IMPROVING EFFECTIVITY OF NEWCASTLE DISEASE VACCINATION AND PRODUCTIVITY, IN INDIGENOUS CHICKEN, THROUGH EFFECTIVE PARASITE CONTROL

A thesis submitted in partial fulfillment of the requirements for Master of Science degree of University of Nairobi [(Applied Microbiology (Virology option)]

Kemboi, David Chebutia (BVM, UoN)

Department of Veterinary Pathology, Microbiology and Parasitology

2014

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Kemboi David Chebutia, BVM (UoN)

Signed ..... Date 14/04/2014

This thesis has been submitted for examination with our approval as University supervisors:

Prof. Lilly C. Bebora, BVM, MSc., PhD Signature ..... Date 15/4/2014

Department of Veterinary Pathology, Microbiology and Parasitology,

University of Nairobi

Prof. Philip N. Nyaga, BVM, MPVM, PhD Signature ..... Date 15/4/2014

Department of Veterinary Pathology, Microbiology and Parasitology,

University of Nairobi

Dr. Lucy W. Njagi, BVM, MSc., PhD Signature ..... Date 14/4/2014

Department of Veterinary Pathology, Microbiology and Parasitology,

University of Nairobi

Prof. Paul G. Mbuthia, BVM, MSc., FRVCS, PhD Signature ..... Date 15/4/2014

Department of Veterinary Pathology, Microbiology and Parasitology,

University of Nairobi

DEDICATION

This work is dedicated to my loving parents Isaac Kipkemboi Ruttoh and Susan Omutondo Ruttoh, my sisters Miriam Chepkoech, Joyceline Chemutai, Florence Cherop and Catherine Chepchirchir and my niece Caitlyn Cabrina Chepkemboi.

ACKNOWLEDGMENTS

I express my sincere gratitude and appreciation to my supervisors; Professor Lilly C. Bebora, Professor Philip N. Nyaga, Dr Lucy W. Njagi, Professor Paul G. Mbuthia and Professor N. Maingi for their invaluable support, inspiring guidance, encouragement and suggestion and their personal commitment during the entire duration of the study.

I wish to express my gratitude to RUFORUM for awarding me a scholarship through Prof. L.C. Bebora and others to pursue this master's degree programme and for funding the entire project.

I appreciate the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi for facilitation and use of laboratoy facilities. The members of staff: Ms. Mary Mutune, Mr. Julius Kibe, Mr. Richard Otieno, Mr. Ezekiel Weda, Mr. Patrick Wahome and Mr. Gordon Otieno for their moral and technical support. Thank you.

I would also like to appreciate Mrs Dorcas Nduati for her assistance in data analysis.

I am greatly indebted to my colleagues and wonderful friends Drs. Hannah Chegeh, Simon Maina, Willie Mwangi. Jared Serem, Jimmy Kuria, Charles Mundia and Isaac Omwenga for their constant technical assistance and moral support during the entire period of the study, God bless you.

Special thanks to my family for their prayers and continuous encouragement

Most of all, praise and thanks be to my Heavenly Father for His grace that this study was undertaken successfully.

Table of Contents

DECLARATION……ii

DEDICATION iiii

ACKNOWLEDGMENTS..... iv

TABLE ON CONTENTS v

LIST OF TABLES xii

LIST OF FIGURES..... xiii

LIST OF APPENDICES xiii

LIST OF ABBREVIATIONS xiv

ABSTRACT..... xvi

CHAPTER ONE: INTRODUCTION 19

1.1 Hypothesis..... 3

1.2 Objectives..... 4

1.2.1 Broad objective 4

1.2.2 Specific objectives..... 4

1.3 Justification 4

CHAPTER TWO: LITERATURE REVIEW 6

2.1 Poultry in Kenya..... 6

2.2 Benefits of poultry..... 6

2.3 Constraints to poultry production 7

2.4 Newcastle disease 7

2.4.1 Pathotypes of Newcastle disease virus 8

2.4.2 Maintenance of the virus in village situation 8

2.4.3 Clinical signs of Newcastle disease 9

2.4.4 Pathology due to Newcastle disease 10

2.4.5 Diagnosis of Newcastle disease 10

2.4.5.1 Isolation and identification of Newcastle disease virus 11

2.4.5.1.1 Direct detection of viral antigens 11

2.4.5.1.2 Virus isolation 11

2.4.5.1.3 Virus biological pathogenicity characterization 12

2.4.5.2 Serological tests for Newcastle disease virus antibodies 12

2.4.5.3 Molecular techniques in diagnosis of Newcastle disease 13

2.4.6 Control of Newcastle disease 13

2.4.6.1 Live vaccines 13

2.4.6.2 Inactivated vaccine 14

2.4.6.3 Thermostable vaccine 14

2.4.6.4 Vaccination programs 15

2.4.7 Immune response to Newcastle disease virus 16

2.5 Poultry parasites 16

2.5.1 Ectoparasites 16

2.5.2 Endoparasites excluding hemoparasites 17

2.5.3 Haemoparasites 18

2.5.4 Diagnosis of poultry parasites 18

2.5.5 Parasite treatment 19

2.6 Immunosuppression and parasites 20

2.7 Egg quality 20

CHAPTER THREE: MATERIALS AND METHODS 22

3.1 Study area 22

3.2 Study chicken 23

3.3 Study design 24

3.4 Collection of data on production parameters and quality of eggs produced in the area 25

3.5 Collection and processing of specimens, in wet and dry seasons 25

3.5.1 Serum samples for determination of antibody titres 25

3.5.2 Swabs and tissues for viral isolation 26

3.6 Hemagglutination inhibition test 26

3.6.1 Preparation of red blood cells for the test……..……..26

3.6.2 Source of viral antigen for the test……....26

3.6.3 Hemagglutination test for determination of working concentration 27

3.6.4 Hemagglutination inhibition (HI) test procedure……..27

3.7 Viral isolation from swabs and tissues 28

3.7.1 Swab processing for viral isolation……...28

3.7.2 Tissue processing for viral isolation..... 288

3.7.3 Viral isolation 28

3.8.1 Establishment of initial parasite burden and initial antibody titers 29

3.8.2 Grouping of remaining chicken per treatment 29

3.8.3 The various treatments administered and vaccination 31

3.8.4 Blood collection and testing 33

3.9 Examination and identification of parasites 33

3.9.1 Faecal collection and identification for helminth eggs and coccidia 33

3.9.2 Examination for presence of parasites on legs and skin of the study chicken before slaughter 34

3.9.3 Post-mortem examination for parasite isolation..... 34

3.9.4 Identification of parasites 35

3.10 Determination of the village chicken productivity..... 35

3.11 Data management 36

CHAPTER FOUR: RESULTS 37

4.1 Data on poultry production systems, farmer demography and quality of eggs produced in the area 37

4.1.1 Management of poultry 37

4.1.2 Constraints of village poultry production 38

4.1.3 Newcastle disease and its control 39

4.1.4 Management and disposal of sick and dead birds 40

4.1.5 Poultry productivity and marketing 41

4.1.6 Socio-cultural activities that depended on poultry 42

4.2 Egg quality 42

4.3 Seasonality of antibody titers against Newcastle disease 42

4.3.1 Seasonality of antibody titers to Newcastle disease during the wet season 42

4.3.2 Seasonality of antibody titers to Newcastle disease during the dry season 46

4.4 Isolation of Newcastle disease virus 47

4.5 Determination of effect of endo- and ecto-parasite control on the chicken's response to Newcastle Disease vaccination and production (weight gains or losses) 48

4.5.1 Pre-treatment examination results 48

4.5.2 Ectoparasites prevalance in treated groups 48

4.5.3 Endoparasites prevalance in treated groups 49

4.5.4 Hemagglutination inhibition test results 50

4.6 Weight gains after vaccination 53

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECCOMENDATIONS 54

5.1 DISCUSSION 54

5.2 CONCLUSIONS 63

5.3 RECCOMENDATIONS 64

REFERENCES 655

APPENDICES 73

LIST OF TABLES

Table 1: Experimental groups, number of chicken, parasite treatments, Newcastle disease vaccination status and anticoccidial treatment 31

Table 2: Hemagglutination inhibition titers of different age groups of chicken during the wet season 43

Table 3: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition test, during the wet season 44

Table 4: Percent occurrence of protective levels of Newcastle disease antibodies during the wet and dry season 45

Table 5: Hemagglutination Inhibition titers of different age groups of chicken during the dry season 46

Table 6: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition test, during the dry season 47

Table 7: Ecto-parasite prevalence in the preliminary, control and treatment groups 49

Table 8: Prevalance of endoparasites in the preliminary, control and treatment groups 50

Table 9: Geometric mean titers of the different groups in relation to weeks pre and post vaccination 51

Table 10: Average weight in grams for the different groups 54

LIST OF FIGURES

Figure 1: Map of Kenya showing the study area - Mbeere Sub county 23

Figure 2: Medicines used for treatment of experimental birds 32

Figure 3: Newcastle disease vaccine used in vaccination of experimental birds.....33

Figure 4: Source of chicken for stocking the farms 37

Figure 5: Type of supplementation given to the chicken during confinment 38

Figure 6: Constraints affecting poultry production..... 39

Figure 7: Method of disposal of dead birds 40

Figure 8: Uses of eggs by farmers 41

Figure 9: Percent protective levels of antibody titers to NDV in the different age groups in the two seasons 45

Figure 10: Geometric mean titers of control group in relation to time 52

Figure 11: Geometric mean titers for vaccinated group with respect to time post vaccination.....52

LIST OF APPENDICES

Appendix 1: Questionnaire on socio-economic importance and productivity of poultry and ND in indigenous chicken in Mbeere sub-county, Kenya.....73

Appendix 2: Formulae for reagents used..... 81

Appendix 3: Individual birds Hemagglutination inhibition results..... ..84

Appendix 4: Seasonal Newcastle disease antibody titer dynamics in village chicken of Mbeere sub-county, Embu County,

..... 87

LIST OF ABBREVIATIONS

Ab	Antibody
ANOVA	Analysis of Variance
CMI	Cell Mediated Immunity
ELISA	Enzyme linked immunosorbent assay
GMT	Geometric Mean Titer
HA	Hemagglutination
HAU	Hemagglutination Unit
HI	Hemagglutination Inhibition
ICPI	Intracerebral Pathogenicity Index
Ig	Immunoglobulin
IVPI	Intravenous Pathogenicity Index
KBS	Kenya Bureau of Statistics
ND	Newcastle Disease
NDV	Newcastle Disease Virus

NVND	Neurotrophic Velogenic Newcastle disease
OIE	*Office International des Epizooties (*World organization for Animal health)
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
RTPCR	Reverse Transcriptase Polymerase Chain Reaction
SPF	Specific pathogen free
WFP	World Food Programme
V/V	Volume by Volume
VVND	Viscerotrophic Velogenic Newcastle Disease

ABSTRACT

Several factors contribute to lack of effective antibody response after Newcastle disease (ND) vaccination in indigenous chicken. Stress has been shown to be one of them and parasites are thought to play a role in inducing stress. Since, in other studies, chicken in Mbeere sub-county of Embu county, Kenya, have been shown to carry heavy loads of various types of parasites, this study was carried out to establish the extent at which this parasitism affects antibody response to ND vaccination. This study also endeavored to establish effect of parasite control on production of the indigenous chicken. Baseline data on chicken productivity, farmer demography, quality of eggs produced by the chicken in the area, and ND antibody levels in the birds (as indication for endemicity of the disease in the area) were also collected.

The study was carried out in 3 phases, using chicken from the same study area – Mbeere. Phase 1 involved collection of data on: chicken productivity, egg-quality, and owner demography through administration of questionnaire to seventeen households in the area. Eggs were purchased from the same households that questionnaires were administered and the egg quality tested, with respect to egg weight, shape and shell hardness. Thirty four eggs were purchased and examined. Phase 2 involved establishment of data on ND antibody titer levels in the chicken for 2 seasons – wet and dry. For each of the two seasons, serum samples were collected from 24 chicken of all ages and sexes for determination of ND antibody titers. The serum samples were tested using hemagglutination inhibition test. In Phase 3, effect of selective parasite control on the chicken' antibody response to ND vaccination, and productivity of the birds, as measured by weight gains, were studied. Seventy two chicken, from a population that was confirmed to be

infected with ecto- and endo-parasites, were bought and divided into 8 groups which were variously treated for parasites. Groups 1 and 8 were controls, while Groups 2 and 5, 3 and 6, 4 and 7 were duplicates that were treated for endo-parasites only, ecto-parasites only and for both endo- and ecto-parasites, respectively. Groups, 5, 6 and 7 were also vaccinated with ND vaccine; the duplicate unvaccinated pair groups served as respective controls. Emphasis of the treatment was on nematodes and cestodes. Thus, birds in all groups except Group 8 were purposively treated for coccidiosis, so as to remove their influence on the immune response; Group 8 serving as overall control group. After vaccination, all the birds were monitored serologically weekly for ND antibody titers for a period of six weeks using hemagglutination inhibition test. As the birds were bled for sera, their respective weights were taken and recorded.

The analysis of the questionnaires showed that all the households interviewed had indigenous chicken, of all ages and sexes, reared on a free-range system. The birds played a significant role in economic, social and cultural affairs of the households. Farmers were aware of existence of parasites in and/or on their chicken, and ND seriousness but 76.5% of farmers interviewed did not treat their chicken against parasites nor vaccinate against ND. The eggs examined were found to be of good quality.

All the chicken (all ages and sexes) had ND titers. The titers were significantly higher in wet than in the dry season ($p<0.05$). The percentage of chicken with protective titer (2^4) were 100 in wet season and 83 in dry season. The continued presence of ND antibody titers in the study chicken indicated endemicity of the disease in the area.

The vaccinated groups 5, 6 and 7 showed a significant rise ($P<0.05$) in ND antibody titer from the start to the end of the experiment, compared to responses of the respective unvaccinated

groups 2, 3 and 4. From the 3rd week post vaccination up to the end of the experiment, group 7 had a significantly higher antibody titer ($P< 0.05$) than the other 2 vaccinated groups, 5 and 6. Titers of all unvaccinated groups (1, 2, 3, 4 and 8) continuously dropped over the experimental period. There was no significant difference in weight gain among the different groups after vaccination ($P>0.05$).

In conclusion, ND was confirmed to be endemic in study area, as shown by the occurrence of high levels of antibodies to ND during both the dry and wet seasons. Chicken parasite control improved immune response to ND vaccination. Chicken-owners are, therefore, encouraged to protect their birds through ND vaccination. This will be more effective if farmers practiced total parasite control before vaccination. This study, however, did not show significant difference in weight gains between the different test groups, as expected. Further research needs to be carried out to check the effect of parasite control on productivity of village chicken.

CHAPTER ONE: INTRODUCTION

Free-range poultry keeping is the most common type of indigenous chicken production system in Kenya. Currently there are 31.8 million poultry, of which 25.7 million (about 83.4%) are indigenous chicken, kept in villages (KNBS, 2009). The indigenous chicken tend to have low production levels, compared to their exotic counterparts. Several factors have been attributed to this low production of free range village chicken; the main one being high mortality (Yongolo *et al.*, 1997). Diseases especially ND (ND) are reported to be the main constraints in production; ND causes mortalities as high as 100% (Njagi *et al*., 2010a). Endoparasites (including hemoparasites) and ecto-parasites, predation, theft and low levels of husbandry practices also contribute to this low output (Maina, 2005; Sabuni, 2009). In many developing countries, ND (ND) is endemic and therefore represents an important limiting factor in poultry production (Alexander, 1998). Therefore, if any major success is to be achieved in improvement of free range village chicken production, it will mostly depend on successful control of ND (Yongolo *et al.*, 1997).

