds trade. There was existence of poultry – wild bird interactions due to the presence of risk factors that encourage direct or indirect contact of poultry and wild birds with swallow, swift, laughing dove, melodious warbler and fly catcher observed to be the bridge species in the study area. The strength of likely possible interactions between wild birds and poultry was very high for Ajaokuta, Ankpa, Dekina, Lokoja, Okene and Olamaboro LGAs, while

the rest LGAs had high strength of interactions. Based on sampling unit, the strength of likely possible interactions between wild birds and poultry was very high in rural poultry while it was high for both live bird markets (LBMs) and backyard poultry. Biosecurity and husbandry practices were poor which may limit poultry production in Kogi State. It is recommended that poultry farmers should avoid or dislodge existent risk factors that may encourage poultry – wild bird interactions around their farms and as well undertake biosecurity and improved husbandry practices for enhanced poultry production in Kogi State.

7.1 Introduction

Surveillance, biosecurity and other control measures may successfully curtail infection of contagious avian diseases in domestic poultry but not in wild birds (Nettles *et al.,* 1985; Komar and Olsen, 2008). The mobility of wild birds and the challenge of tracking different populations, make it of great importance to identify the environmental factors that favor the occurrence and sharing of avian pathogens between domestic poultry and wild birds (Gilbert *et al*., 2008; Si *et al.,* 2013).

Biosecurity refers to all the management practices aimed at excluding or reducing the potential for the transmission and spread of diseases to animals, humans or an area initially free from the disease causing agents (Halifa, 2008). The two main objectives of biosecurity are bio-exclusion and bio-containment with three components consisting of isolation, traffic control and sanitation (AICP, 2008; USAID, 2009).

The low levels of biosecurity in backyard poultry, rural poultry and LBMs management systems in Nigeria impact negatively on commercial poultry production (Pagani *et al.,*

2008; Abdu, 2010). Strict biosecurity measures in addition to vaccinations are strategic prevention and control policies adopted to control some contagious poultry diseases which vaccinations alone are not enough to control under field conditions (Abdu, 2007; FAO, 2008). Good husbandry practices such as adequate feeding, housing and stocking to avoid overcrowding, good ventilation, proper disposal of wastes, cleaning and disinfection of poultry premises help to keep out infections and their spread (Jordan, 1990; Pagani *et al.,* 2008). Appropriate adoption of biosecurity can as well helps to minimize or eradicate the harmful interactions between wild birds and poultry as they are known to compete for food, water, air, and shelter in an uncontrolled free range environment thereby curtailing the sharing of disease pathogens (FAO, 2008; Pagani *et al.*, 2008).

7.2 Statement of the Research Problem

Wild birds are capable of excreting abundant viruses especially AIVs in their feces before and after the onset of clinical signs or even asymptomatically (Chen *et al*. 2006). Kilpatrick *et al.* (2006) reported that migratory wild birds were the primary spreading agents of the HPAI H5N1 virus in Europe, Middle East and Africa. In addition, Si *et al.* (2013) reported that exchange of avian pathogens is enhanced if poultry markets or rearing free range poultry farms are close to wild birds or waterfowls' habitats such as lakes, wetlands, breeding or over-wintering sites. The interaction of domestic poultry with wild birds has been shown to be a possible source of avian diseases in domestic flocks (Si *et al.,* 2013).

Poor husbandry practices, illiteracy and poverty are hinderances to adequate implementation of biosecurity measures in poultry production in the under developed

countries of Asia and Africa (FAO, 2008). In Nigeria, majority of poultry are rural on free range and live poultry are sold in unhygienic, non structured environment which make institution of biosecurity difficult (Pagani *et al*., 2008).

Environmental factors are strongly linked with the introduction and spread of most avian diseases. Free range duck farming and rice – paddy cultivation were the environmental factors that were found to be associated with risks of HPAI H5N1 outbreaks in Southeast Asia (Gilbert *et al*., 2008). In Nigeria and neighbouring West African countries, plant phenology and land-surface topography were the associated risk factors of HPAI H5N1 outbreaks (Williams *et al*., 2008), while in the Indian subcontinent; land use patterns, presence of wetlands, backyard poultry and poultry husbandry practices as well as the density of human populations were the risk factors associated with outbreaks (Adhikari *et al*., 2009).

7.3 Justification of the Study

The presence of wetlands, abundant wild birds, backyard poultry farms, LBMs and vast number of rural poultry on free range in Kogi State are risk factors that may enhance the spread of AI, ND and other avian diseases. Hence, it is important to assess and identify the risk factors that influence the dynamics of interactions between wild birds and domestic poultry which may in turn lead to the introduction and spread of AI, ND and other important poultry diseases so as to be able to institute preventive and control measures.

The outbreaks of most avian diseases even in the face of vaccinations have led to a search for risk factors for the occurrence of such diseases as alternate way of prevention. The

knowledge of such risk factors will help in the design and institution of appropriate biosecurity at the national, community and poultry farm levels to potentiate existent prevention by vaccination.

The LBM is a medium of interaction between different species of poultry and wild birds that are mixed together in the same cages or environment for sale. Most rural poultry and some backyard (semi commercial) poultry find their ways to the LBM where they are offered for sale and the LBM had been shown to be one of the major routes of disease introduction and spread (Webster, 2004; Pagani *et al*., 2008). In Kogi State, most people sourced birds for rearing from the LBMs, a practice which needs to be discouraged based on facts.

The knowledge of interaction pathways will assist in setting priorities for mitigating actions and in developing the necessary precautionary measures against future disease outbreaks in domestic poultry by dislodging the risk of domestic poultry infections through contact with wild birds.

7.4 Aim of the Study

The aim of the study was to assess poultry - wild bird interactions, biosecurity and husbandry practices in poultry production in Kogi State, Nigeria.

7.5 Objectives of the Study

The objectives of the study were to:

1. Assess the existence of poultry – wild bird interactions in Kogi State.
2. Assess the biosecurity and husbandry practices which favor poultry - wild bird interactions that may lead to the spread of AI and ND in Kogi State.

7.6 Research Questions

1. Are there poultry – wild bird interactions in Kogi State?
2. Are there biosecurity risks and husbandry practices that favor poultry – wild bird interaction which may lead to spread of AI and ND in Kogi State?

7.7 Materials and Methods

7.7.1 Study area

The study area is as described in section 3.7.2

7.7.2 Sample size and sampling method

Using Snedecor and Cochran (1989) method, the alpha level was set at 5% and 50% (0.5) was used to estimate the population proportion. A total of 110 respondents comprising of 72 rural and backyard poultry farmers as well as 36 poultry sellers were selected at random from a list of 140 registered poultry farms and 65 registered LBMs that was obtained from avian influenza control project (AICP) Desk office in Kogi State and used for the study.

7.7.3 Administration of questionnaire

Structured questionnaire was designed, pre-tested on 10 respondents and modified to cover 37 variables and risk questions (Appendix I). The period of questionnaire trials was used to seek consent permission of most respondents with the help of the National Animal Disease Information System (NADIS) agents in each of the LGAs. A total of 108 questionnaire were then administered during the survey by interview to backyard poultry farmers, live bird marketers and rural poultry farmers in each of the 12 LGAs surveyed to assess the species of poultry being kept, the level of biosecurity and husbandry practices,

spillage of feed during feeding of poultry, presence of water body or fish pond on the farm or household, presence of wetland or river in the area, wild birds seen around farms and their local names as well as intervention for disease prevention and control.

The presence of possible factors that encourage mutual interactions between wild birds and poultry were noted based on the observations of the researcher to validate the answers given by respondents. Observation of poultry – wild bird interactions was done around rural households, poultry farms, wetlands and within LBMs for evaluation of the responses of respondents by the researcher.

7.7.3.1 *Assessment of wild bird - poultry interactions*

The assessment of wild birds-poultry interactions were based on the absence of adequate housing or poultry on free range, presence of water body or fish pond on the farm, spillage of feed during feeding, presence of tall grasses or trees for roosting of wild birds, disposal of poultry litter and dead poultry in refuse dumps, area as transit route for live bird trade as well as presence of major rivers or wetlands in the study area. These factors that encourage wild bird-poultry interactions are grouped into direct or indirect factors and scored according to the method of David and Bruce (2008). Direct factors were scored 16 (very high risk of interaction) while indirect factors were scored 4 (low to high risk of interaction) based on their levels of influence on contact of poultry and wild birds. The number of respondents that answered yes to the presence of either of the direct or indireect factors was multiplied by the score for each factor to get the weighted score for the LGAs or the sampling units. In addition, names (English and Native) of wild birds commonly seen around poultry farms and households were collated to draw out the abundant and contact bridge species between poultry and other wild birds.

7.7.3.2 *Assessment of biosecurity and husbandry practices that may favor the spread of avian influenza and Newcastle disease*

The risk factors that may enhance the spread of AI, ND and other avian pathogens were assessed based on failures in the level of biosecurity. Biosecurity measures were assessed through the presence of fence on the poultry farm, LBMs and rural households or any means by which human and vehicular movement could be restricted; presence of footbath on the farm or disinfection of the LBMs, proper hand wash and disinfection after handling poultry; disposal of poultry litter and dead poultry as well as ways by which sick poultry were handled by the farmers and poultry sellers.

The LGAs surveyed were grouped in 3 agric zones designated as zones A, B and C (KGSADP, 2009). Zones A and B constitute areas of high commercial poultry activities including the State capital situated within the central and western flanks (West and Central senatorial districts) of Kogi State which include Adavi, Ajaokuta, Ijumu, Kabba/Bunu, Lokoja, Mopamuro, Ogori-Mangogo and Okene LGAs. Zone C constitute areas with mostly rural poultry and few backyard poultry farms situated within the eastern flank (East senatorial district) of Kogi State which include Ankpa, Dekina, Ofu and Olamaboro LGAs. Zones A and B had similar production characteristics which were compared with that of zone C in terms of biosecurity measures and the odd ratios of risk factors calculated, risk factors with $p \leq 0.05$ was taken as significant relative to the zone concerned.

Similarly the sampling units surveyed were rural household poultry, backyard poultry farms and LBMs. Rural households and backyard poultry have almost similar

management characteristics which were compared with the slight different features of LBMs.

7.7.4 Data analyses

The data generated from the questionnaire were checked for completeness, entered in Excel format (Microsoft ^{R} Excel 2003) after validation and the errors in data entry were corrected. The data were then recruited into SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA) and analyzed based on LGAs and sampling units by descriptive statistics and reduced into tables.

7.8 Results

7.8.1 Results of assessment of wild bird – poultry interactions

A total of nine direct and indirect factors that pointed towards possible interactions between wild birds and poultry were considered. Strength of possible interactions was rated as low to high and very high. The LGAs with possible interactive factors of a total sum of up to 400 and above were rated as very high, areas with factors of a total sum less than 400 were rated as high while areas with sum of factors less than 200 were rated as low (Tables 7.1). Also, sampling units with the sum of weighted factors up to 2,000 and above were rated as very high, those with the sum of weighted factors less than 2,000 were rated as low to high possibility of interactions (Table 7.2). Of the factors considered, scavenging by wild birds on poultry farms/households had the highest score of 1,344 followed by presence of rivers/wetlands with 1,296; poultry on free range with 1,136 and spillage of feed/broadcasting of grains with 878; disposal of litter or carcass in refuse dump with 368; area as transit route for live bird trade and presence of trees for roosting of wild birds each with 288; area bordered by AIVs infected State with 216;

Table 7.1: Scoring of possible risk factors given by respondents that are indicative of likely wild bird-poultry interactions in the surveyed Local Government Areas of Kogi State.

Local Government Area	Scavenging by wild birds on farm/household (Score = 16)	Poultry on free range (Score = 16)	Spillage of feed/broadcasting of grains (Score = 16)	Fish pond on farm/household (Score = 16)	Presence of rivers/wetland (Score = 16)	Disposal of litter/carcass in refuse dump (Score = 4)	Transit route for live bird trade (Score = 4)	Border to AIVs infected State (Score= 4)	Trees for roosting of wild birds (Score = 4)	Total score
Adavi	6 (16)= 96	6(16)= 96	5(16)= 80	1(16) = 16	0 (16)= 0	9 (4)= 36	9(4)= 36	0(4)= 0	7(4)= 28	388
Ajaokuta	9(16)= 144	6(16)= 96	4(16)= 64	1(16) = 16	9(16)= 144	9(4)= 36	9(4)= 36	0(4)= 0	9(4) 36	572*
Ankpa	5 (16)= 80	6(16)= 96	7(16)= 112	0(16) = 0	9(16)= 144	9(4)= 36	9(4)= 36	9(4)= 36	5(4)= 20	560*
Dekina	6 (16)= 96	6(16)= 96	8 (16)= 128	1(16) = 16	9(16)= 144	9(4)= 36	2(4) = 8	9(4)= 36	3(4)= 12	572*
Ijumu	6 (16)= 96	6(16)= 96	3(16) = 48	1(16) = 16	0(16)= 0	8(4)= 32	0(4) = 0	9(4)= 36	6(4)= 24	348
Kabba/Bunu	6(16)= 96	6(16)= 96	3(16)= 46	1(16) = 16	0(16)= 0	8(4)=32	0(4) = 0	0(4)= 0	6(4 = 24	312
Lokoja	9(16)= 144	6(16)= 96	5(16)= 80	1(16)=16	9(16)=144	6(4)=24	9(4)=36	0(4)= 0	5(4)= 20	560*
Mopamuro	4(16)= 64	6(16)= 96	4(16)=64	2(16)=32	0(16)=0	7(4)= 28	0(4) = 0	0(4)= 0	4(4 = 16	300
Ofu	9(16)=144	6(16)= 96	6(16) = 96	0 (16) = 0	9 (16)= 144	6(4)= 24	8(4)= 32	0(4)= 0	9(4)= 36	572*
Ogori/Mangogo	6(16)= 96	7(16)=112	2(16) = 32	0(16) = 0	0(16) = 0	7(4)= 28	8(4)= 32	9(4)= 36	6(4)= 24	360
Okene	9(16)= 144	5(16)= 80	4(16)= 64	1(16)= 16	0(16)= 0	7(4)= 28	9(4)= 36	9(4)= 36	3(4)= 12	416*
Olamaboro	9(16)= 144	5(16)= 80	4(16)= 64	0(16) = 0	9 (16) = 144	7(4)=28	9(4)= 36	9(4)= 36	9(4)= 36	568*
Overall	1,344	1,136	878	144	1,296	368	288	216	288	5,528

Scores: 400 and above = Very high risk of interactions (*)
Below 400 = Low to high risk of interactions

Table 7.2: Scoring of possible risk factors given by respondents that are indicative of likely wild birds-poultry interactions in the surveyed sampling units of Kogi State