Studies by Kasiiti (2000) and Njagi *et al*. (2010a) showed that the ND virus (NDV) is present in healthy village chicken and that hens that survive outbreaks or have antibodies from previous exposure to ND may maintain the virus endemicity in respective areas. Other factors associated with virus endemicity include: carrier chicken, village poultry population dynamics, other poultry species, wild birds, and heterogeneity of the virus (Awan *et al*., 1994; Njagi *et al*., 2010b). Management practices, including confinement, mode of disposal of poultry waste and carcasses and recovery rates of chicken from disease outbreaks also favor maintenance of virus

in village populations (Njagi *et al*., 2010b). Nyaga *et al.* (1985) indicated that ND outbreaks are reported during the cold and dry periods of the year with peaks in April, June-July and September-November periods, meaning that antibody titers to NDV virus can be found in chicken all year round.

Many studies on ND epidemiology and control have been focused on commercial (exotic) chicken due to the considerable loss experienced, with respect to the high expenses incurred in raising these birds (Njagi *et al.*, 2010a). There is little research done on domestic village poultry (Yongolo *et al.*, 1997). Study on these village birds is necessary since there are large differences in management between commercial and village poultry; it will not suffice to simply transfer established epidemiological and control practices of ND from commercial sector to village environment (Yongolo *et al.*, 1997).

Vaccination has been shown to be the only sure way of preventing ND in poultry (Alders and Spradbrow, 2001). However, in some instances, there has been no significant prevalence change to antibody levels, indicating that there may be other contributing factors (Nyaga *et al.*, 1985). Immune-suppression may be one major cause of this; stress being the major cause of immuno-suppression (Otim, 2005; Njagi *et al*., 2010a). This may explain some of the ND vaccination failure cases which have been experienced in the field; where disease has occurred in vaccinated chicken (Kasiiti, 2000).

Village chicken have a great diversity of ecto- and endo-parasites (including hemoparasites) (Maina, 2005; Sabuni, 2009). Studies by Sabuni (2009) have put the prevalence rate of ecto- and endo-parasites in indigenous chicken in Eastern province, Kenya at a range of 90-96%. Parasites have been shown to induce stress through competition with the chicken for food, blood sucking

and irritation (Arends, 2003). The stress level is worsened by presence of a wide range of bacterial and viral conditions, coupled with poor nutrition and walking over long distances in search of food. Since stress has been reported to cause immune-suppression (Njagi *et al.*, 2010a), there is a possibility that the heavy parasite burden could lower the chicken's immune response in case of ND vaccination. This is important since very little has been done to study the effects of stress on effectiveness of ND vaccination. Controlling ecto- and endo-parasites may thus reduce stress and hence improve the efficacy of ND vaccination. Parasitism has also been reported to cause absolute loss of proteins (Tizard, 1996) and this may lead to reduced levels of immunoglobulin and further contribute to reduced immune response.

In a controlled study in Zimbabwe, Hønning *et al.* (2003) showed that parasites reduced the rise in ND antibody titer in vaccinated chicken. However, they worked on only one type of parasite, *Ascaridia galli*, a helminth, and the presence of other types of parasites may still be present and causing stress. It will be significant to determine the effect on the response to vaccination when all parasites are controlled since no such a study has been carried out yet. Results from this study will help in coming up with vaccination strategies of chicken in order to improve the health and productivity in village chicken.

1.1 Hypothesis

Parasite control in village chicken does not enhance immune response to ND vaccination.

1.2 Objectives

1.2.1 Broad objective

To determine the degree of improvement of ND vaccination immune response and productivity, in indigenous chicken, through effective parasite control

1.2.2 Specific objectives

1. To collect baseline data on poultry production systems, farmer demography and quality of eggs produced in Mbeere sub-county.

2. To establish the existing antibody titers to ND in chicken and recover ND virus in dry and wet seasons.

3. To determine the effect of endo- and ecto-parasite control on the chicken's response to ND vaccination and productivity (weight changes).

1.3 Justification

Indigenous chicken constitute the highest number of poultry kept in Kenya by small scale farmers. Improvement of health and productivity of these chickens, therefore, would go a long way towards poverty alleviation. Diseases have been rated as a major cause of low output in the village chicken with ND rated the most important disease because of its high mortality, rapid spread and contagious nature. Effective control of ND is by vaccination. However, cases of vaccination failure or lack of effective vaccine protection have been reported and a number of factors, including immune-suppression, have been associated with this phenomenon. Village birds have been shown to have high prevalence of parasites and these parasites have been shown to be a major cause of stress leading to immune-suppression (Arends, 2003). Little has been

done to determine the effect of this stress and the extent of parasite burden on response to ND vaccination as well as chicken and productivity of village chicken. This study will therefore fill the gaps identified by controlling these parasites through treatment and vaccinating the birds.

CHAPTER TWO: LITERATURE REVIEW

2.1 Poultry in Kenya

There are 31.8 million domestic poultry in Kenya, of these 25.7million (about 83.4%) are of indigenous type, while the rest are semi-commercial and commercial chicken (KNBS, 2010). These chicken are a source of protein in form of meat and eggs. Local indigenous chicken produce 54% and 75% of the total meat and egg, respectively, and their products fetch relatively higher prices compared to the commercial ones (Njue *et al.*, 2002).

Chicken are reared under different production systems. In intensive production system, farmers keep exotic commercial broilers and layers, while in semi-intensive production system, genetically improved strains and crosses of local breeds with Rhode Island Red and Light Sussex are kept. Traditional, least-capital-intensive system, where birds are left free- ranging in the daytime and confined during the night, is normally used for indigenous village chicken (Njue, 1997). These village chicken are normally more resistant to local diseases than the exotic breeds and scavenge for their own feed with little or no supplementation (Bebora *et al.*, 2005).

2.2 Benefits of poultry

Free range indigenous village chicken serves an important multipurpose function in the village economy. Poultry farming is practiced by 80% of the small scale and subsistence farmers, mainly women. Chicken and eggs have two major uses, namely: sell to generate income and home consumption, while manure is used in farms and gardens as fertilizer (Mwakapuja, 2009). Chicken also are used for cultural functions and ritual and in poor households, they are kept as convertible and accumulative assets that are available and easily traded for payments and other

income requirements (Anderson, 2001). The sector provides employment to a number of groups in the communities such as farmers, traders, transporters; and restaurant, hotel and eateries' workers in villages and towns (Mwakapuja, 2009).

2.3 Constraints to poultry production

Diseases are a major hindrance to village chicken productivity. Apart from Newcastle disease (ND), other causes of mortalities and low productivity in village chicken include coccidiosis, fowl pox, fowl typhoid, fowl cholera, infectious coryza, chronic respiratory disease and endo- and ecto-parasites (Cumming, 1992; Maina, 2005; Sabuni, 2009). Mortalities due to diseases in birds kept under free-range system, where the only disease control measure exercised is vaccination against ND, is about 11% (Stotz, 1993). This is because ND is the major constraint causing huge losses. Other constraints include poor management practices, in particular poor nutrition and housing; predation; lack of formalized market; theft, low genetic potential; and poor husbandry. These result in high mortalities and reduced production. However Bebora *et al*. (2005) reported that some indigenous village chicken have a laying capacity comparable to that of the exotic breeds; a potential that can be nurtured to develop good lines of indigenous chicken, without necessarily cross-breeding them with exotic breeds.

2.4 Newcastle disease

Newcastle disease (ND) is a viral disease of many kinds of poultry and wild birds characterized by marked variation in morbidity, mortality, signs and lesions. The virus is in the order Mononegavirales, family Paramyxoviridae and genus *Avulavirus* (Alexander, 1997).

2.4.1 Pathotypes of Newcastle disease virus

Newcastle diseasevirus (NDV) is classified into five pathotypes based on the predominant signs in affected chicken, which in turn are affected by the strain (Beard and Hanson, 1984). These are viscerotrophic velogenic (Doyle) form that causes an acute lethal infection characterized by hemorrhagic lesions in the digestive tract (Doyle, 1927), neurotrophic velogenic (Beach) form that causes, acute lethal infection characterized by respiratory and neurological signs (Beach, 1942), mesogenic (Beaudette) form that causes a less pathogenic form with mortalities in young birds, lentogenic (Hitchner) form that causes a mild or inapparent respiratory infection (Hitchner and Johnson, 1948); and the asymptomatic (enteric) form that mainly involves infection with lentogenic virus strain which causes no overt disease (Mcferran and Mc Craken, 1988).

2.4.2 Maintenance of the virus in village situation

The viral host range is wide and has been reported to affect 250 avian species with domestic chicken and turkey being severely affected (Alexander, 1998). Nyaga *et al.* (1985) indicated that ND outbreaks are reported during the cold and dry periods of the year with peaks in April, June-July and September-November periods affecting all ages of chicken. Another study by Kasiiti (2000) indicated that ND is widespread in village chicken and ducks in Kenya. The study by Njagi *et al.* (2010b) showed that the virus is present in healthy hens and that those surviving outbreaks or have antibodies from previous exposure to ND may maintain the virus endemicity in the village chicken. Thus, carrier chicken, village poultry population dynamics, other poultry species, wild birds and heterogeneity of the virus are some of the risk factors that have been associated with the maintenance of NDV (Awan *et al*., 1994; Njagi *et al*., 2010b). Management

practices, including confinement of birds, mode of disposal of poultry waste and carcasses, and recovery rates of chicken from disease outbreaks also favor maintenance of virus in village populations (Njagi *et al*., 2010b). Confinement of birds, lack of feed supplementation, cold temperatures, winds, all of which induce stress in birds, are some of the identified risk factors associated with occurrence of ND in village indigenous chicken. Restocking of farms with chicken from the market and neighborhood flock has also been shown to be a major risk factor for ND outbreaks (Njagi, 2008). Nyaga *et al.* (1985)'s observation that ND outbreaks are reported during both cold and dry periods of the year means that antibody titers to NDV virus can be found in birds all year round.

2.4.3 Clinical signs of Newcastle disease

Infection with viscerotropic velogenic strain leads to a disease with high morbidity and mortality approaching 100%, with the observed clinical signs being depression, listlessness, respiratory distress and dyspnoea, prostration and death. The neurotropic velogenic strain, mainly reported in United States of America, is characterized by mortality of about 50% in adults and 90% in young ones. There would be sudden onset of severe respiratory disease later followed by neurological signs. Egg production falls dramatically, but diarrhea is usually absent (Alexander, 2003). Other typical signs include greenish diarrhea, muscular tremors, torticollis, paralysis of the legs and wings, opisthotonus, inappetance, coughing and oedema around the eyes and head (Alexander, 2003). Mesogenic strains usually have low mortality, except in young and susceptible birds that may be considerably affected by exacerbating conditions. It usually causes respiratory disease and a marked drop in egg production with nervous signs present or absent (Alexander, 2003).

Lentogenic strains do not cause disease in young fully susceptible birds and such strains are used as vaccine candidates (Alexander, 2003).

2.4.4 Pathology due to Newcastle disease

Gross lesions and organs affected depend on the strain. There is no pathognomonic lesion associated with any form of the disease; in some cases, gross lesions may be absent. Hemorrhagic lesions in the intestines present in Viscerotrophic velogenic ND distinguish it from Neurotrophic velogenicND viruses (Hanson, 1988). These hemorrhagic lesions are particularly prominent in the mucosa of the proventriculus, ceca and small intestines. They are markedly hemorrhagic and appear to result from necrosis of the intestinal wall or lymphoid tissues such as caecal tonsils and Peyers patches (Alexander, 2003). Gross lesions are not always present in the respiratory tract but when observed, they include mucosal hemorrhage and marked congestion of the trachea. Air-sacculitis may be present and chicken and turkeys infected in lay with velogenic viruses usually have egg yolk in the abdominal cavity (Alexander, 2003). The histopathology of ND is as varied as the clinical signs and gross lesions (Alexander, 2003).

2.4.5 Diagnosis of Newcastle disease

The three main areas of diagnosis are detection, characterization of the virus and epidemiology. Clinical diagnosis based on history, signs, and lesions may establish a strong index of suspicion for ND, but laboratory confirmation should always be pursued in order to identify the strain (OIE, 2000).

2.4.5.1 Isolation and identification of Newcastle disease virus

2.4.5.1.1 Direct detection of viral antigens

Immunofluorescence and immunoperoxidase techniques can demonstrate the presence of NDV in tissues, using thin sections (Alexander, 2003; Njagi *et al*., 2012).

2.4.5.1.2 Virus isolation

Because of their extreme sensitivity and convenience, ten to twelve (10-12) day-old specific pathogen free (SPF) embryonated eggs are readily used for cultivation of NDV. This is done by inoculation on to the chorioallantoic membrane or into the allantoic sac. Virulent ND viruses can also be propagated in cell culture (Alexander, 2003). Samples for isolation include antibiotic treated cloacal and tracheal swabs or trachea and bone marrow (Alexander, 2003). For cultivation in eggs, finely ground tissues, organs and faecal samples treated with antibiotics are centrifuged at 1000g for 10 minutes and 0.2ml of the supernatant inoculated into the allantoic sac. The eggs are incubated at 37^{o}C and examined daily by candling. While eggs dying on the first day are discarded, those, which die thereafter up to day 7 of incubation, are chilled at 4^{o}C and the allanto-amniotic fluid harvested. The presence of the virus is detected by hemagglutination test (Alexander, 2003).

2.4.5.1.3 Virus biological pathogenicity characterization

Widespread presence of lentogenic strains in feral birds and use of such viruses as live vaccines means that isolation of NDV is rarely sufficient to confirm a diagnosis of disease hence further virus characterization such as pathogenicity testing and nucleotide sequencing is necessary (Alexander, 2003). Mean death time in eggs, Intracerebral Pathogenicity Index (ICPI) and Intravenous Pathogenicity Index (IVPI) are the *in vivo* tests used for assessment of pathogenicity (Alexander, 2003).

2.4.5.2 Serological tests for Newcastle disease virus antibodies

Numerous serological tests may be used to detect antibodies in serum but give little information on the infecting NDV strain. Hemagglutination inhibition has been most commonly used and shown to have accurate results (Alexander, 2003). Office International des Epizooties (OIE) states that a titer may be regarded as positive if there is inhibition at serum dilution of 2^3 or more against 4HA units or 2^4 or more against 8HA units (OIE, 2000). Hemagglutination inhibition (HI) can also be used for measuring immune status of vaccinated birds where mean levels of HI titers ranging from 2^4 - 2^6 after a single live vaccine to 2^9- 2^{11} following multiple vaccination programs are expected. Other serological tests used include: virus neutralization test (using known ND antiserum), agar gel immunodiffusion and enzyme linked immunosorbent assay (ELISA) and fluorescent antibody technique (OIE, 2000). Since laboratory services for diagnosis of NDV are not always available in rural areas, a sensitive, simple, inexpensive and specific field test for rapid and accurate diagnosis is necessary for immediate control measures to combat the disease and avoid further dissemination (Njagi, 2008). While commercial rapid kits are available, they have not been regularly used for indigenous chicken in Kenya. The enzyme linked

immunosorbent assay has gained application for the viral diagnosis of both antigens and antibodies in recent years (Yolken, 1982). Serological tests have not been used to detect the response of vaccination to ND in indigenous chicken following parasite control in Kenya.

2.4.5.3 Molecular techniques in diagnosis of Newcastle disease

Apart from being fast and sensitive, molecular based techniques can also achieve all the three aspects of diagnosis, referred-to above, in a single test. Most of these techniques involve polymerase chain reaction (PCR) and reverse transcriptase-PCR (RTPCR) (Alexander, 2003).

2.4.6 Control of Newcastle disease

Vaccination is the only sure way of preventing ND in poultry (Alders and Spradbrow, 2001). However, it should be emphasized that in no circumstance can vaccination be regarded as an alternative to good management practice, biosecurity or good hygiene in rearing domestic poultry (Alexander, 2003). Live vaccines and inactivated vaccines are currently used in countries that vaccinate against ND (OIE, 2000).

2.4.6.1 Live vaccines

Live vaccines are sold as freeze dried, are relatively cheap, easy to administer and can be used for mass vaccination (Alexander, 2003). These have been divided into lentogenic and mesogenic groups with their preferred mode of administration being intranasal installation, eye drop or beak dipping for lentogenic vaccines while mesogenic vaccine requires intramuscular injection. Drinking water and aerosol administrations can also be used (Alexander, 2003). Hitchner B1 and La Sota vaccines are used in most countries and are derived from the mesogenic strain of NDV. Since infection is by live vaccines, the virus stimulates local immunity soon after

application; as it multiplies, the virus may also be spread from vaccinated to non vaccinated birds (Alexander, 2003). It should be noted, however, that some mesogenic vaccines may cause disease; this is more so in young birds, especially if there is a dual infection with exacerbating organisms (Otim, 2005). The live vaccines are also heat labile and this can be a disadvantage under village management system where transport and cold storage facilities are often inadequate (Otim, 2005).

2.4.6.2 Inactivated vaccine

Inactivated vaccines are produced from infective allantoic fluid of virulent NDV treated with B-propiolactone or formalin to kill the virus and then mixed with adjuvant. The vaccine is applied by either subcutaneous or intramuscular injection. The vaccines induce high levels of protective antibody over long duration of application and can be used in situations unsuited for live vaccines. However, these vaccines are expensive to produce and administer (Alexander, 2003).

2.4.6.3 Thermostable vaccine

Two types of vaccine are considered thermostable; Australian V4 and I_2 . Australia V4 has both thermostability and immunogenicity and was specifically developed to be used in village chicken. It is administered in coated pelleted feed (Spadbrow, 1992). The possibility of natural transmission of this vaccine strain from vaccinated to non-vaccinated birds makes it suitable under village conditions; however in Tanzania, V4 has not indicated success in feed administration (Foster *et al*., 1999).

Strain I_2 produced by allantoic cavity inoculation also shows immunogenicity and thermostability. It's produced by harvesting the allantoic fluid and freeze drying or storing at 4^oC

before being dispatched. Refrigeration is not required for transport of this vaccine hence suitable under village conditions (Spadbrow, 1992).

2.4.6.4 Vaccination programs

Vaccination programs should be tailored to suit the prevailing disease situation, availability of vaccines, maternal antibodies, size of flock, presence of other organisms, expected life of the flock, past vaccination history and cost (Alexander, 2003). This is to avoid too little vaccination, over vaccination, mis-timing of vaccination, all of which have serious consequences (Alexander, 2003). Village chicken flocks are particularly comprised of multi-species, small size, mixed ages and are distributed in households over large areas (Mukiibi, 1992; Spadbrow, 1999). Poverty and limited education of the rural people contribute to the poor health status of the poultry (Spadbrow, 1999). Frequently, local customs and circumstances lead to vaccination problems (Alexander, 2003).

The control of ND in village indigenous chicken, like in commercial sector, requires a multifaceted approach. Australian V4 vaccine is a thermostable vaccine with good immunogenicity and it has been specifically developed for use in village chicken in tropical countries. It can be administered in pelleted feed or via eye drop. In addition to heat resistance V4 vaccine virus can be transmitted to non vaccinated chicken, disseminating the immunization and making it suitable for use under village conditions (Alexander, 2003).

2.4.7 Immune response to Newcastle disease virus

Two forms of immune responses against NDV exist. These are: cell mediated immune response and humoral immunity. The initial immune response after infection is cell mediated; this can be detected as early as 2-3 days after infection (Alexander, 1997). Humoral immunity develops within 6-10 days and peaks at 3-4 weeks post infection (Alexander, 1997). These antibodies remain detectable up to 1 year after recovery from infection or vaccination (Russell and Koch, 1993; Njagi *et al*., 2010b). The antibody titer of log mean 2^5 has been reported to provide 100% protection from challenge (Allan *et al*., 1978). Passive immunity occurs with maternal antibodies being passed in the egg yolk then to the chick and these confer protective immunity (Allan *et al.*, 1978; Njagi *et al.*, 2008). On vaccination, antibodies are produced in the respiratory tract and the Harderian gland, which is the main site of Ig-A antibody formation in chicken (Russell and Koch, 1993).

2.5 Poultry parasites

2.5.1 Ectoparasites

Poultry ecto-parasites are members of the animal phylum Athropoda characterized by possession of externally segmented bodies, jointed appendages and chitinous exoskeleton. Lice, flies, and fleas are members of the class Insecta and orders Phthiraptera (lice), Siphonaptera (fleas) and Diptera (flies) while mites and ticks belong to the class Arachnida, order Acarina (Arends, 2003). These parasites cause low mortality but significantly hinder and lower production (Sabuni, 2009). Poultry that are seriously infested by common ecto-parasites exhibit irritation and react by scratching and preening. Blood sucking parasites e.g. chicken mites get onto birds only to feed and are more difficult to detect; examination of roost, beddings, walls and cracks,

crevices and beneath manure clods is necessary for detection (Arends, 2003). Lice species affecting chicken include *Menacanthus stramineus, Menopon gallinae, Cuclotogaster heterographus, Lipeurus caponis, Gonoides gigas* and *Goniocoites gallinae* (Msoffe and Cardona, 2009). *Echidinophaga gallinacea* (stick tight flea) is the only flea commonly affecting poultry (Msoffe and Cardona, 2009). A study by Sabuni (2009) in Eastern province indicated that four ecto-parasites namely, lice, mites, fleas and ticks occur at a high prevalence, with an overall prevalence of 90-97%.

2.5.2 Endoparasites excluding hemoparasites

These poultry parasites other than hemoparasites include nematodes, cestodes, trematodes and protozoan species (Maina, 2005). A wide array of internal parasites can be found in indigenous free-range chicken which are due to availability of intermediate hosts (Arello, 1998). Most farmers are not aware of the existence of parasitism in poultry and hence they do not deworm their chicken (Ndegwa *et al.*, 1998).

Maina (2005) reported prevalence of endoparasites at 90% in traded poultry in Eastern province. These included nematodes, cestodes and protozoan species. The most common gastrointestinal parasites were nematodes, including: *Heterakis, Gongylonema, Tetrameres, Acuria, Ascaridia* species, among others; *Raillietina echinobathrida*, was the most prevalent cestode. Others included *Syngamus trachea* (Maina, 2005).

Protozoa of the genus *Eimeria* cause coccidiosis in poultry. These parasites are cosmopolitan but are of little importance in free-ranging family poultry occurring at low numbers. They may be asymptomatic in presentation or may cause decreased growth, diarrhoea, and high mortality

(Msoffe and Cardona, 2009). The most pathogenic *Eimeria* species are *E.tenella, E.necatrix* and *E.brunetti;* E.cervulina and *E.maxima* also cause disease but are less pathogenic (Soulsby, 1982).

2.5.3 Haemoparasites

Haemoparasites are found in poultry mainly in the tropics and include species of *Plasmodium, Leucocytozoon, Hemoproteus, Aegyptinella, Eperythrozoon* and *Trypanasoma* (Msoffe and Cardona, 2009). Poultry haemoparasites are of little or no immediate concern but a heavy infestation may lead to anaemia and death (Soulsby, 1982). Transmission is by arthropod vectors, including mosquitoes and biting midges as well as other flies and the poultry soft tick (*Argas persicus)* (Msoffe and Cardona, 2009). A study by Sabuni (2009) in Eastern province recorded haemoparasite prevalence of 79.2%, including those of three genera: *Plasmodium*, *Leucocytozoon* and *Hemoproteus* . *Plasmodium gallinaceum* was the most prevalent of the haemoparasites.

2.5.4 Diagnosis of poultry parasites

Ectoparasites can be diagnosed by clinical signs such as scratching and preening, through detection of the parasites on/in skin, head, plumage, roost, beddings, walls (cracks, crevices) and beneath manure clods. Scaly leg mites can be diagnosed by examining skin scraping(s); after digested with potassium hydroxide (Arends, 2003).

Helminth infections can be diagnosed by identifying the eggs in the faeces using simple floatation technique and also using a modified McMaster method. Performing a direct post-mortem examination and detecting the worms can also be done (Permin and Hansen, 1998; Soulsby, 1982).

Diagnosis of coccidiosis can be done by post-mortem examination of the bird (s). Faecal examination (to detect oocysts) can also be used for diagnosis (Soulsby, 1982).

Diagnosis of haemoparasites is made by preparation and examination of blood smears. Thin blood films are prepared from fresh blood, air dried within 5-10 seconds, fixed in methanol for 5 minutes, stained with 10% Giemsa for 10 to 15 minutes, washed with tap water, dried by blotting , cover slipped and examined under the light microscope at high magnification (X100) (Msoffe and Cardona, 2009).

2.5.5 Parasite treatment

Synthetic pyrethroids such as permethrin, organophosphates, carbamates and pyrethroids insecticides are the main chemicals used for ecto-parasite treatment by direct application to poultry, litter or buildings (Arends, 2003). Tetrachloroviphos and ivermectin among others have also been used (Permin and Hansen, 1998). Scaly leg of chicken can be treated by smearing vaseline jelly with insecticides or dipping the leg in paraffin (kerosene) and then gently brushing the leg (Msoffe and Cardona, 2009).

The most common drugs used for treatment of endoparasites (other than haemoparasites) include fenbendazole, flubendazole, levamisole, mebendazole, piperazine and thiabendazole (Permin and Hansen, 1998). Tucker *et al*. (2007) showed that albendazole at a dosage of 20 mg/kg is both safe and effective in the treatment of birds for *Ascaridia galli*, *Heterakis gallinarium, Capillaria obsignata* and *Raillietina cesticillus.* Glycomides, quinolones, clopidols, sulphonamides, iobedine, amprolium, zoalene, nitromide, nicarbazin and monsein are used for treatment and prevention of coccidiosis (Soulsby, 1982).Treatment of haemoparasites is usually not cost effective but can be done with anti-malarial compounds (Msoffe and Cardona, 2009).

2.6 Immunosuppression and parasites

Parasites cause stress, which has been associated with immunosuppression (Njagi, 2008). Permin and Hanson (1998) emphasized the importance of parasite prevalence of 90-100% in lowering the humoral immunity. The synthesis of immunoglobulins is reduced in animals severely affected by parasites, owing to an absolute loss in protein (Tizard, 1996). This might result in reduced antibody response as reported by Hørning *et al*. (2003).

A controlled study carried out on effect of *Ascaridia galli* on ND vaccination showed that 5 weeks after vaccination parasite infested birds had lower level of antibody titters than those treated against parasites (Hønning *et al.*, 2003). Reported ND vaccination failure in village chicken might be explained as a result of an immune-suppression by parasitism (Hønning *et al.*, 2003). Hence, parasites may interfere with the effectiveness of vaccines used to control important poultry diseases, such as ND in village birds. Control of all parasites may be better than control of *Ascaridia galli* only, hence the need for this study.

2.7 Egg quality

Egg quality can be assessed by using shell integrity, texture and shape. Assessment of shell integrity includes: weight, shape and hardness of the shell - being free from cracks, having hard shells (thin shells and lack of shells being indicators of poor shell quality). The egg shell texture should be smooth without roughness , pimples, pinholes and/or mottleness . Normal eggs have smooth oval shape; abnormalities in eggs include flat sided eggs and flat sided eggs (EMEA, 2001)

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

Three studies were carried out in Mbeere Sub-county, Kenya (Figures 1). The area was purposively selected based on the large population of free-range chicken of 165,090 (KNBS, 2009). Other agricultural activities practiced in the district include; cattle, sheep and goat keeping and millet, sorghum and green gram production. Mbeere Sub county lies between latitudes $0^{o}20'$ and $0^{o}50$ South and longitudes $37^{o}16'$ and $37^{o}56'$ East, at altitude 500 to 1200 meters above sea level. Long rains fall between mid-March and June while short rains occur in October to December. Dry periods are between January and early March; and between August and September. The daily temperature ranges from 20-30^{o}C (Onduru *et al.*, 2002).

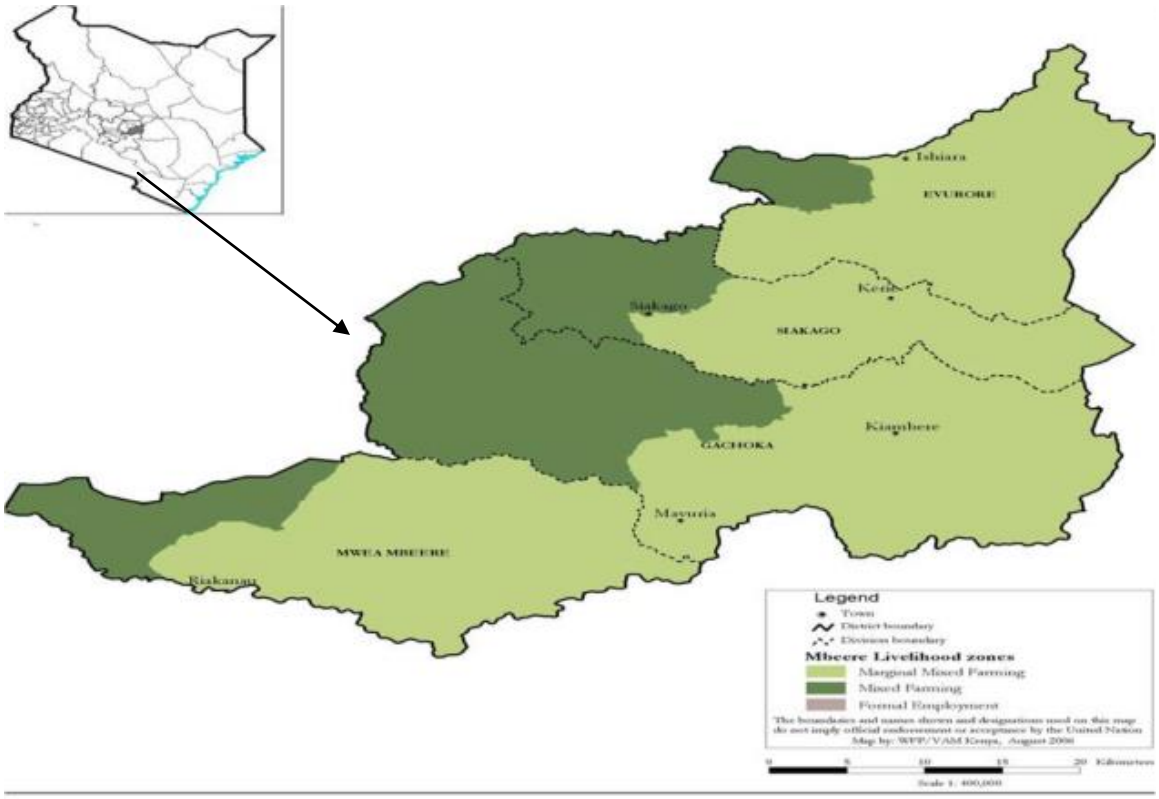


Figure 1: Map of Kenya showing the study area - Mbeere Sub County (Source WFP/YAM Kenya, February 2006)

3.2 Study chicken

Chicken of various age groups: chicks (under 2 months of age), growers (2-8 months of age) and adults (over 8 months of age) were used for the checking of levels of antibody titers during the wet and dry seasons while only growers were used for the checking of effect of parasite control on immune response following ND vaccination; the age groupings were done following the criteria given by Sabuni (2009). Determinations of respective ages were done subjectively, guided by farmers' information. The identified birds were bought and transported alive in cages to the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of

Veterinary Medicine, College of Agriculture and Veterinary Sciences, Kabete Campus, for respective experiments. .