Sampling unit	Scavenging by wild birds on farm (Score = 16)	Poultry on free range (Score = 16)	Spillage of feed/broadcasting of grains (Score = 16)	Fish pond on farm (Score = 16)	Presence of river/ wetland (Score = 16)	Trees/roosting of wild birds (Score = 4)	Border to AIVs infected State (Score = 4)	Disposal of litter/carcass in refuse dumps (Score = 4)	Transit for live bird trade (Score = 4)	Total score
Backyard	35(16)= 560	0 (16)= 0	22(16)=352	8(16)=128	18(16)=288	28 (4)=112	18 (4)=72	20 (4)=80	26 (4)=104	1,696
Live bird market	15 (16)=240	36(16)=576	32(16)=512	0 (16)= 0	18 (16)=288	9 (4)=36	18(4)=72	36 (4)=144	27(4)=108	1,976
Rural household	34 (16)=544	36(16)=576	36(16)=576	1 (16)=16	18 (16)=288	32 (4)=128	18 (4)=72	36 (4)=144	27(4)=108	2,452*
Overall	1,344	1,152	1,440	144	864	276	216	368	320	6,124

Scores: 2,000 and above = Very high risk of interactions (*)
Below 2,000 = Low to high risk of interactions

while the presence of fish pond on the farm/household had the lowest weighted score of 144 (Tables 7.1 and 7.2). The strength of likely possible interactions between wild birds and poultry was very high for Ajaokuta, Ankpa, Dekina, Lokoja, Okene and Olamaboro LGAs, while the rest LGAs had high strength of interactions (Table 7.1). Based on sampling unit, the strength of possible interactions between wild birds and poultry was very high in rural poultry while it was high for both LBMs and backyard poultry (Table 7.2). During the study, it was observed that most backyard poultry farmers sourced day-old chicks and rearing stock from the South-west or from the LBMs (Plate VI). The movement of rural poultry using motor bike, bicycles and commuter buses across LBMs within the State and across the State was also seen. Rivers and wetlands around human dwellings being used for rice farming with the presence of scaenging poultry and wild birds were also seen (Plate VII). The respondents gave the native names of some wild birds such as swallow, swifts, laughing dove, pigeons, black kite, warbler and fly catcher that are commonly found in their areas that were seen around households, backyard poultry farms and wetlands scavenging for food with poultry (Plate VIII and Table 7.3). Rural poultry were seen scavenging around refuse dumps and interacting with various domestic animals including pigs (Plate IX). These observations of likely interactive pathways of wild birds and poultry have been mapped as shown (Figure 7.1).

Table 7.3: Wild birds frequently seen around households, poultry farms and wetlands with their native names in Igala, Ebira and Okun, the three major Languages spoken by the people of Kogi State, Nigeria.

Common name	Native name (*Igala)*	Native name (*Ebira)*	Native name (*Okun)*
Black stork	*Ẹwẹ-omi*	*Inomi – enyi*	*Ẹyẹ-omi*
Vulture	*Ugwunu*	*Uba*	*Egunyẹyẹ*
Swallow	*Akpadede*	*Epandede*	*Apandede*
Swift	*Aja*	*Irepe*	*Asa*
Dove	*Ikede*	*Arivadi*	*Arubadi*
Cattle egret	*Ichakolo*	*Ane*	*Amioro*
Buzard	*Uji-omaga*	*Obono*	*Gangan*
Pigeon	*Oketebe*	*Arekuku*	*Ẹyẹ le*
Kite	*Ugbono*	*Irepe*	No equivalent
Hawk	*Ukokolo*	*Ukokoro*	No equivalent
Owl	*Ogugu-nokwu*	*Igugu*	No equivalent
Cuckoo	*Obututu*	No equivalent	No equivalent

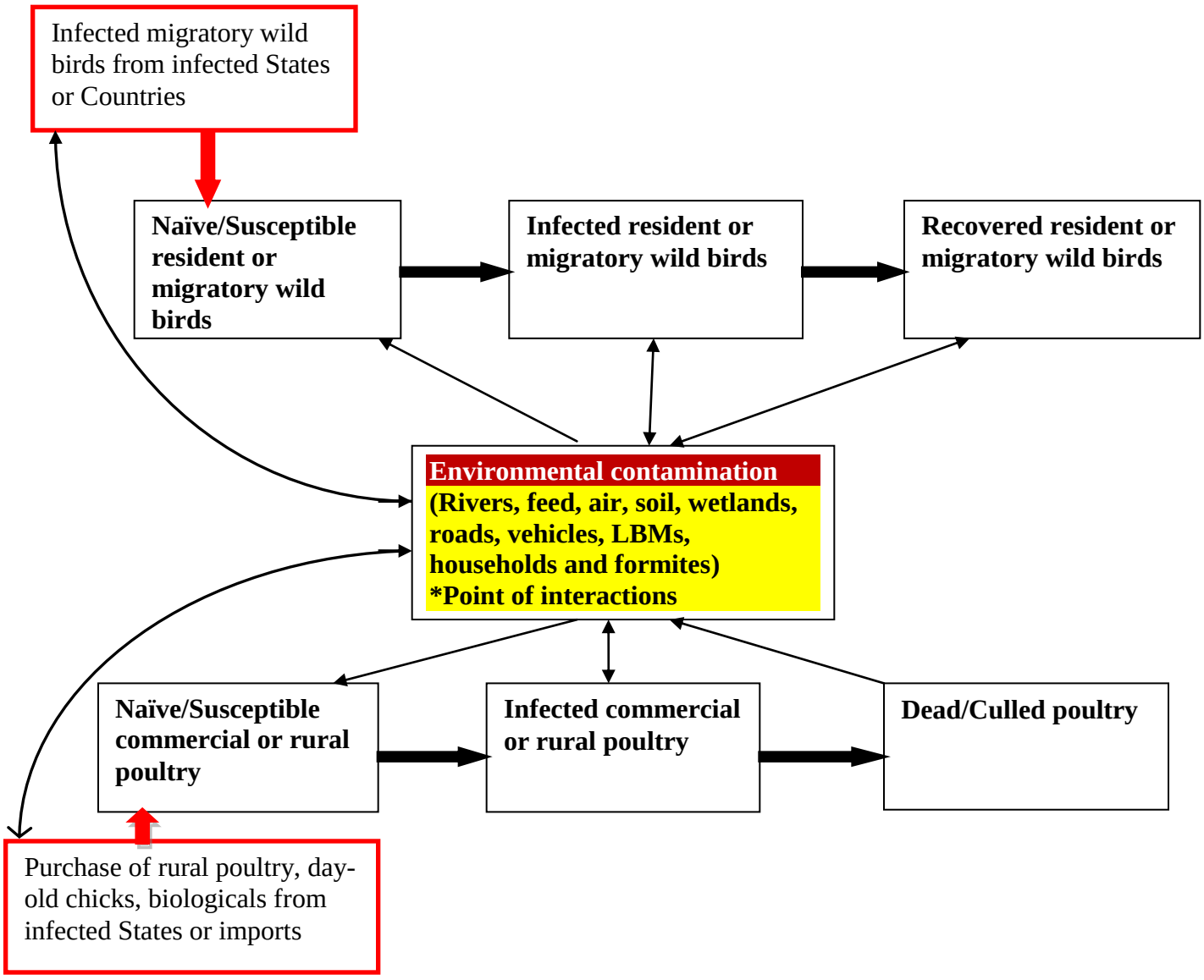


Figure 7.1: Wild bird – poultry interaction pathways and infection cycle based on favorable risk factors in Kogi State. Red solid arrows indicate introduction of HPAI viruses, black solid arrows indicate spread and progression of infections, single headed black arrows indicate direction of infections while double headed arrows indicate cross infections between the chains.