The minimal sample size was calculated using the method described by Martin *et al* (1987), as follows:

$$N = 4pq/L^2$$

Where; N= sample size, p= prevalence (50%), q= 1-p and L= Limit of error on prevalence taken at 10%

$$= \frac{4 * 0.5 * 0.5}{0.1^2} = 100$$

For statistical, animal welfare and financial reasons, 8 birds were used for each group for the seasonality and experiment to demonstrate effect of parasite control on ND vaccination response, this was in line with the accepted recommendation for use of animals for an experiment that state that six animals are the minimum animal welfare and statistically viable number that can be used (EMEA, 2001)

Forty eight (48) chicken were used for the seasonality study; 24 for each season, including the three age groups, while 72 were used for experiment to demonstrate effect of parasite control on ND vaccination response - 8 chicken per group, for the 8 groups used, and 8 birds for the initial assessment of parasite burden.

3.3 Study design

The study was carried out in 3 phases in line with the 3 study objectives given earlier, in Section 1.1.2. Studies on antibody titer determinations and collection of questionnaire data (i.e. the

first two objectives) were cross-sectional and sampling was purposive and convenient (based on reachable willing owners, regardless of the number of chicken kept; so long as the birds were kept on free-range system, had no history of ND vaccination, and no parasite control/treatment was exercised), study for the 3rd objective was longitudinal.

3.4 Collection of data on production parameters and quality of eggs produced in the area

Data collection was done using semi-structured questionnaires; a sample of which is given in Appendix 1. Seventeen (17) farmers were interviewed. Each farmer was interviewed separately.

Thirty four (34) eggs were also bought randomly from farmers who had them. These were taken to Kabete for quality assessment, with respect to weight, shape and hardness of the shell.

3.5 Collection and processing of specimens, in wet and dry seasons

3.5.1 Serum samples for determination of antibody titers

Individual farms were used for determining ND antibody titers of birds in the wet and dry seasons, where a maximum of 2 birds from each farm were sampled to a total of 24 chicken. At the Department of Veterinary Pathology, Microbiology and Parasitology, the birds were kept in cages; bled for serum production, swabbed from oro-pharyngeal region and cloaca, and tissues collected (spleen, trachea and cecal tonsils) for viral isolation.

About 10 cc of blood was collected for serum as the birds were being euthanized for collection of tissues for virus isolation. Blood was collected into sterile universal bottles (without anticoagulant) and placed in a slanting position at 4^{o}C overnight. The samples were then centrifuged at 3000 rpm for 10 minutes, serum harvested into serum vials and stored at -20^{o}C

until analyzed. Antibody titer determination was done using hemagglutination inhibition test (HI). The exercise was done twice – in wet and in dry season respectively.

3.5.2 Swabs and tissues for viral isolation

The swabs and organs, from same bird, were placed together in 2 ml viral transport medium comprising minimum essential medium with penicillin and streptomycin (at a concentration of 2000 international units and 2000 micrograms per ml, respectively) and stored at -20^{o}C until viral isolation was done.

3.6 Hemagglutination inhibition test

3.6.1 Preparation of red blood cells for the test

Five milliliters of blood was collected from 3 chicken belonging to the Department of Veterinary Pathology, Microbiology and Parasitology; the birds were kept in isolation and had never been vaccinated for any disease. The blood was collected in Elsevier's solution and centrifuged at 1000 rpm for 10 minutes. The supernatant and buffy coat were removed and the red blood cells (RBCs) washed three times in phosphate buffered saline (PBS), pH 7.2. Red blood cells were re-suspended in phosphate buffered saline to a concentration of 1% v/v and stored at 4^{o}C until use.

3.6.2 Sources of viral antigen for the test

The Newcastle disease virus used for the test was acquired from the virology laboratory, Department of Veterinary Pathology, Microbiology and Parasitology and had not been characterized to know the type. .

3.6.3 Hemagglutination test for determination of working concentration

Hemagglutination test (HA) was carried out in accordance with the OIE (2000). Briefly, 0.025ml of PBS was dispensed into each well of a plastic U bottomed microtiter plate and 0.025ml of harvested virus grown in eggs placed into the first well. Two fold dilutions were made using 0.025ml volume transferred from well to well across the plates. A volume of 0.025ml 1% v/v chicken RBCs was dispensed in each well including control well and plate mixed by tapping gently. The plate was left at room temperature for 40 minutes for the RBCs to settle. Hemagglutination end point was determined by tilting the plate and observing for the presence or absence of peculiar central button shaped settling of RBCs. The titer was taken as the well with the highest dilution giving complete hemagglutination and no tearing (OIE, 2000). This was taken as one (1) hemagglutination unit. Four hemagglutination units were used to carry out the hemagglutination inhibition (HI) test.

3.6.4 Hemagglutination Inhibition (HI) test procedure

The HI test against NDV using 4 hemagglutinating units was done using the procedure described by OIE (2000). Briefly, the test was carried out by running two fold dilutions of equal volumes (0.025ml) of PBS and test serum (0.025ml) in a V-bottomed microtiter plate. Four (4) hemagglutinating units (HAU) of virus were added to each well and the plate was left at room temperature for a minimum of 30 minutes. Finally 0.025ml of 1% (v/v) chicken RBCs was added to each well and, after gentle mixing, the RBCs were allowed to settle for 40 minutes at room temperature. The HI titer was taken as the highest dilution of serum causing complete inhibition of hemagglutination; this was identified as the one showing peculiar central button shaped settling of RBCs. Validity of the results was assessed against a negative control serum included

in the test (OIE, 2000) The HI titers were determined in all chicken, and the geometric mean titter (GMT) of each group calculated.

3.7 Viral isolation from swabs and tissues

3.7.1 Swab processing for viral isolation

The swab contents were expressed into 2mls of the transport medium, centrifuged at 3500 rpm for 10 minutes and the supernatant transferred to a sterile bijoux bottle. Samples were stored at -20°C until virus isolation was done.

3.7.2 Tissue processing for viral isolation

Tissues (spleen, trachea, cecal tonsils) were finely ground, mixed with 2mls of minimum essential media with antibiotics then centrifuged at 3500 rpm for 10 minutes. The supernatant was placed into sterile bijoux bottles. Samples were stored at -20°C until virus isolation was done.

3.7.3 Viral isolation

Specific pathogen free (SPF) embryonated eggs obtained from Muguga poultry farm, 10-12 days old, were inoculated, respectively, with 0.2 mls of a mixture of supernatant from finely ground organs and respective swabs on the chorio-allantoic membrane, incubated at 37°C and examined regularly for signs of death which includes collapse of blood vessels and lack of movement of the embryo (Alexander, 2003). Since no egg died after 7 days, the eggs were chilled at 4°C and their amniotic fluids harvested and tested for HA (Alexander, 2003).

3.8 Establishment of initial parasite burden and initial antibody titers

Seventy two (72) chicken with no previous history of vaccination or worm control, bought from the market in the study area, were used in this study. Growers of all sexes (2 – 8 months of age) were used for the study. The birds were transported to Kabete campus, where they were left to acclimatize for 3 weeks and introduced on growers mash gradually until they were used to the feed. Before start of the experiment, 8 birds were euthanized to determine the initial types and loads of parasites in the birds. Total examination was done and the birds were found to harbour internal worms such as: roundworms (*Gongylonema* species, *Heterakis* species, *Tetrameres americana*) and flatworms (*Raillietina echinobathrida*), coccidia, and lice of the genera *Menopon* and *Menacanthus*). Thus, apart from the intended treatments for endo- and ecto-parasites, separately and collectively, an additional baseline treatment for coccidia was given. This was to make sure that all the experimental chicken were free of coccidia at start of the experiment – all the groups were treated except one control group (8). The coccidia treatments were done within the 3 weeks acclimatization period and continued through the experimental period. The anticoccidial drug used was Intracox® (Tolrazuril 25mg) which had been found earlier to be effective against coccidia (unpublished research).

The initial antibody titers of these birds were also determined using the eight chicken sera. The titers ranged from 1:32 to 1:256, with a geometric mean titer of 90.51.

3.8.2 Grouping of remaining chicken per treatment

In this experiment, the remaining sixty four (64) chicken were divided into eight (8) groups of 8 birds each, with varying treatments and vaccination status, as given in Table 1. The birds were weighed before the start of the experiment. The birds were wing tagged (for identification) and

the numbers picked randomly using Ms excel and placed into the eight (8) groups (thus the selection was by random assignment). There were two (2) control groups: numbered 1 and 8. The difference between the two was that Group 1 birds were treated against coccidia while Group 8 birds remained untreated; Group 8 birds serving as overall controls. Groups 2 to 4 were controls for treatment groups. Groups 5 to 7 were the treatment groups that were used to determine the antibody responses to NDV vaccine after treatments for endoparasites and ecto-parasites, either separately (Groups 5 and 6, respectively) or combined (Group 7). All the 8 birds per group were bled, for serum, from the wing vein at intervals of 1 week for 6 weeks. Weight gains or losses were also recorded as indicators of production (growth).

Table 1: Experimental groups, number of chicken, parasite treatments, Newcastle disease vaccination status and anticoccidial treatment

Group of chicken	No. of chicken	Endoparasite treatment	Ectoparasite treatment	ND vaccination	Anticoccidial treatment
1	8	- (none)	- (none)	-(none)	+
2	8	Albendazole treatment	-(none)	-(none)	+
3	8	-(none)	Sevin+permethrin	-(none)	+
4	8	Albendazole	Sevin+permethrin	-(none)	+
5	8	Albendazole treatment	-(none)	+	+
6	8	-(none)	Sevin+permethrin	+	+
7	8	Albendazole	Sevin+permethrin	+	+
8	8	- (none)	- (none)	- (none)	-

Key: ND: Newcastle disease No. : Number + vaccinated/treated for coccidia

- not vaccinated/not treated for coccidia

3.8.3 The various treatments administered and vaccination

The experimental treatments included: Albendazole for helminths and Sevin and permethrin for ecto-parasites, while Intracox® was used for coccidia (Fig 2). Albendazole was administered one week prior to vaccination at a dosage of 20mg/kg body weight via drenching; 0.2ml based on the weight of the heaviest chicken; this was repeated after four weeks. Sevin and permethrin were

administered by dusting the individual birds and the cages. This was repeated after a month, while Intracox® (Tolrazuril 25mg) was administered orally at a dose of 1ml per 1000ml of water for 2 days.

Vaccination was done using a locally available vaccine AVIVAX- F (Fig 3) following the manufacturer's recommendation. A layers vaccination regime was used, i.e., primary vaccination on day 0, a booster 14 days later, followed by another booster 1 month later, with respect to groups as shown on Table 1. The vaccine was administered using the intranasal route.

Figure 2: Medicines used for treatment of experimental birds

Figure 3: Newcastle disease vaccine used in vaccination of experimental birds

3.8.4 Blood collection and testing

Blood (2.5-3cc) was collected from all the eight birds in each group through the brachial vein into universal bottles (without anticoagulant); serum was then separated and stored at -20^{o}C until analyzed. Blood was collected pre-vaccination and then at weekly intervals after vaccination, for 6 weeks. The serum was analyzed and tested using HI test.

3.9 Examination and identification of parasites

3.9.1 Faecal collection and identification for helminth eggs and coccidia

For each of the groups, faecal samples were collected twice a day in the morning and evening, put into sample bottles and labeled for identification. The samples were later processed in the laboratory using the McMaster technique, with saturated sodium chloride solution as the floating medium. Identification of helminth eggs and coccidial oocysts was done using a standard microscope under ×10 objective magnification (Soulsby, 1982).

3.9.2 Examination for presence of parasites on legs and skin of the study chicken before slaughter

Before necropsy, each bird was examined thoroughly for presence of parasites on the legs and skin and general health status. If present, the parasites were collected, taken to the Parasitology laboratory for identification..

3.9.3 Post-mortem examination for parasite isolation

After external examination, the birds were sacrificed by cervical dislocation and post-mortem carried out as described by Charlton (2006); any lesions seen were recorded. To isolate the gastrointestinal helminths, the whole digestive tract was removed carefully and subdivided into trachea, oesophagus, crop, proventriculus, gizzard, duodenum, small intestines, caeca and large intestines. All sections were opened longitudinally with a pair of scissors. The serosal layer of the crop and oesophagus was examined macroscopically to check for *Gongylonema* species. The serosal surface of the proventriculus was examined carefully for the presence of embedded *Tetrameres* spp. The keratinized layer of the gizzard was removed for detecting the presence of *Acuaria* species. After opening the intestine, the mucosa was scraped, and washed so as to recover the smaller helminths. All the larger helminths were picked up with forceps, the residual contents were examined under a stereo-microscope at 40 times magnification, and all the smaller parasites were collected and transferred to 70% alcohol (Gibbons *et al.*, 1996).
The air sacs were checked for the presence of air sac mites and if present the entire air sac was placed into a container containing 70% alcohol for identification.

Ectoparasites were examined according to the method described by Sabuni (2009). Briefly, the whole skin, head and legs were taken from the bird into a container containing 70% alcohol. The skin parasites were collected from the alcohol and from the skin and preserved in 70% alcohol. In birds suspected for infestation with mites, deep scrapings from the legs were collected using a scalpel blade to a slide and digested with 10% potassium hydroxide before being examined under a microscope at x10 objective.

3.9.4 Identification of parasites

To count the cestodes, only the numbers of scolexes were considered, while for the nematodes the entire worm was considered. For identification, the helminths were mounted in drops of lactic acid, and the scolexes of cestodes were cleared with lactic acid. All the helminths were then examined under light microscope and were identified on the basis of helminthological keys described by Soulsby (1982) and Permin and Hansen (1998).

Ectoparasites were cleared with lactic acid and fixed on microscope slide before detailed morphological examination and identification were done, using a light microscope at x10 magnification.

3.10 Determination of the village chicken productivity

As the birds were treated (with/without vaccination), they were weighed at weekly intervals (as blood for serum was also collected) as a measure for respective growth rates. The weighing was done in the morning, before feeding the birds, using a digital weighing balance.

3.11 Data management

The data collected was stored in a spreadsheet program (excel). Descriptive analysis was conducted on the production data collected from individual households.

The immune status of the birds and comparison was done using analysis of variance (ANOVA).General linear model for repeated measures was used to evaluate association between parasite control, ND vaccination response and weight gains. A confidence level of 95% was as a cut off to show level of significance.

CHAPTER FOUR: RESULTS

4.1 Data on poultry production systems, farmer demography and quality of eggs produced in the area

4.1.1 Management of poultry

All the farmers' interviewed kept local breed of chicken under the free range system. The day to day management of the poultry was carried out by women (88.0%) with assistance from the husbands and children (12.0%).

The farmers used more than one source of chicken for stocking; markets (64.7%) were the most common sources of the farmed chicken. Other sources included hatching from the flock (41.0%), purchase from neighbors (41.0%) and gifts from neighbors (5.0%) (Figure 4).

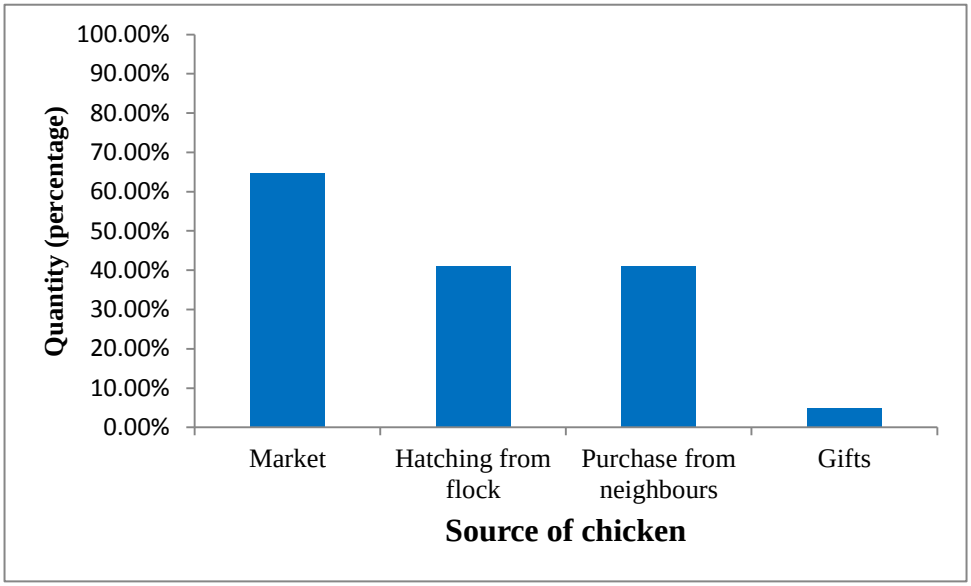


Figure 4: Source of chicken for stocking the farms

All birds were housed at night. Majority of farmers (88.0%) housed all age groups together while 12.0% of them housed their birds separately, according to age. Wooden house (52.9%), mud-walled house (41.0%), kitchen, iron sheet and wire mesh houses (6.1%) were the different types of housing used. Sixty five (65.0%) of the households confined their chicken at some particular time of the year, mainly during the planting season (18.0%), while the rest confined chicks only to protect them from predators. During the confinement period, the birds were supplemented with a variety of supplements with most farmers using more than one supplement at a given time ,cereal grains (76.5%), commercial feed (23.5%) and kitchen leftovers (17.6%) were the supplements used (Figure 5).

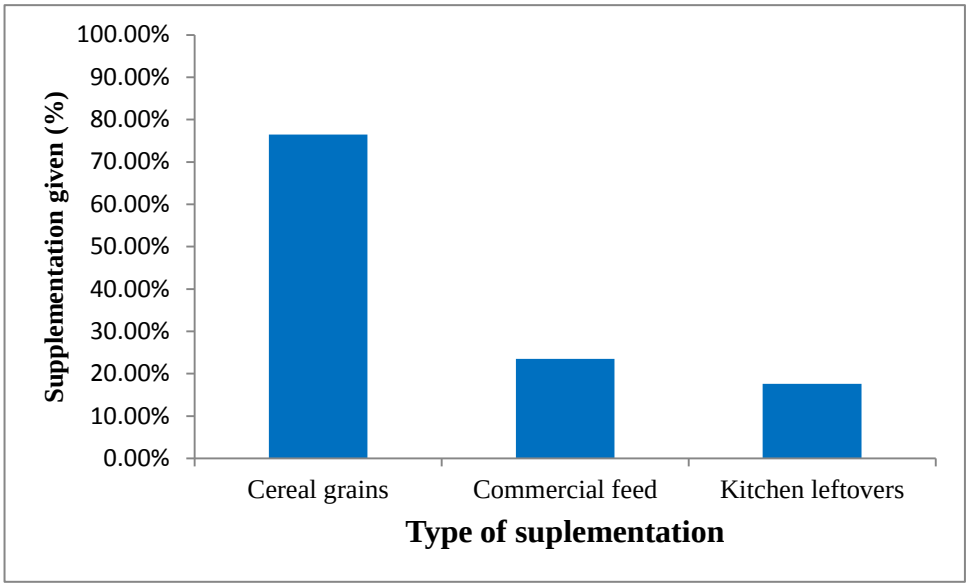


Figure 5: Type of supplementation given to the chicken during confinement

4.1.2 Constraints of village poultry production

Diseases (88.2%) were observed to be the major constraint in poultry production; ND being the major one (82.4%). Other diseases included: fowl pox (17.6%), fowl typhoid (11.7%), lameness,

coccidiosis and chronic respiratory disease (5.0%); some farmers considering more than one disease as a major constraint. Other problems included parasites (70.6%), predation (52.9%), lack of feed (17.6%) and accidents (11.8%) with most farmers stating more than one constraint to poultry production (Figure 6).

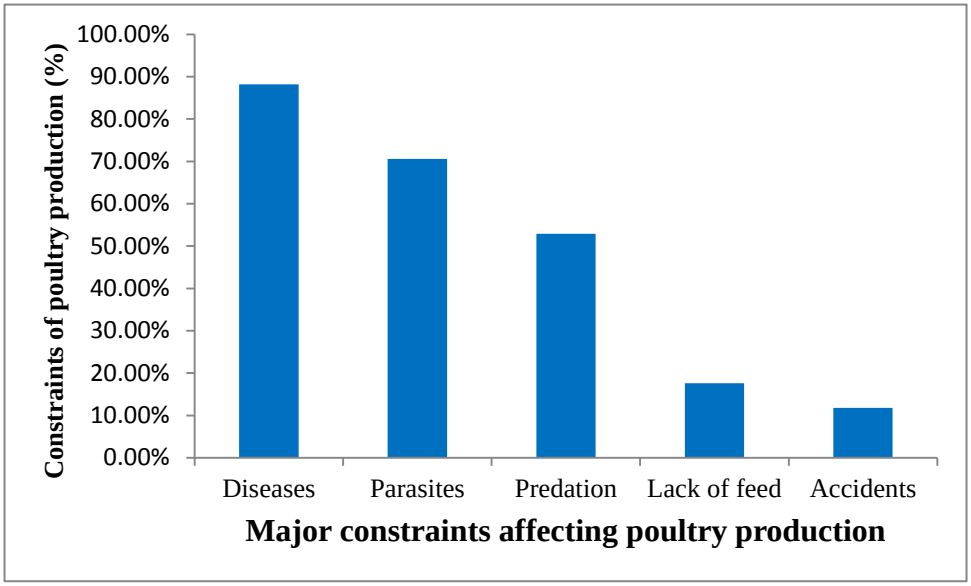


Figure 6: Constraints affecting poultry production

Only 23.5% of the farmers vaccinated against ND. It was the only disease that the farmers vaccinated against and the vaccination was done by Animal Health Assistants (75.0%) with few doing it on their own or calling a veterinary doctor (25.0%). Forty seven percent (47.0%) of the farmers isolated new bird entrants to their flock.

4.1.3 Newcastle disease and its control

All the farmers interviewed seemed to understand ND, which they locally called '*Kivuruto*'. They described its major signs as drowsiness, inactivity and greenish watery diarrhoea. Some farmers also mentioned ruffled feathers, weakness and dullness as additional signs. The farmers mentioned that the disease had a high mortality affecting all ages. Drought, lack of feed and wind

were the common factors associated with the disease. Other factors associated with the spread of the disease included: poor hygiene, mixing with sick birds, birds eating intestines from sick or dead birds, birds coming in contact with dogs, contaminated feed and water.

4.1.4 Management and disposal of sick and dead birds

Killing and consuming was the most common method of handling sick birds (41.2%). However 29% of the farmers interviewed did nothing to the sick birds while some (29.8%) treated using antibiotics and herbal medicine, mostly *Aloe* spp. and pepper.

Some farmers used more than one method of disposal of dead birds depending on the situation, burying of the carcass (41.2%) was the common method of disposal of the carcass (Figure 7). Some farmers threw away the carcass to the bush (36.2%), fed to the dogs (17.6%) or burned the carcass (5.0%). In some cases the farmers applied more than one method of disposal.

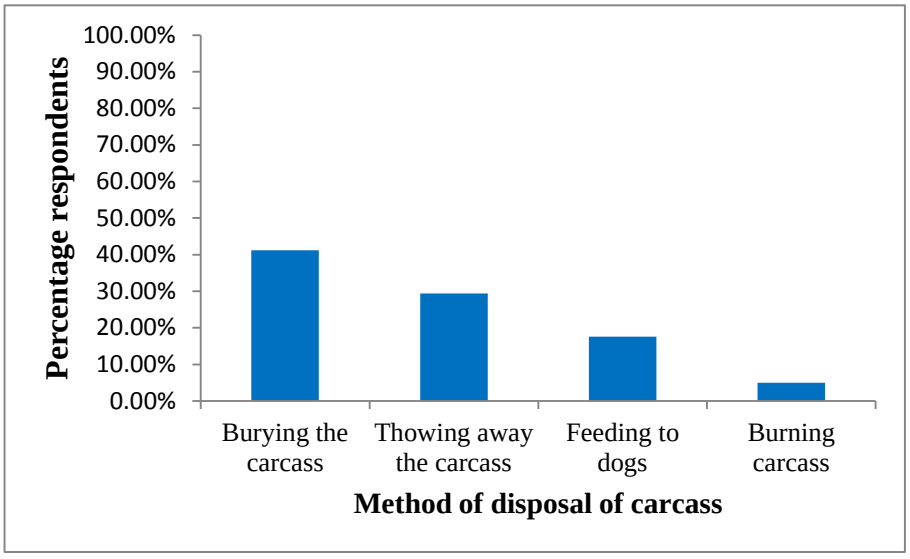


Figure 7: Methods of disposal of dead birds

4.1.5 Poultry productivity and marketing

Most farmers reported 6-7 months as the average time their chicken started laying, with chicken laying between 15 to 30 eggs per clutch and having 2-3 clutches in a year. Seventy eight percent (78%) of the farmers reported that their hens sat on 8 to 12 eggs per incubation time with most of the eggs hatching.

Between July to December was the period most farmers reported a drop in egg production with 76% of them attributing this to lack of feed and the rest attributing it to disease outbreaks.
Eggs had more than one use to the farmers: home consumption (94.0%), selling (59.0%) to get money, hatching (94.0%) and as gifts (35.0%) being the main uses of eggs (Figure 10). Markets (41.0%), hotels (17.6%), homes (17.6%) and village shops (11.8%) were given as the places where the eggs were sold (Figure 8).

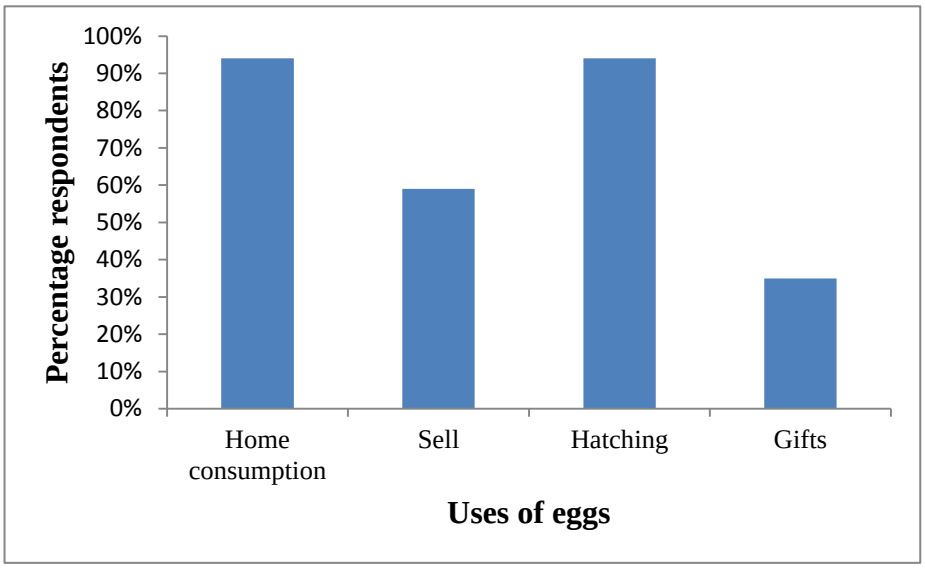


Figure 8: Uses of eggs by farmers

Kenyan shillings (Kshs) 10 was the average price of eggs while the average prices of chicken ranged from Kshs 350 to Kshs 800 for cocks; Kshs 300 to Kshs 700 for hens; Kshs 200 to 600 for pullets; Kshs 150 to 600 for cockerels and Kshs 50 to 250 for chicks. Market, hotels and restaurants, neighbors and brokers were the main customers for the chicken with most farmers having no preference on the criteria for selecting the chicken(s) to sell.

4.1.6 Socio-cultural activities that depended on poultry

Farmers reported that poultry was an important part of most social and cultural activities in ceremonies such as weddings, church events, family gatherings, baby showers and Christmas celebrations. Poultry also served as gift(s) to neighbors and as church offering(s).

4.2 Egg quality

A total of twelve (12) eggs were bought during the wet season (November 2011) and their weights were measured. The weights varied from 38.5 to 48.3g with the average weight being 42.42g. For the dry season, a total of 22 eggs were bought during the month of March 2012 and the weights and circumferences measured. The weights ranged from 34.4g to 54.1g, with a mean of 42.1g while the circumferences ranged from 11.6cms to 13.8cms with a mean circumference of 12.4 cms.

4.3 Seasonality of antibody titers against Newcastle disease

4.3.1 Antibody titers to Newcastle disease virus during the wet season

All the 24 birds tested positive for antibodies against ND with the titers ranging from 1:16 (2^4) to 1:256 (2^8) (Table 2).

Table 2: Hemagglutination inhibition titers of different age groups of chicken during the wet season

Age groups	Number of samples	NDV antibody titer								
		1:2	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**	**GMT**
Chicks	7	-	-	-	1	-	4	1	1	70.66
Growers	8	-	-	-	1	2	4	-	1	53.82
Adults	9	-	-	-	-	1	5	3	-	74.65
TOTAL	**24**	**0**	**0**	**0**	**2**	**3**	**13**	**4**	**2**	**65.85**

Key: **NDV** = Newcastle disease virus

GMT means Geometric mean titer

All of the serum samples were positive for antibodies against ND virus. The chicks had titers ranging from 1:16 (2^4) to 1:256 (2^8) while growers had titers ranging from 1:16 (2^4) to 1:256 (2^8) with majority having titers of 1:64. Adults had titers ranging from 1:32(2^5) to 1:128 (2^7) with majority (5) having titers of 1:64 (2^6).

The geometric mean titer for the group was 65.875.

Comparing the geometric mean antibody titers, there was a significant difference ($P<0.05$) between growers (53.82) and chicks (70.66) and between growers and adults (74.65), with the growers having a lower antibody titer, but no significant difference between chicks and adults ($P>0.05$).