Plate VI: Backyard poultry in cages with wild birds (arrows) scavenging on feed spillage (**A**) around backyard poultry farm. The arrival of day-old chicks from hatcheries in Ibadan (south-west Nigeria) just before collection by farmers (**B)**



Plate VII: River Niger in Lokoja (**A)** where rural poultry and wild birds often mix together at the bank in search of food and water. A vast area of wetland in Ajaokuta LGA cultivated year round with rice and often visited by rural poultry and wild birds (**B**).



Plate VIII: Wild birds (pigeons and doves) roosting on rooftop (**A)** and scavenging around household where rural and backyard poultry are kept (**B**).



Plate IX: Rural poultry on free range scavenging in the same area with piglets (arrow) (**A**). Rural poultry scavenging around a refuse dump (arrow) where poultry litters are discarded (**B**)



Plate X: The nature of live bird markets in Kogi State with poultry kept in baskets, iron and wooden cages (A and B) adjoining other commodities in the market with no fence for movement restriction. Transportation of poultry inside locally made baskets to live bird markets using commuter vehicle and motocycle (C and D).

7.8.2 Results of assessment of biosecurity and husbandry practices

Biosecurity was generally poor as 80 (74.1%) of the respondents had no fence or any means of movement restriction to their poultry facilities. On the basis of LGAs, all the 9 (100%) poultry farmers and sellers from Ofu LGA did not have any means for movement restriction; for Olamaboro and Ijumu LGAs it was 8 (88.9%); for Ajaokuta and Kabba/Bunu LGAs it was 7 (77.8%); for Ankpa, Dekina, Lokoja, Mopamuro, Ogori/Mangogo and Okene LGAs it was 6 (66.7%) while it was 5 (55.6%) for Adavi LGA respectively (Figure 7.2). On the basis of sampling unit, 9 (25.0%) of the backyard poultry farms did not have any means for the restriction of movement, 36 (100 %) of the LBMs did not have any means of movement restriction, 35 (97.2%) of the rural poultry households did not have any means of movement restriction (Table 7.4).

Also, 95 (88.0%) of the respondents had no footbath on their poultry farms or did not undertake disinfection of their poultry facilities. On the basis of LGAs, all the 9 (100%) respondents from Ofu, Okene and Olamaboro LGAs did not have footbath or carry out disinfection of hands and poultry facilities; it was 8 (88.9%) for Adavi, Ajaokuta, Ankpa, Dekina, Ijumu Kabba/Bunu and Lokoja LGAs while it was 6 (66.7%) of the respondents for Mopamuro and Ogori-Mangogo LGAs respectively (Figure 7.2). Based on sampling units, 23 (63.9%) of the backyard poultry farms did not have footbath, 36 (100%) of the LBMs did not undertake disinfection of the markets, cages and hand washing after handling birds as well as 36 (100%) of the rural poultry households did not undertake proper cleaning and disinfection of their households (Table 7.4).

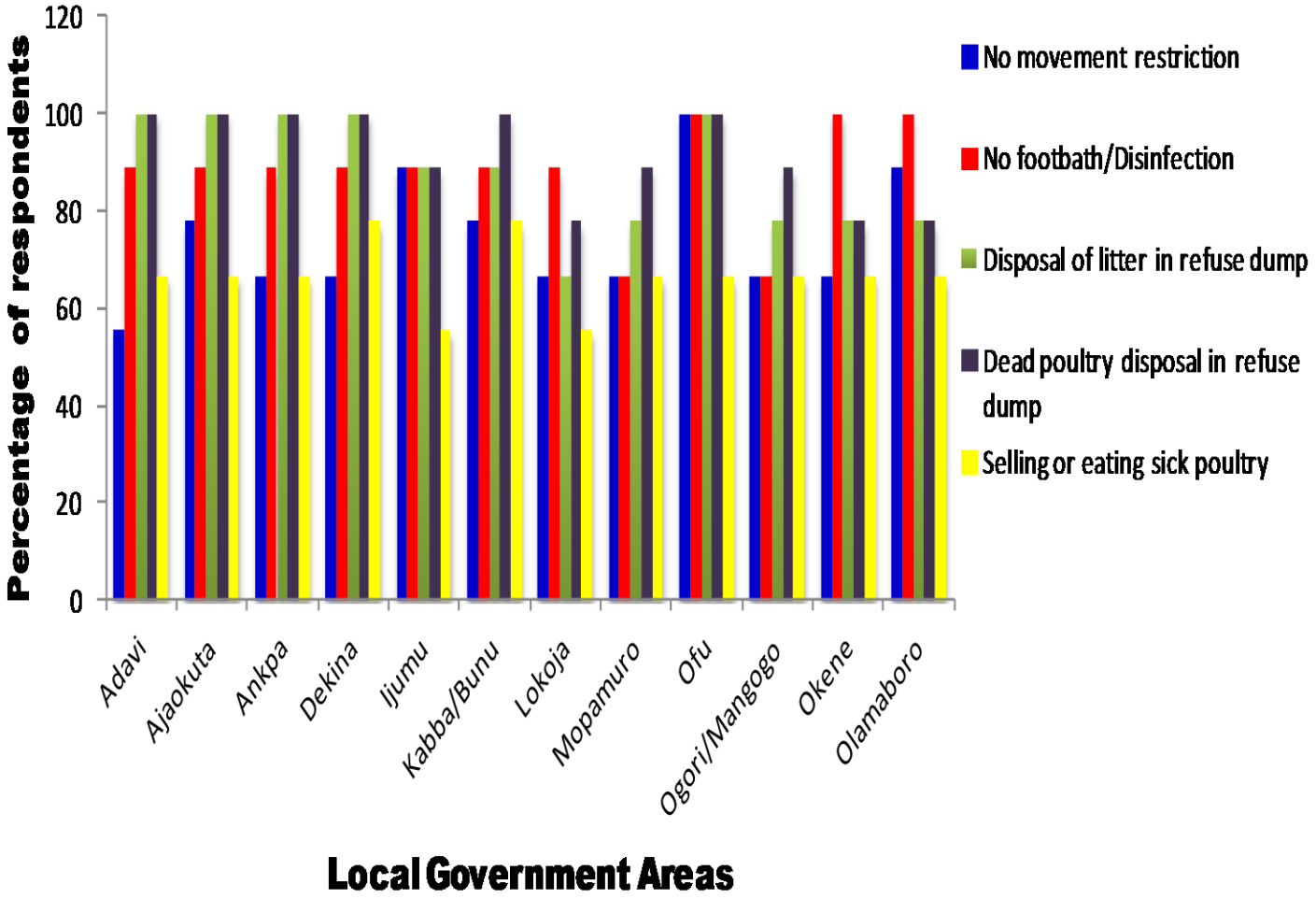


Figure 7.2: Assessment of risk factors that enhance the spread of avian influenza, Newcastle disease and other avian pathogens in the surveyed Local Government Areas of Kogi State.

Table 7.4: Assessment of risk factors that enhance the spread of avian influenza, Newcastle disease and other avian pathogens in the surveyed poultry sampling units in Kogi State, Nigeria.