All the birds (100%) showed serological evidence of specific immunity (Table 3) that is 1:8 (2^3) and above. Using the criterion that a titer of 2^4 to2^7 is considered protective, 100% of the birds had protective levels of antibodies (Table 4 and Figure 9).

Table 3: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition test, during the wet season

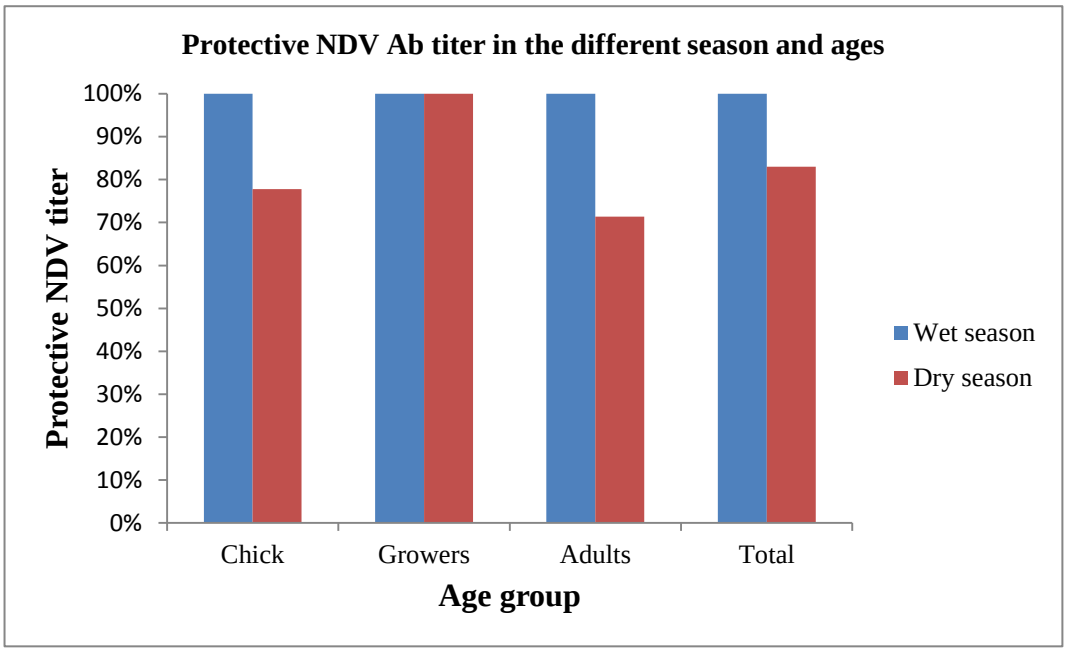
Age groups	**Number of Samples**	**Specific immunity**	**Non specific immunity**	**Percentage specific immunity**
Chick	7	7	-	100
Growers	8	8	-	100
Adults	9	9	-	100
Total	24	24	-	100

Key: **GMT** – Geometric mean titers

NB: Specific immunity is indicated by titers of 1:8 (2^3) and above

Table 4: Percent occurrence of protective levels of Newcastle disease antibodies during the wet and dry season

Age groups	Wet season		Dry season	
	Protective NDV Ab titer ($2^4$to 2^7) (%)	Non protective NDV Ab titer	Protective NDV Ab titer ($2^4$to 2^7) (%)	Non protective NDV Ab titer (%)
Chicks	100	-	77.8	21.2
Growers	100	-	100	-
Adults	100	-	71.4	28.6
Total	100	-	83	17



Key: NDV: Newcastle diseaseND, Ab: antibody

Figure 9: Percent protective levels of antibody titers to NDV in the different age groups in the two seasons

4.3.2 Antibody titers to Newcastle disease virus during the dry season

All the 24 birds tested positive for antibodies against ND with the titers ranging from 1:4 (2^2) to 1:128 (2^7) (Table 5).

Table 5: Hemagglutination Inhibition titers of different age groups of chicken during the dry season

Age groups	Number of Samples	NDV antibody titer								
		1:2	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**	**GMT**
Chicks	9	-	1	1	2	4	1	-	-	20.16
Growers	8	-	-	-	2	-	3	3	-	58.69
Adults	7	-	-	2	2	-	2	1	-	26.25
TOTAL	**24**	**0**	**1**	**3**	**6**	**4**	**6**	**4**	**-**	**31.08**

Key: NDV = Newcastle disease virus

GMT = Geometric mean titer

The chicks had antibody titers ranging from 1:4 (2^2) to 1:64 (2^6) while growers had titers ranging from 1:16 (2^4) to 1:128 (2^7). Adults had titers ranging from 1:8 (2^3) to 1:128 (2^7).

The geometric mean antibody titer for the different age groups during dry season was 31.08. Comparing the geometric mean antibody titers, there was a significant difference ($P<0.05$) between growers (58.69) and chicks (20.16) and between growers and adults (26.25), with the growers having a higher antibody titer than the chicks and adults, but no significant difference between chicks and adults ($P>0.05$).

Over ninety five percent (95.8 %) of the birds showed serological evidence of specific immunity, that is 1:8 (2^3) and above (Allan and Gough, 1974); this included 100% of the growers and adults and 88.8% of the chicks (Table 6). There was a drop in the percentage of protected birds to 83.0 % during the dry season from the 100% in the wet season (Table 4 and figure 9).

Table 6: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition test, during the dry season

Age groups	**Number of Samples**	**Specific immunity**	**Non-specific immunity**	**Percentage-specific immunity**
Chick	9	8	1	88.8
Growers	8	8	-	100
Adults	7	7	-	100
Total	24	23	1	95.8

Key: Protective levels: Levels of antibodies that protect the birds from getting ND ($2^4$to 2^7)

There was a significant difference ($P<0.05$) in the mean antibody titers between the two seasons with the wet season being higher than the dry season. Adults and chicks had significantly higher antibody titers in the wet season than dry season ($P<0.05$) while growers had no significant difference in levels of antibody titers between the two seasons.

4.4 Isolation of Newcastle disease virus

No virus was isolated from swabs and tissues from the birds from the initial inoculation in embryonated eggs.

4.5 Determination of effect of endo- and ecto-parasite control on the chicken's response to Newcastle Disease vaccination and production (weight gains or losses)

4.5.1 Pre-treatment examination results

The eight (8) birds sacrificed before the start of the experiment showed the following: (1) that 62.5% of the birds had lice and this was the only ecto-parasite isolated (Tables 7 and 8), and (2) they had 100% endo-parasite prevalence, Individual prevalence per the species showed that 62.5% of the total birds had tapeworms (62.5%), 100% of the total population had caecal worms and 37.5 % of the total population had *Gongylonema* species.

4.5.2 Ectoparasites in treated groups

Groups 2, 4, 5 and 7 were treated for ecto-parasites using a combination of permethrin and sevin (Cabaryl). At post-mortem, all the birds in the treated groups had no ecto-parasites found on examination while untreated groups 1, 3, 6 and 8 still had high carriages of ecto-parasites ($P<0.05$) (Table 7).

Table 7: Ecto-parasite prevalence in the preliminary, control and treatment groups

Group	Overall prevalence (%)	Prevalence by species (%) *M.stramineus*	*M.gallinae*	*G.gallinae*
Preliminary findings				
	62.50	100	0	0
Non vaccinated groups				
1	100	100	25	37.5
2	0	0	0	0
3	100	100	30	0
4	0	0	0	0
8	60	100	0	0
Vaccinated groups				
5	0	0	0	0
6	100	100	0	0
7	0	0	0	0

Key: *M.stramineus= Menacanthus stramineus, M.gallinae = Menopon gallinae, G. gallinae = Gonoicoites gallinae*

4.5.3 Endoparasites in treated groups

Groups 3, 4, 6 and 7 were treated using albendazole (20mg/kg body weight), after treatment all the birds had no helminths with the exception of *Gongylonema inguivicola* species that was embedded in mucosa of the crop (Table 8). The non treated groups still had varied helminth species with a high parasite carriage compared to the treated (Table 8).

Table 8: Prevalane of endoparasites in the preliminary, control and treatment groups

Group	Overall prevalence (%)	Prevalence by species out of the total number (%)				
Preliminary findings		**TA**	**G.I**	**S.S**	**H.I**	**R.E**
	100	0	37.5	100	0	62.5
Non Vaccinated						
1	100	0	37.5	100	0	87.5
8	100	20.0	80.0	60.0	60.0	60.0
2	80.0	20.0	40.0	100	60.0	80.0
3	0	0	0	0	0	0
4	33.3	0	33.0	0	0	0
Vaccinate d						
5	100	0	33.0	100	33.0	100
6	20.0	0	20.0	0	0	0
7	50.0	0	50.0	0	0	0

Key: TA=*Tetrameres americana* GI= *Gongylonema inguivicola* S.S= *Subulura suctoria*

HI= *Heterakis isolonche* RE= *Raillietina echinobothrida*

4.5.4 Hemagglutination inhibition test results

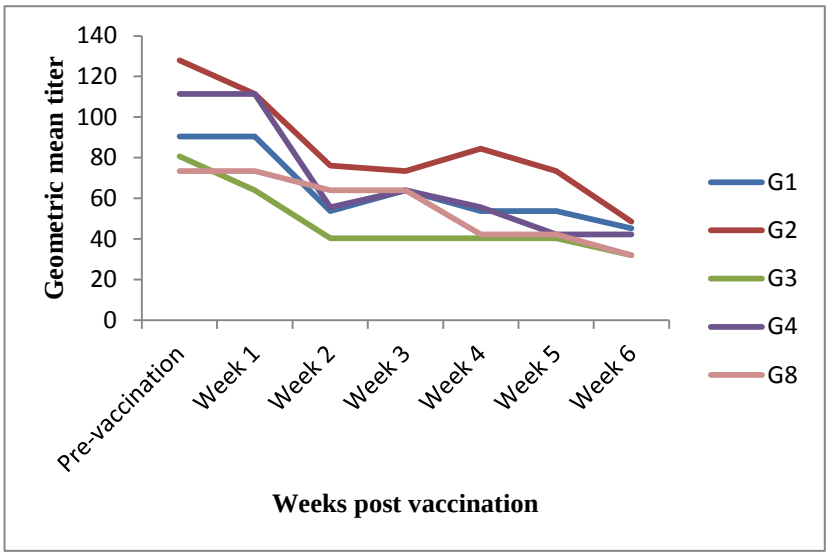
All the chicken used in this study were positive for specific NDV antibodies pre-vaccination, with the individual GMT of the groups shown in Table 9. Throughout the vaccination period, the vaccinated groups 5, 6 and 7 showed a significant rise ($P<0.05$) in the antibody levels compared to the respective control groups. Thus, while the antibody titers in the control groups were dropping, titers in the vaccinated groups were rising. From week 3 post vaccination, group 7 had

a significantly higher antibody level (P<0.05) than the other vaccination groups; this prevailed all the way to the end of the experiment (Figures 10 and 11). The vaccinated groups showed a steady rise up to week 3, where group 7 acquired a higher level compared to the other two groups 5 and 6 respectively. Group 6 had a lower level than the other two vaccinated groups but was level with group 5 from week 4 to week 6.

Control groups 1, 2, 3, 4 and 8 had a drop in the pre vaccination NDV antibody titers to week 6, though this was not statistically significant at the end of the 6 weeks compared to the prevaccination (P>0.05) (Table 9).

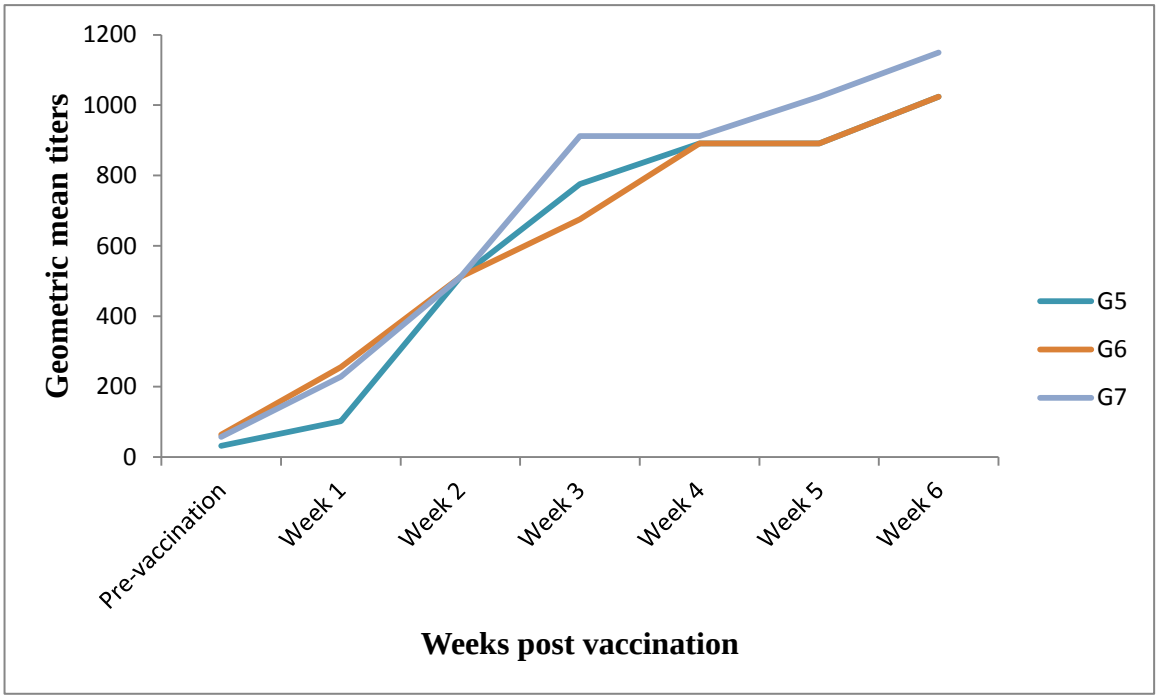
Table 9: Geometric mean titers of the different groups in relation to pre and post Vaccination periods

Group		**Prevaccination**	**Post vaccination**					
			Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
G1	GMT	91	91	54	64	54	54	45
	SD	165	165	42	78	82	75	78
G2	GMT	128	111	74	74	84	74	49
	SD	70	28	53	53	45	43	18
G3	GMT	81	64	40	40	40	40	32
	SD	37	49	18	18	18	18	0
G4	GMT	111	111	56	64	56	42	42
	SD	78	78	97	48	40	45	23
G5	GMT	32	256	612	776	891	891	1024
	SD	49	194	0	280	228	627	560
G6	GMT	64	256	517	776	891	891	1024
	SD	35	171	280	280	228	228	560
G7	GMT	57	228	512	912	912	1024	1149
	SD	91	140	280	561	209	503	627
G8	GMT	74	74	64	64	42	42	32
	SD	51	51	48	48	46	46	49



Key: G1= Group 1, G2= Group 2, G3= Group3, G4= Group 4, G8= Group 8

Figure 10: Geometric mean titers of control group in relation to time



Key: G5= Group 5, G6= Group 6, G7= Group 7

Figure 11: Geometric mean titers for vaccinated group with respect to time post vaccination

4.6 Weight gains after vaccination

The birds in each group had a gain in weight attaining a higher average weight at the end of the experiment than at the beginning. There was no significant difference in weight gain among the different groups (Table 10)

Table 10: Average weight in grams for the different groups

	Average weights					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	525±66	585.8±159	578±142	553.5±134	706±164	788±191
2	520±96	522±106	536.2±126	551.2±150	676.2±202	710.6±200
3	466.7±160	506±65	540.7±80	595±87	798.7±92	838±27
4	458.3±115	526.2±155	565.8±167	613.2±138	704.2±131	799.3±149
5	455±124	537.2±182	481.4±201	535.4±193	672.2±274	678.6±281
6	545±145	493.8±127	469±137	544±153	710.2±160	779.8±166
7	533.3±124	651.2±181	596.2±175	638.7±163	767.5±169	779.3±174
8	457±33	532.8±82	529.6±74	548.6±76	724.8±112	761.4±136

There was no significant difference in association between the prevalence of ectoparasites after treatment with the weight gains and geometric mean titer with time (P=0.8237). There was also no significant difference in the prevalence of endoparasites after treatment with the weight gains and geometric mean titer with time (P=0.0878), and no significant difference in association between overall parasite prevalence after treatment, the mean antibody titers and the weight (P=0.2261)

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECCOMENDATIONS

5.1 DISCUSSION

The study showed that domestic indigenous poultry production has a number of constraints with the major ones being diseases, parasites, lack of feed, predation and accidents, similar to what was reported by Njagi *et al*. (2010a) in the same area. ND was reported as the major disease constraint, as previously reported in other countries (Awan *et al*., 1994; Zeleke *et al.,* 2005; Otim, 2005). Njagi *et al*. (2010a) also reported similar findings in the area five years back, indicating there has been no dynamic change. Factors such as management and handling of sick birds, disposal of carcasses, confinement, introduction of new birds from the market and wind have been associated as the major risk factors in ND outbreaks (Njagi *et al.* 2010a) and it has also been reported that live bird markets are a major source of infected poultry (Alexander, 1998). These practices are common in the area and had also been reported earlier by Njagi *et al*. (2010a), with sale and salvage of sick birds, throwing carcasses to the bush or feeding to dogs, introducing new entrants to flock without isolation, and confining birds of all ages together during certain times of the year being the main ones that could lead to spread and maintenance of the ND virus in the flock. Some farmers were observed to rear ducks together with chicken during the questionnaire interview and in so doing increasing the risk of ND outbreaks since ducks have been shown to shed ND without showing any clinical signs (Njagi *et al*., 2012). This indicates that minimal, if any, extension services are practiced in the area; hence the problems still remain the same.

Vaccination is not carried out by most village farmers as was the case during the study. Similar to previous report by Njagi (2008) and also by Otim (2005) in Uganda, this increases the risk of

the birds coming down with ND, as vaccination is the only sure method of preventing ND (Alexander, 2003).

No farmer associated wild birds with ND outbreaks even though most homesteads had wild birds nesting and interacting with the chicken. Wild birds have been previously associated as possible sources of ND infections (Olabode *et al.,* 1992).

The indigenous chicken, in this study, had good productivity with the birds having between 2-3 clutches per year, each of 15-30 eggs. This observation agrees with that of Bebora *et al*. (2005) who compared indigenous hens with commercial layer birds brought for show exhibition at the Agricultural Society of Kenya Show, Nairobi, assessing their egg-laying capacity using pliability of their pubic bones. They found that some of the studied indigenous hens were very close to, while others had higher laying capabilities than the respective exotic ones. Lack of feed was quoted as a major factor affecting egg production, especially during the period between July and December; it resulted in drop in egg production, and was due to confinement of the birds in the planting season. This has not been reported in previous studies in the area. Most of the eggs produced were for home consumption, with a few being sold mainly in the local markets, in village shops, and directly to neighbors. If managed well, these birds could contribute and assist in improvement of nutrition and human health in rural population. The fact that chicken prices ranged from Kshs 50 for a chick to Kshs 800 for an adult cock, could easily contribute towards improving the economic status of the farmers; leading to poverty alleviation . Apart from income, chicken have socio-cultural importance to the community in ceremonies such as weddings, church events, family gatherings, baby showers and Christmas celebrations. Poultry also served as gift(s) to neighbors and as church offerings, thus, prevention of poultry losses is important in empowering the farmers and improving their social status. These socio-cultural

practices can also be a source of spread for the virus, between homesteads during outbreaks. It is, therefore, important to educate the farmers on this, as part of the control measures for ND viral spread.

Overall the birds had high levels of antibody titer during both the wet and dry seasons, an indication of endemicity of the virus in the village chicken in Mbeere, as previously reported by Njagi *et al*. (2010b), who worked in the same area , Otim (2005) in Uganda and Zeleke *et a*l. (2005) in Southern and Rift Valley districts in Ethiopia. Using Allan and Gough (1974) criterion which states that "a titer of 1:8 and above is generally accepted as indicative of specific immunity", most birds had specific immunity meaning they had come in contact with the NDV. The wide range of NDV titer, shown in this study, may be due to natural infection which is known to produce higher antibody titers than vaccination (Luc *et a*l., 1992). The continued hatching of chicks, which was the common re-stocking practice in this area, seemed to continuously provide susceptible chicken which could be infected by those that survived previous ND outbreak(s); an observation which was also reported by past researchers (Martin, 1992). This may have been the case during the wet season where the farmers reported an outbreak of a disease similar to Newcastle and serum samples from the birds showed high ND antibody titers; implying that chicken in this area got infected at different times producing a near cyclic pattern of disease, hence maintaining an endemic situation throughout the year (Otim, 2005; Njagi, 2008). Free range management system that allows the uninterrupted cycle of infection as the virus passes from one chicken to another may also be a cause of this endemicity, as suggested by Zeleke *et al*. (2005). The chicken may also acquire infection(s) from wild birds and in some instances from ducks, which some farmers kept together with chicken in Mbeere. Ducks have been shown to harbor and shed the NDV without showing any clinical signs of the

disease (Njagi, 2008). Methods of disposal of sick and dead birds such as throwing to the bush and feeding to dogs, practiced by some of the farmers in the area, which was also recorded by Njagi *et al*. (2010a) in the same area and Otim (2005) in Uganda, also aided in the spread of the disease and maintenance of the endemic status of the disease .

Both wet and dry seasons have been associated with ND outbreaks in Kenya (Nyaga *et a*l., 1985). Start of wet season has been associated more with outbreaks (Jintana, 1987); in Vietnam (Nguyen, 1992) and Uganda (Mukiibi, 1992) higher seasonal incidence and severity of ND is reported in dry season. This is in line with Martin (1992) suggestion that outbreaks are often associated with change in season especially between wet and cold weather. In this study, ND outbreak may have occurred during the wet season hence the higher antibody titers during the wet than the dry season. This is supported by Awan *et al*., (1994)'s conclusion that ND is associated with periods of stress, which could be due to change in climate and lowered resistance at the beginning of wet season, due to inadequate feed. The lowered resistance, in the current study was indicated by decrease in antibody titers in the dry season; coupled with increase in the number of susceptible birds. Concentration of HI antibodies has been reported to decline within 3-4 months of non-stimulation (Otim, 2005).

From this study, the maintenance of the cyclicity of the disease in Mbeere chicken can, therefore, be linked to two factors; availability of susceptible population of chicken and lowered immunity, as manifested by lowered antibody titers. This is supported by the observation that was made during the study period. In the wet season (November, 2011), the number of birds per homestead was low, with some homesteads having as few as two birds; most of them being adults. This low number could be attributed to an outbreak of ND that had caused high mortalities, all the 24 birds that were screened for ND antibodies, within this season, turned positive with high antibody

titers. Contrary to this, during the dry season (March 2012), the number of birds had increased significantly, the flock composition constituted mostly growers and chicks and most of the hens were either brooding or incubating. This could be explained by the fact that dry season was the harvesting time; there was, therefore, abundant grain harvest. With improved nutrition, there was increased egg laying and hatching; enabling the farmers to restock their flocks that had gone down during the wet season. Restocking resulted in increased number of chicks which ended up being susceptible to the disease, as observed during the study in November 2011. This fuelled ND outbreaks and maintained the virus within the recovered chicken; which was used as source of infection for the next cycle. The maternal antibodies, if any were passed to the chicks, waned off within 3-4 months (Otim, 2005).

Village chicken scavenge for their feed with little supplementation, this scavenging behaviour encourages the spread of ND. Otim (2005) associated socio-cultural activities in rural households with ND outbreak. The ND dynamics appear to depend on regional and community activities and need to be controlled regionally rather than country wide.

Adults and chicks had significantly higher titers compared to growers during the wet season and significantly lower titers during the dry season; similar to what Njagi *et al.* (2010a) reported. This was possibly so because chicks could have gotten maternal antibodies from immunized hens through the eggs or through body contact during feeding and drinking (Mwakapuja, 2009). The low antibody levels in the two groups during the dry season may be due to the low levels of antibodies in adults that will correspond to the low levels in eggs and hence chicks. Huchzermeyer (1993) noted that brooding hens and hens with chicks that were kept segregated may also escape infection, this may, however, be due to lack of exposure to the virus.

The lack of isolation of the virus from the birds may be due to neutralization (clearance) of the virus by the high levels of protective antibodies (Alexander, 1997). Healthy looking birds may harbour virulent NDV but if they have high antibody titer this may prevent them from having clinical disease (Njagi *et al.*, 2010a). Njagi *et al.* (2012) suggested that ducks with protective levels of antibodies ($2^4$to 2^7) may not develop clinical disease but instead remain virus carriers; however, when immunosuppressed, these experimental ducks were shown to shed the virus. This could also happen in chicken and birds with antibodies that may shed and act as source of infections to other susceptible birds during periods of stress. The non-isolation of the virus from the oropharyngeal and cloacal swabs, trachea, caecal tonsils and spleen could also be due to the viral titer having been very low, to the extent that it could not be detected by the isolation method that was used (Alexander, 1997).

This study showed that the indigenous chicken from the study area had a wide range of parasites as reported by Maina (2005) and Sabuni (2009). Various species of lice, *Menacanthus stramineus, Menopon gallinae* and *Goniocoites gallinae,* were the only ecto-parasites found on the birds. This does not quite match what Sabuni (2009) found: Apart from lice, he also documented fleas and mites from chicken in the study area. The chicken, in the current study, also had a high prevalence of endoparasites (100%), similar to the findings of Maina (2005). She reported a prevalence of 90% with nematodes of the species; *Heterakis, Gongylonema, Tetrameres, Acuaria* and *Ascaridia* and a cestode; *Railletia echinobathrida* being the isolated species. In this study, *Railletia echinobathrida* (cestode), *Heterakis isolonche, Subulura suctoria, Tetrameres americana* and *Gongylonema inguivicola* (roundworm species) were recovered. No *Acuaria* and *Ascaridia galli* were recovered; this may be attributed to the fact that most farmers reported using piperazine (Ascarex®) for deworming – it was the only dewormer used

(unpublished questionnaire study results). Piperazine has a narrow range of activity, which acts on *Ascaridia galli* and a few other round worms but not on tapeworms and caecal worms (Arends, 2003).

A combination of sevin® (Cabaryl) and permethrin, was used to treat against ectoparasites. It was very effective; eliminating all the ectoparasites; this underscores the effectiveness of the combined drug method. Application of the treatment on monthly basis should be recommended to avoid re-infection. Albendazole at a dose of 20mg/kg body weight, repeated after a month, was shown to be effective and safe against most of the helminths, both nematodes and cestodes. This is in accordance with a finding by Tucker *et al.* (2007) who showed it to work against *Ascaridia galli, Heterakis gallinarium, Capillaria obsignata* and *Raillietina cesticillus.* In this study it was found to effective on more worms including *Subulura* and *Tetrameres* species. The drug had mimimal effect against *Gongylonema inguivicola*. It is possible that the anthelmintic may not have reached in adequate doses where the parasite was, since *G. inguivicola* is normally found under the mucosa of the oesophagus and crop. The mucosa may have protected it from being exposed to lethal dose of the anthelmintic.

Strict biosecurity cannot be effectively implemented in domestic village chicken hence vaccination is the ideal method for ND control (Otim, 2005). However certain factors affect immunity of the chicken and may adversely affect post vaccination immune response. Immunosuppressants such as infectious bursal disease, aflatoxicosis and parasites can affect immune response and can lead to vaccination failure (Otim, 2005; Permin and Hansen, 1998). There was a significant difference between the humoral response of vaccinated and respective non vaccinated groups in this study indicating that vaccination boosted the immune response of

the birds and is the sure method of controlling NDV, an observation supported by Alders and Spradbrow (2001). Where treatment was done, separately or combined, the HI titers were significantly higher than those of the respective control birds. Also, combined ecto- and endo-parasite treatment resulted in chicken that had significantly higher HI titers than in cases where ecto- and endo-parasite infections were treated separately. Hørning *et al*., (2003) had earlier showed that control of helminth parasite improved ND vaccine immune response; he however did not control ecto-parasites as in this study. Permin and Hansen (1998) showed that heavy ascarid parasitism lowered the humoral immunity to ND. This study has gone further to demonstrate the lowering of immunity caused by both ecto- and endoparasites. The synthesis of immunoglobulins is reduced in animals severely affected by parasites, owing to an absolute loss in protein (Tizard, 1996). This might result in reduced antibody response as seen in this study and that by Hønning *et al*. (2003). There was no significant difference between chicken that had only ecto-parasite control and those that had only endo-parasite control done, with respect to immune response, indicating that ecto- and endo-parasites may have similar effect on the immune response.

There were different responses to vaccination among individual birds in the vaccinated groups; this may, however, be an idiosyncrasy because local chicken ecotypes have divergent responses towards ND vaccine, with some demonstrating high selection for antibody response to vaccine as well as early response (Beard and Hanson, 1984) hence the variation.

A decline in the levels of antibody titers for the control groups is a natural phenomenon, since not vaccinated; this has also been reported to take about 3-4 months by Otim, (2005).This study showed a rise in antibody level in groups where both ecto- and endo-parasites were controlled than where only ecto- and endo-parasites were controlled, respectively, but there is need for the

study to be repeated and be carried out for longer period, so as to be able to check if there was a significant change in the antibody levels later on.

There was no significant association between the parasite prevalence, geometric mean titer and weight gains indicating that total parasite control improved the immune response against ND but had no significant difference to the weight gains and productivity. This may be attributed to the change in feed and management system of the birds from free range to caged system. Indigenous chicken also take time to grow and a longer time may be needed to assess the effect of the parasite control and vaccination on weight gains.

Faecal samples taken at the beginning of the experiment showed that all the birds had coccidiosis at a lower level (+) but later there was a flare up leading to some mortality. This flare up may be due to confinement that led to stress and icreased build up of infective sporulated oocysts as reported by Alexander (1998), since the birds had been used to free range system. Other factors that could have contributed to this infection are transport, change in weather, change in feed and handling during vaccination and bleeding (Alexander, 1997).

This study has thus demonstrated presence of various types of ecto- and endo-parasites in Mbeere chicken and endemicity of ND in the area. It has also shown that total parasite control improves the birds' immune response to ND vaccination. This information will enable formulation of parasite control strategies that will result in effective vaccination for the Mbeere chicken, which will, in turn, result in increased production and alleviation of poverty in the rural areas, in line with vision 2030.