Variable factor	Backyard farm (%)	Rural household (%)	Live bird market (%)	Total (%)
No movement restriction	9 (25.0)	35 (97.2)	36 (100.0)	80 (74.1)
No footbath/Disinfection	23 (63.9)	36 (100.0)	36 (100.0)	95 (88.0)
Disposal of poultry litter in refuse dump	20 (55.6)	36 (100.0)	36 (100.0)	92 (85.2)
Disposal of dead poultry in refuse dump	27 (75.0)	36 (100.0)	36 (100.0)	99 (91.7)
Selling or eating sick poultry	0 (0.0)	33 (91.7)	36 (100.0)	69 (63.9)
Overall	79 (43.9)	176 (97.8)	180 (100.0)	435 (80.6)

On disposal of poultry litter, 92 (85.2%) of the poultry farmers and poultry sellers would dispose litter in refuse dump. Based on LGAs, all the 9 (100%) respondents in Adavi, Ajaokuta, Ankpa and Dekina LGAs disposed litter in refuse dump; for Ijumu and Kabba/Bunu LGAs it was 8 (88.9%); for Mopamuro, Ogori-Mangogo and Olamaboro LGAs it was 7 (77.8%) while it was 6 (66.7%) for Lokoja and Ofu LGAs respectively (Figure 7.2). On the basis of sampling unit, 20 (55.6%) of the backyard poultry farmers would dispose litter in refuse dump while 36 (100%) each of the poultry sellers and rural poultry farmers would dispose it in refuse dump respectively (Table 7.4).

Similarly, 99 (91.7%) of the respondents would dispose dead poultry in refuse dump. On the basis of LGAs, all the 9 (100%) respondents in Adavi, Ajaokuta, Ankpa, Dekina, Kabba/Bunu and Ofu LGAs would dispose dead poultry in refuse dump; 8 (88.9%) of the respondents in Ijumu, Mopamuro and Ogori/Mangogo LGAs would dispose dead poultry in refuse dump while 7 (77.8%) of the respondents in Lokoja, Okene and Olamaboro LGAs would dispose dead poultry in refuse dump respectively (Figure 7.2). Based on sampling units, 27 (75.0%) of the backyard poultry farmers would dispose dead poultry in refuse dump while 36 (100%) each of the poultry sellers and rural poultry farmers would dispose dead poultry in refuse dumps (Table 7.4).

However, on the handling of sick poultry, 69 (63.9%) of the respondents would sell out or eat sick poultry. Based on LGAs, 7 (77.8%) of the respondents from Dekina and Kabba/Bunu LGAs would eat or sell out sick poultry; 6 (66.7%) of the respondents from Adavi, Ajaokuta, Ankpa, Mopamuro, Ofu, Ogori-Mangogo, Okene and Olamaboro LGAs would eat or sell out sick poultry while 5 (55.6%) of the respondents from Ijumu and Lokoja LGAs would eat or sell out sick poultry (Figure 7.2). On the basis of sampling units, 36

(100%) of the poultry sellers would sell out sick poultry, 33 (91.7%) of the rural poultry farmers would eat or sell out sick poultry while none of the backyard poultry farmers would eat or sell sick poultry (Table 7.4).

The risk factors that may enhance the spread of AI and ND were compared through univariate analysis across three agric zones grouped as A+B vs C. The odds of occurrence of the risk factors in the agro-zones, A+B were more than that of zone C but were not significant except for the presence of rivers or wetlands (Table 7.5).

Moreover on production and management systems, 32.4% of poultry were kept under intensive management while 67.6% of poultry kept under extensive management system were both rural and commercial poultry (Table 7.6). Also, various species of poultry were seen in rural rural households and LBMs but in backyard farms, only ckickens of different types were seen (Figure 7.3).

Table 7.5: Univariate analysis of risk factors for possible poultry – wild bird interactions, spread of avian influenza, Newcastle disease and other avian pathogens in Kogi State, Nigeria

Variable factor	A + B n = 72 (%)	C n = 36 (%)	OR (95% CI)	P value
No movement restriction	51 (70.8)	29 (72.2)	0.586 (0.222-1.546)	0.276
No footbath and disinfection of premises/ cages	61 (84.7)	34 (94.4)	0.532 (0.071-1.560)	0.143
Disposal of litter in refuse dump	61 (84.7)	31 (86.1)	1.123 (0.362-3.501)	0.848
Disposal of dead poultry in refuse dump	67 (93.1)	34 (94.4)	1.274 (0.233-6.882)	0.782
Eating or sales of sick poultry	51 (70.8)	30 (83.3)	2.082 (0.754-5.692)	0.157
Scavenging of wild birds on farms	55 (76.4)	29 (80.6)	1.281 (0.477-3.441)	0.623
Poultry on free range	48 (66.7)	24 (66.7)	1.00 (0.428-2.337)	1.00
Wild bird roosting on trees around farms & LBMs	46 (63.9)	26 (72.2)	1.470 (0.613-3.520)	0.386
Spillage of feed on farms & LBMs	24 (33.3)	10 (27.8)	0.769 (0.320-1.852)	0.558
Water body or open fish pond	8 (11.1)	1 (2.8)	0.229 (0.027-1.903)	0.140
Presence of rivers or wetlands	18 (25.0)	30 (83.3)	15.00 (5.376-41.852)	0.002*

OR = Odd ratio p-value = Measure of statistical significant level.

Table 7.6: Production and management systems used by poultry farmers in Kogi State, Nigeria.

	Production system		
Sampling unit	Extensive (%)	Intensive (%)	Total (%)
Backyard farm	1 (0.9)	35 (32.4)	36 (33.3)
Live bird market	36 (33.3)	-	36 (33.3)
Rural household	36 (33.3)	-	36 (33.3)
Total	73 (67.6)	35 (32.4)	108 (100)

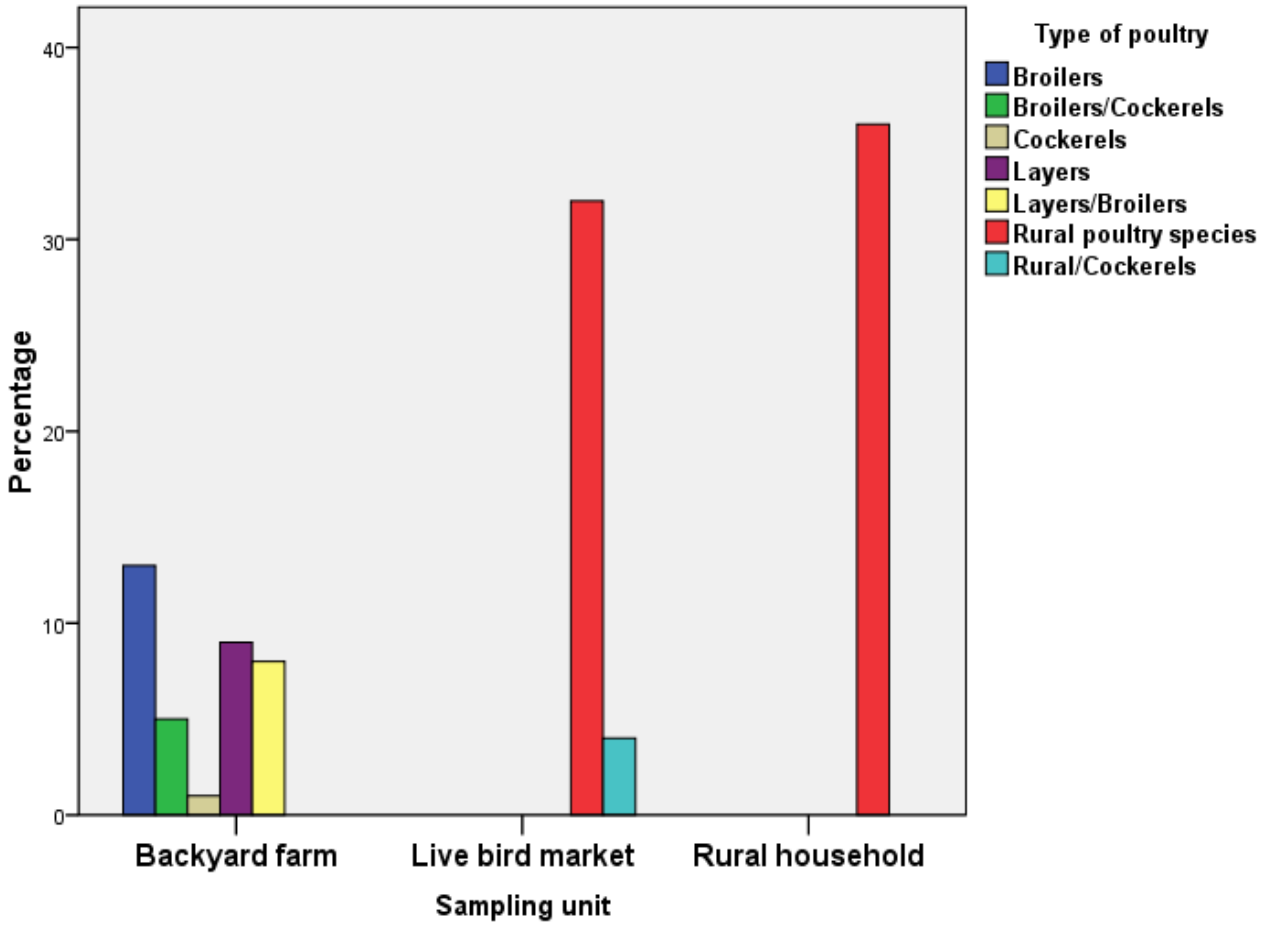


Figure 7.3: Types of poultry found in the various sampling units surveyed in Kogi State, Nigeria.

7.9 Discussion

Most of the LGAs surveyed, especially those with wetlands or major rivers were seen to have very high level of wild birds – poultry interactions. Si *et al*. (2013) reported that the distribution of human population and high poultry production in addition to close proximity to rivers or wetlands were useful factors that influenced interactions pathways between poultry and wild birds. The study area though a State within the hintherland, lies within the Niger – Benue confluence, lower Niger/Anambra river flood plain and lower Benue flood plain that are frequented by resident and migratory birds (Abdu, 2010). It was reported that the distribution of outbreaks of H5N1 HPAI in Europe, Asia and Turkey was associated with the presence of wetlands and lakes where migratory birds used to congregate and breed during over wintering (Kilpatrick *et al*., 2006).

Si *et al.* (2013) further explained that wetlands had high prevalence of AIVs and other avian pathogens transmission due to favorable biotic and abiotic conditions such as high density of naïve hosts, cool and wet environment that enhance pathogens survival for faeco-oral transmission. The HPAI (H5N1) virus is reported to survive in water or faeces for long periods at low temperatures with the viruses remaining infective in water for up to 207 days at 17 ˚C and up to 102 days at 28 ˚C as well as in liquid faeces for up to 35 days at 4 ˚C or 7 days at 20 ˚C (Stallknecht *et al.,* 1990). The indication of high level of scavenging by wild birds on poultry farms and poultry on free range as observed in this study as well as indiscriminate disposal of poultry litter or dead birds in refuse dump create a platform for direct contact, interaction and circuit sharing of AI, ND and other pathogens between wild birds and domestic poultry.

Poultry are important links between wild birds and humans in the occurrence of some zoonotic diseases especially AI as seen in the emergence of HPAI H5N1 from its precursor goose virus in southern China with it wider global spread (Si *et al.,* 2013). The traditional system of poultry husbandry as observed during the study favors close contact with humans hence, posing a threat to human health. This will be of much concern in the event of an outbreak of HPAI in Kogi State which may impact negatively on human health as well as prevention and control activities. The search for disease pathogens of poultry especially AI, has gone beyond wild birds that explore water habitats to land based wild birds and mammals. Vakuru *et al.* (2012) due to the reoccurrence of HPAI H5N1 outbreaks in 2008 after about 9 months of the absence of reported cases in Nigeria undertook a survey for AIVs in 8 States and identified possible potential bridge species of wild birds in which AIVs could have hidden before the resurgence.

The scavenging of wild birds on poultry farms, households and around LBMs coupled with the spillage of feed during feeding of poultry or drying of feed ingredients on poultry farms and households bring wild birds directly into close contact with poultry for possible interactions in competing for food, water and space. Rural poultry on free range usually act as a bridge between wild birds and backyard poultry, especially those kept on extensive management system. From observation and analysis of the answers by respondents, swallow, swift, laughing dove, black kite, warbler and fly catcher were indentified as the contact bridge species between poultry and wild birds. These species of wild birds are seed and insect eaters and were frequently seen in backyard farms and most households scavenging for food. Burns et al. (2012) had identified some of these species of wild birds as important bridges between domestic poultry and migratory water birds elsewhere.

The presence of open fish pond or water body on a poultry farm may also lead to both direct and indirect contact between poultry and wild birds. Wild birds most often suffer from water deprivation, some times traveling over long distance in search of this essential need of life and during migration, they disperse pathogens in faeces along their pathways, water courses and overwintering sites (Kilpatrick *et al*., 2006; Jourdain *et al.*, 2007).

Biosecurity is poor from the stand points of all the components considered relating to traffic control, isolation, cleaning and disinfection. Movement restriction is a means by which human and vehicular traffic control is enforced. In poultry facilities in backyard farms, LBMs and households there is need to restrict access to the entire farm and poultry rearing area using fence and farm gates in order to keep out infectious pathogens from the poultry flocks (USAID, 2009). The near absence of movement control in most of the farms, households and all the LBMs give access to people and wild birds for direct contact with poultry and is a pointer to the ease of unhindered introduction and spread of disease causing pathogens by humans, animals, vehicles and formites during outbreaks of AI, ND and other contagious poultry diseases in the study area.

The use of footbath, disinfection of hands, poultry farms and LBMs was almost nonexistent despite the high advocacy the practice received at the onset of HPAI out breaks in 2006 in Nigeria. The Federal Ministry of Agriculture in collaboration with USAID and other foreign donor agencies held several workshops aimed at training farmers, poultry sellers and other stakeholders involved in the poultry production chain on the importance of disinfection and as well made available disinfectants to live bird marketers and farmers at no cost to be able to practice biosecurity (Pagani *et al.*, 2008). The paucity in the use of disinfection by poultry farmers and poultry sellers was an indication that the people did not understand the value

and usefulness of the practice in keeping pathogens away from their flocks hence, were not ready to invest into the practice shortly after the government withdrew its incentive.

Disposal of poultry litter and carcasses relate to cleaning (containment or exclusion) component of biosecurity and were greatly flawed. A greater number of poultry farmers and poultry sellers disposed their litter in the refuse dump which might lead to lateral spread of AI, ND and other infections to other in-contact poultry on free range and wild birds thereby resulting in perpetuation of infection cycles. In some cases, the poultry litter is used as crop manure without composting and might pose a danger to human health for the crop farmers especially during HPAI outbreaks. The practice of feeding dead poultry to fish carries the same risk of perpetuating infection cycles especially for AIVs that are known to survive in water and later become an infective source to free range poultry and wild birds that may drink from the pond.

The handling of sick poultry by poultry farmers and poultry sellers relates to isolation (containment or exclusion) component of biosecurity which was also observed to be poor. A greater majority of the poultry farmers and poultry sellers would not do anything if their poultry were sick. This will lead to great losses and spread of AI, ND and other pathogens which may hamper productivity and disease reporting. The study did not probe further to know if the inaction of most of the stakeholders to sick poultry was due to the prohibitive cost of disease intervention measures or for any other reason. Some of the poultry farmers and poultry sellers would eat sick birds or sell out sick birds which are risky practices and pose danger to public health. Continuous holding of sick poultry for sale in LBMs may lead to the spread of disease agents especially, AI and ND to other apparently healthy poultry

held together in the same cage or space with a rebounding lateral spread of infections to household rural poultry when birds are bought and carried home.

The study revealed rural poultry production to be highest which confirmed the fact that a vast majority of poultry production in Nigeria are rural poultry (Abdu *et al*., 2002; Adene and Oguntade, 2006; Ameji, 2010). This has a major drawback in the institution and application of improved farming practices as well as biosecurity due to smaller flock size and high cost of investing into such management practices. The management system also shows that more chickens including backyard and rural poultry are kept under free range management while a low percentage of chickens are under intensive management similar to earlier reports (Sonaiya, 1990; Adene and Oguntade, 2006; Ameji *et al*., 2012). Birds kept under free range are a threat to themselves and other susceptible commercial poultry as sources of AI, ND and other infectious diseases they may contract from their free association with wild birds during competition for shelter, feed and water. It is well known that poultry under extensive management do not receive adequate housing, healthcare and nutrition (Sonaiya, 1990; Adene and Oguntade, 2006). This has major drawbacks on rural poultry productivity and economic returns.

The odd ratios of biosecurity risks as calculated between the contiguous zones were not significant, an indication that there was no difference in the occurrence of the interactive and risk factors for the spread of AI, ND and other avian pathogens between these zones. However, the presence of wetlands or major rivers was significant in causing the occurrence of wild bird-poultry interaction and the spread of AI, ND and other avian pathogens in zone C than in zones A + B combined. This shows that wild bird interactions with poultry occur

more in zone C and are likely to play more role in the spread of avian pathogens in zone C than the other zones.

It was concluded that there is existence of poultry-wild bird interactions in the study area due to the presence of wetlands; major rivers; spillage of feed during feeding of poultry; rural and some backyard poultry on free range; observation of birds roosting on trees around farms, households and LBMs. Biosecurity and husbandry practices were poor which may limit poultry production in Kogi State.

CHAPTER EIGHT

CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

From this study, it was concluded that:

1. There was evidence of AI antibodies in poultry with the seroprevalence of AI (H5) by HI being 4.2% while the seroprevalence of AI (NP) by ELISA was 30.8%.
2. There was evidence of AI antibodies in wild birds in Kogi State with the seroprevalence of AI (NP) by ELISA being 4.5% but the seroprevalence of AI (H5) by HI test was zero. This the first report of AI (NP) antibodies in wild birds in Kogi State.
3. Black stork (*Ciconia nigra*) from Lokoja LGA was the only species of wild birds that had AI (NP) antibodies.
4. There was evidence of ND antibodies in poultry from backyard farms, rural households and LBMs with an overall seroprevalence of ND by HI test being 75.9% while using ELISA it was 68.1%.
5. There was evidence of ND antibodies in wild birds in Kogi State with a seroprevalence of ND by HI test being 29.1% while using ELISA, it was 27.1%.
6. Fourteen (29.2%) free flying wild birds from six (28.6%) LGAs out of the 21 LGAs of Kogi State had detectable levels of ND antibodies in their sera by HI test.
7. Four species of free flying wild birds, black kite, swallow, swift and laughing dove were the only species out of the 50 species of free flying wild birds surveyed in Kogi State that had ND antibodies. This is the first report of ND antibodies in wild birds in Kogi State.

8. There were no AIVs in Kogi State as the viruses were not detected in poultry and wild birds by RT-PCR.
9. There was circulation of NDVs in Kogi State as the viruses were detected in poultry and wild birds by RT-PCR in poultry from all the LGAs surveyed and in wild birds from 4 LGAs in Kogi State with overall prevalence of 60.5%.
10. The highest detection of NDVs in poultry from the sampling units was from LBMs with 62.5%.
11. The detection of NDVs in wild birds surveyed in Kogi State was only in swallow and swift species of wild birds with a prevalence of 60.0%. This is the first report of NDVs in these species of wild birds in Nigeria.
12. Among the surveyed LGAs, Dekina, Ofu, Ankpa and Olamaboro LGAs which are contiguous areas have higher prevalence of NDVs over other LGAs in Kogi State.
13. There is very high likelihood of wild birds – poultry interactions in 6 LGAs and high likelihood in the other 6 LGAs surveyed.
14. Biosecurity was poor with much risk factors that may enhance the spread of AI and ND including:
    - The lack of movement restrictions in all LBMs as well as by most of the backyard and rural poultry farmers.
    - There was absence of footbath and disinfection of farms, households and LBMs by most of the backyard poultry farmers, rural poultry farmers and fowl sellers.
    - Most of the poultry farmers and fowl sellers disposed litter and dead poultry in refuse dump as well as most of them would sell or eat sick poultry.

8.2 Recommendations

The following recommendations were made based on the findings of the study:

1. Continuous surveillance for AI and ND in other species of wild birds should be carried out to know the true status of the diseases as well as further studies by research institutes to isolate and characterize AIVs and NDVs circulating in poultry and wild birds in Kogi State.
2. Black stork should be screened from commercial poultry as they are likely carriers of AIVs.
3. Poultry farmers and fowl sellers should be educated on the danger of eating or selling sick poultry as well as keeping different species of poultry in the same compartment to prevent the spread of AI and ND.
4. Farmers should screen black kite, swallow, swifts and laughing dove from commercial poultry.
5. Vaccination against ND should be stepped up in backyard poultry farms as well as instituted in rural poultry.
6. Biosecurity should be instituted and encouraged in backyard poultry farms, rural households and LBMs by undertaking movement restriction, sanitation and avoiding the disposal of poultry litter and dead poultry in refuse dumps.
7. Henceforth, health and management practices should focus on rural poultry improvement to increase their productivity as well as curbing their negative influence on commercial poultry farming.

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APPENDICES

Appendix I: Sample of the structured questionnaire used for observation and to collect data from poultry farmers and poultry sellers in the surveyed Local Government Areas of Kogi State.

I. Farm/LBM/Wetland Identification

LGA---------- Date----------

Town/Village---------- Lat.---------- Long.----------

1. Name of flock owner/marketer----------

2. Address of farm/market/household/wetland----------

II. Demographic characteristic of backyard poultry farm/LBM/rural household poultry

3. Spp/No.: Chickens ( ) Turkeys ( ) Geese ( ) Ducks ( ) G/fowls ( ) Pigeons ( ) Quails ( ) Ostriches ( ) Wild birds ( ) Others----------

4. Types of birds: Breeders ( ) Layers ( ) Broilers ( ) Cockerels ( ) Local ( ) others ( )

5. Production system: Commercial ( ) Backyard ( ) Rural ( ) LBM ( ) Wild range ( ) Mixed farming ( )

6. Housing: Battery cage ( ) Deep litter ( ) Free range ( ) others ( )

III. Disease and Management History

7. Diseases seen often on the farm/household/market: ND ( ) IBD ( ) Cox ( ) Fowl typhoid ( ) Colibacillosis ( ) Fowl pox ( ) Others_____

8. Diseases vaccinated against: ND ( ) IBD ( ) Fowl pox ( ) AI ( ) Marek's ( ) Cox ( ) None ( ) Others_____

9. How far is the poultry from residential area: <500m ( ) <1km ( ) <3km ( ) ≥3km ( ) Comment [ ]

10. Where is the source of your feed: Commercial ( ) Toll mill ( ) Self mill ( ) Comment [ ]

11. If toll or self milling, do you dry ingredients on the farm/household/market: Yes ( ) No ( ) Comment [ ]

12. How do you dispose poultry litter: Thrown in refuse dump ( ) Used as manure ( ) Buried ( ) Burnt ( ) Comment [ ]

13. How do you handle sick poultry: Isolate/call a Vet ( ) No treatment ( ) Self medicate ( ) Sold out ( ) Eaten ( )

14. How do you handle dead poultry: Thrown in refuse dump ( ) Eaten ( ) Buried ( ) Burnt ( )

15. Give the local name of the wild birds seen around your area_____English name_____

IV. Risk factors that encourage poultry – wild bird interactions or spread of avian pathogens

S/No	Risk question	Yes	No	Researcher's observation	
				Yes	No
16	Are there any water body or fish pond on the farm/household/LBM				
17	If yes, do you make use of the water in poultry facility				
18	Do the same poultry attendants attend to fish				
19	Is the fish pond drained into large water body or river				
20	Is the farm/household/market fenced				
21	Can you restrict movement of visitors within poultry facilities/rearing area				
22	Does the farm/household/market share fence with another farm/household/market				

23	Does the farm/household/market have footbath or hand washing disinfection				
24	Are there tall trees and grasses around the farm/ household/market				
25	If yes, do wild birds roost on the trees				
26	Do attendants spill feed during feeding of poultry				
27	Do wild birds come around your farm/ household/market to scavenge feed				
28	Do you have a feed store on the farm/ household/market				
29	Do wild birds and rodents have access to the feed store				
30	Do wild birds or rodents have access to feed ingredients being dried				
31	Do your workers live on the farm/household/market				
32	Do your farm workers have birds in their houses				
33	Do your farm workers visit LBM to sell eggs or live poultry				
34	Do traders visit your farm/household to buy eggs or live poultry				
35	Is there a major river/wetland in your area?				
36	Is your area bordering another State where poultry production/diseases such as AI and ND are known?				
37	Is there a major travelers' route through your area where live birds are transported?				

Appendix II: Preparation of 20 ul Master Mix for Avian influenza viruses detection Polymerase Chain Reaction

Reagent	Concentration	1x (µl/sample)	71x (Total Mix, µl)
Nuclease Free H_2O		4.7	333.7
PCR Buffer	10x	5.0	355.0
$MgCl_2$	50 mM	2.5	177.5
dNTPs	10 mM	2.5	177.5
DTT	10 mM	2.5	177.5
M52C (Primer)	25 µM	0.75	53.3
M253R (Primer)	25 µM	0.75	53.3
Nuclease Inhibitor	5 U/µl	0.5	35.5
Reverse Transcriptase	10 U/µl	0.3	21.3
Ampli Taq	5 U/µl	0.5	35.5
RNA template		5.0	
Final Volume		25.0	1420.1

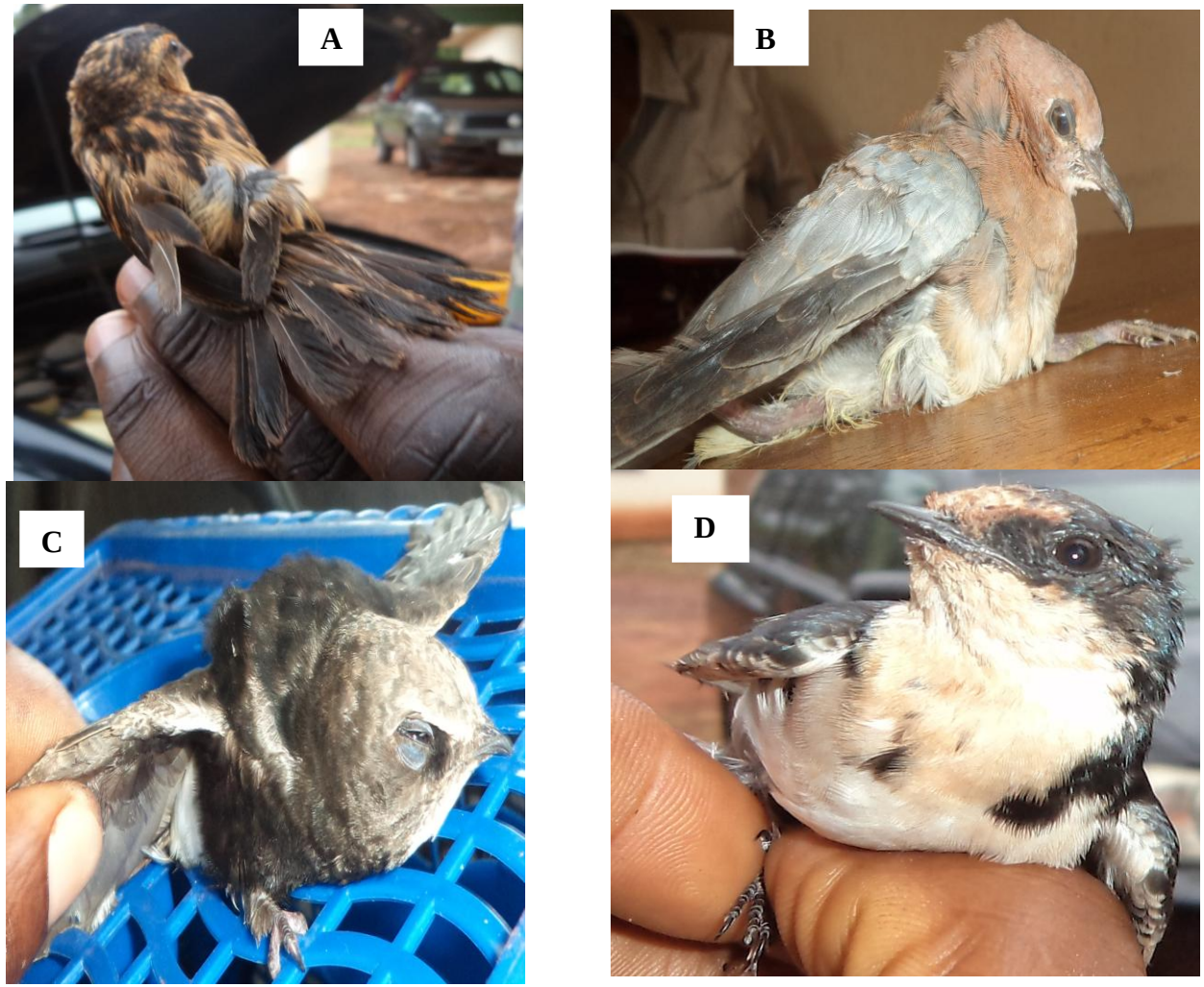
Source: Avian Influenza Regional Laboratory, NVRI, Vom, Plateau State, Nigeria.

Appendix III: Preparation of 20 ul Master Mix for Newcastle disease viruses detection Polymerase Chain Reaction

Reagent	Concentration	1x (µl/sample)	40x (Total Mix, µl)
Nuclease Free H_2O		11.5	460
PCR Buffer	5x	5.0	200
dNTPs	10 mM	0.5	20
APMV-1 (Forward)	10µM	1.0	40
APMV-1 (Reverse)	10 µM	1.0	40
Reverse transcriptase enzyme		0.5	20
Nuclease Inhibitor	15 U/µl	0.5	20
RNA template		5.0	200
Final Volume		25.0	1000

Source: Avian Influenza Regional Laboratory, NVRI, Vom, Plateau State, Nigeria.

Appendix IV: Photographs of some of the wird birds, A = melodious warbler, B = laughing dove, C = swallow, D = swift, E = black stork, F = fly catcher, G = black kite and H = laughing dove surveyed in Kogi State, Nigeria.




E


F


G


H