5.2 CONCLUSIONS

Based on the findings, it can be concluded that:

- Indigenous poultry are normally kept on free range system in the study area in Mbeere sub-county. These birds play a significant role in the economic, social and cultural well being of the community.
- is the most significant constraint in poultry production in the region.
- The indigenous poultry have good productivity with good quality eggs.
- In all seasons birds have high titers, the antibodies tend to wane off during the dry season making the birds susceptible to introduction of velogenic strain of NDV.
- Parasite control results in improved immune response to ND vaccination; a comprehensive parasite treatment giving better results than partial treatments (for ecto- or endo-parasites only).
- If regular comprehensive parasite control is exercised birds will respond well to ND vaccination, hence be protected from the disease; they will be free from stress, caused by the parasites; and their productivity will improve. The resultant effect of this will be more income to the farmers, which will eventually translate to improved economy for the country as a whole. The farmers and their families will also get enough proteins, will be healthier, and hence be able to work better to build the country's economy.
- Albendazole at a dosage of 20 mg/kg body weight repeated monthly is both safe and effective in elimination of most helminths with the exception of *Gongylonema inguivicola* and hence manufacture of r poultry formulation will be beneficial.

- Treatment, by dusting, of the ecto-parasites using a combination of sevin® and permethrin is effective against all the ecto-parasites. Application of the treatment on monthly basis should be recommended to avoid re-infection.

5.3 RECOMENDATIONS

- Vaccination is recommended during the start of dry season to maintain high levels of antibodies and prevent outbreaks, especially in chicks and adults.
- Education of flock owners on disease transmission and prevention and discouraging them from restocking their farms with chicken from the market.
- Treatment, by dusting, of the ecto-parasites using a combination of sevin® and pemethrin repeated monthly should be recommended for total control.
- Albendazole at a dosage of 20 mg/kg body weight repeated monthly by drenching should be recommended for use in helminth control.
- Total parasite control before ND vaccination should be recommended for effective immune response to the vaccine.

REFERENCES

Alders, R.G and **Spradbrow, P.B (2001**). Controlling Newcastle disease in village chicken: Field Manual. Canberra, Australian centre for international Agricultural research. Monogram **83**: 112.

Alexander, D. J. (1997). Newcastle disease and other Paramyxoviridae infections. In: Diseases of poultry, 10th edition. B W Calnek, H J Barnes, C W Beard, W M Reid and H W Yoder, editors. Iowa State University Press, Ames, Iowa. pp. 541 – 570.

Alexander, D.J (1998). Newcastle disease and other Paramyxoviridae in; A laboratory manual on isolation and identification of Avian pathogens, 4th edition. D.E Swayne, J.R Glisson, J.E Pearson and M.W Reed, eds. American Association of Avian pathologist, Kennel square, P.A pp 156-163.

Alexander, D.J (2003). Newcastle disease, other Paramyxoviridae and Pneumovirus Infections. In; Diseases of Poultry 11th Edition. Saif, Y.M., Barnes, H.J., Glossons, G.R., Fadly, M.A McDougald, D.J., and Swayne, D.E (Eds), (pp. 63-100), Iowa State press, Ames.

Allan, W.H and Gough, R. H (1974). A standard hemagglutination inhibition test for Newcastle disease; a comparison of macro and micro methods. *Veterinary Records*. (**95**): 120 – 123.

Allan, W.H, **Lancaster, J.E** and **Toth**, **B (1978).** Newcastle disease vaccine-there production and use. Animal production and health services. No 10. FAO. Rome.

Anderson ,S (2001). Animal genetic resources and sustainable livelihoods. In: International Livestock Research Institute Special Issue on Ecological Economics. Article no. 3.

Arello, S.E (1998). The Merck's Veterinary Manual, 8th edition. Merck's and Co. Whitehouse station, USA. 1917-1923.

Arends, J.J (2003). External parasites and poultry pests in: Diseases of Poultry. 11th edition. Saif, Y.M, Barnes, H.J, Glissen, J.R, Fadly, A.M, McDougald, L.R and Swayne, D.E (Eds). Iowa state press, A Blackwell publishing company, Ames, Iowa pp 905-930

Awan. M. A., Otte, M. J. and James, A. D. (1994). The epidemiology of Newcastle disease in rural poultry: a review. *Avian Pathology*, **23**: 405-423.

Beach, J.R (1942). Avian pneumoencephalitis. Proceedings of US livestock situation Association, **46**. 203.

Beard, C. W and **Hanson**, **R.P (1984).** Newcastle disease. In: Diseases of poultry. M.S Hofstad, H.J Barnes, B.W.Carnek (eds).

Bebora, L.C, Mbuthia, P.G, Macharia, J.N, Mwaniki, G. Njagi, L.W and **Nyaga, P.N (2005)**. Appraisal of indigenous chicken potential in egg production. *The Kenyan Veterinarian*, **29**:10-13.

Charlton, B.R (2006). Necropsy of the fowl; In: Avian disease manual. 6th edition, A.J Bermudez, M. Boulianne, D.A Halvolson, J.S Schrader, L.J Newman, J.E Sander and P.S Wakennel (eds) American Association of Avian pathologists, pp 232-233.

Cumming, R.B (1992). Village Chicken Production: Problems and potential. In: Proceedings of Newcastle Disease in Village Chicken.

Doyle, T.M (**1927).** A hitherto unrecorded disease of fowls due to filter-passing virus. *Journal of Comparative Pathology and Therapeutics*, **40**: 144- 149.

Foster, H.A, Chitukulo,H.R, Tuppa, E. Mwanjala, T and Kusila (1999). Thermostable Newcastle vaccine in Tanzania. *Veterinary Microbiology*, **68**: 127- 130.

EMEA (The European Agency for the Evaluation of Medicinal Products) Veterinary Medicines and Information Technology (2001). Efficacy of anthelmintic: Specific recommendations for poultry. http://www.eudra.org/emea.html

Gibbons, L.M, Junes, A and Kahlil, L.F (1996). Laboratory Manual for Eight International course on identification of parasites of economic importance. CABI institute of Parasitology U.K.

Hanson, R.P (1988). Heterogenecity within strains of Newcastle disease virus: Key to survival. In D. Alexander (Ed). Newcastle disease Kluwer Cademic publishers; Boston, M.A 113-130.

Hitchner, S.B and **Johnson, E.P (1948**). A virus of low virulence of immunizing fowl against Newcastle disease. *Veterinary Medicine*; **43**: 525-530.

Hørning, G, Rasmussen, A, Permin, A and **Bisgaard, M (2003).** Investigation of influence of helminths parasites on vaccination of chicken against Newcastle disease virus under village conditions. *Tropical animal health and production* **35**: 415-424.

Huchzermeyer, F. W. (1993) Why is velogenic Newcastle disease endemic in some countries and not in others. *Zimbabwe Veterinary Journal*, 24:111 – 113.

Jintana, D. (1987). Thailand; Poultry production. In: Newcastle disease in poultry.Cupland, J.W (Eds). A new food pelleted . ACIAR monograph. No. **5**. ACIAR, Canberra, 108-109

Kasiti, J.L (2000). Isolation of Avian paramyxovirus from village chicken and wild birds in Kenya. Msc thesis. University of Nairobi.

KNBS (Kenya National Bureau of Statistics) (2009). Kenya population census report.

Luc, P.V., Hong, N.T and Chinh, V.T (1992). Levels of anti-Newcastle disease virus antibodies in industrial poultry at various ages and seasons. *Journal of Agricultural and Food Industrial Organization* (*Agr.Food Ind*.,) **9**:348-350.

Maina, A.N (2005). Prevalence, intensity and lesions associated with gastrointestinal parasites of indigenous chicken in Kenya. Msc thesis, University of Nairobi.

Martin P A J (1992). The epidemiology of Newcastle disease in village chickens. *In*: P.B Spadbrow (Ed). In: Proceeding of the 39th international workshop on Newcastle disease in village chickens. Control with thermostable oral vaccines, 1991 (pp 40-45). Kuala Lumpar, Malaysia: Australia centre for international Agricultural research (ACIAR).

Mcferran, J.B and **McCracken, R.M (1988).** Newcastle disease. In D.J Alexander (Eds). Newcastle disease, Boston, M.A: Kilswer Academic publisher, 168-183.

Msoffe, P.L and **Cardona, C.J (Eds) (2009).** Poultry Disease Handbook for Africa.

Mukiibi, M.G. (1992). Epidemiology of Newcastle and the need to vaccinate local chicken in Uganda. In P.B Spadbrow (Ed). Proceeding of the 39th international workshop on Newcastle

disease in village chicken. Control with thermostable oral vaccines, 1991 (pp 155-158). Kuala Lumpar, Malaysia: Australia centre for international Agricultural research (ACIAR).

Mwakapuja, S.R (2009). Disease surveillance on village chicken vaccinated against Newcastle disease in rural communities in Morogoro Tanzania. Msc dissertation Sokoine University of Agriculture, Morogoro, Tanzania.

Nairobi provincial livestock officer (2007). Livestock population figures. *Annual report.*

Ndegwa, J.M, Kimani, C.W, Siaba, D.N, Mukisira, E.A and **Rejong, R (1998).** Characteristics of rural production in different agro ecological zones in Kenya. In the Proceedings of the 6th Biennial KARI scientific conference. Agricultural research development for sustainable resources management and increased production. 9th-13th November, pp 540-547.

Nguyen, T.D. (1992). Poultry production and Newcastle disease in Vietnam. In P.B Spadbrow (Ed). Proceeding of the 39th international workshop on Newcastle disease in village chicken. Control with thermostable oral vaccines, 1991 (pp 171-173). Kuala Lumpar, Malaysia: Australia centre for international Agricultural research (ACIAR).

Njagi, L.W (2008). Endemicity of Newcastle disease virus in village indigenous chicken and the role of carrier ducks. PhD thesis, University of Nairobi.

Njagi, L.W, Nyaga, P.N, Mbuthia, P.G, **Bebora, L.C, Michieka, J.N, Kibe, J.K** and **Minga, U.M (2008).** Newcastle disease virus and antibody levels in matched sera, ovules and mature eggs in indigenous village hens. *The Kenya Veterinarian,* **32** (1): 1-6.

Njagi, L.W., Nyaga, P.N., Mbuthia, P.G., Bebora, L.C., Michieka, J.N., and Minga, U.M. (2010a). A retrospective study of factors associated with Newcastle disease outbreaks in village indigenous chicken. *Bulletin of Animal Health and Production in Africa,* **58(1)**. http://ajol.info/index.php/bahpa.

Njagi, L.W, Nyaga, P.N, Bebora, L.C, Michieka, J.N, Mbuthia, P.G., Kibe, J.K and **Minga, U.M (2010b).** Prevalence of Newcastle disease virus in Village indigenous chicken in varied agro-ecological zones in Kenya. *Livestock Research for Rural development*, **22 (5)**:

Njagi L W, Nyaga P N, Bebora L C, Mbuthia P G and Minga U M (2012). Effect of Immunosuppression on Newcastle Disease Virus Persistence in Ducks with Different Immune Status. ISRN Veterinary Science,

Njue, S.W, Kasiti, J.L, Macharia J.M, Gachere, S.G, and Mbugua, H.C.W (2002). Health and Management improvement of family poultry product in Africa-Survey results from Kenya. Pp 39-45. In characteristic and Parameters of family poultry production in Africa. IAEA. Vienna. Austria.

Njue, S.W, Kasiti, J.L, Macharia M.J, Gacheru, S.G and Mbugua, H.C (2002). Health Management Improvements of family poultry production in Africa- Survey results from Kenya pp 39- 45. In. Characteristic and parameters of family poultry production in Africa. IAEA. Vienna, Austria.

Nyaga, J.M, Nyaga, P.N and Kariuki, D.P (1985). Epidemiology of Newcastle disease in Kenya. *Bulletin of Animal health and production in Africa***, 33.** 249-251.

Office international des Epizooties (OIE) (2000). Newcastle disease. In: Manual of standards for diagnostic tests and vaccines, 4th ed. OIE, Paris. Pp. 221-232.

Olabode, A.O, Lamorde, A,G Shidala, N.N and Chikeudu, A.A (1992). Village chicken and Newcastle disease in Nigeria. Australian Centre for International Agricultural Research proceedings no **39** pp 159-160.

Onduru, D.D, Gachimbi, L, Maina, F, Muchena, F.N and A. der Jager (2002). Sustaining Agricultural Production in semi-arid areas of Eastern Kenya. *A Case study of Mbeere District. Integrated Nutrient Management to Attain Suitable Productivity (INMASP)* report No. Ke-03

Otim M O (2005). Aflatoxicosis, infectious bursal disease and immune response to Newcastle disease vaccination in rural chicken. PhD thesis. Royal college

Permin, A and Hansen, J.W (1998). Epidemiology, diagnosis and control of poultry parasites. FAO. Rome pp 1-157.

Russel, P.H and Koch, G (1993). Local antibody- forming cell response to Hitchner B1 and Ulster strain of Newcastle disease virus. *Veterinary immunology and immunopathology*, **37**; 165-180.

Sabuni, A.Z (2009). Prevalence, intensity and pathology of ecto and hemo parasites infection in indigenous chicken in Eastern, Province of Kenya. Msc thesis, University of Nairobi.

Soulsby, E.J.L (1982). Helminths, arthropods and protozoa of domestic animals. 7th Edition. London: Baillire and Tindall, East Sussex, UK.

Spadbrow (1992). Newcastle disease in village chicken. Control with thermostable oral vaccine. Proceeding of an international workshop, Kuala lumpar, Malaysia. ACIAR, Canberra.

Spradbrow, P.B (1999). Epidemiology of Newcastle disease and economics of its control. In, Proceedings of a workshop on poultry as a tool of poverty eradication and promotion of gender equality, F. Dolberg and P.H Peterson (Eds), Tune, Denmark 165- 173.

Stotz, D (1993). Farm Management Handbook of Kenya IV: Production techniques and economies of small holder livestock production system in Kenya. Ministry of Livestock Development, Animal Production division, Nairobi. Pp 140.

Tizard,I,R (1996). Veterinary Immunology, An introduction. 5th ed. W. B. Saunders Company. Philadelphia, Pennsylvania. USA. Eds. pp 280-283.

Tucker, C.A, Yazwinski, T.A, Reynolds, L, Johnson, Z and Keating, M (2007). Determination of the anthelminthic efficacy of albendazole in the treatment of chicken naturally infected with gastrointestinal helminths. *Journal of Applied Poultry Research* **16**: 392-396.

Yolken, R.H (1982). Enzyme Immunoassay for detection of infectious agents in body fluids, current limitations and future prospects. *Review of infectious Diseases*, **4**: 35-68.

Yongolo, M.G, Macau, M and Minga, U.M (1997). Newcastle disease and infectious bursal disease among free range village chicken in Tanzania. Msc Dissertation, Sokoine University of Agriculture, Morogoro. Tanzania.

Zeleke, A, Sori, T, Gelaye, E and Ayelet, G (2005). Newcastle Disease in village chicken in Southern and Rift Valley Districts in Ethiopia. *International Journal of Poultry Science*, **4 (7):** 507-510.

APPENDICES

Appendix 1: Questionnaire on socio-economic importance and productivity of poultry and Newcastle disease in indigenous chicken in Mbeere sub-county, Kenya

Questionnaire No..... Date of interview..... Name of interviewer.....

A) BACKGROUND INFORMATION

1. Name of the homestead ……..……..
2. Location.....Sub- location.....Village.....
3. Number of chicken owners in the homestead: (1) male (….), (2) female (….)
4. Name of person interviewed (respondent).....Sex 1=male 0=female
5. Respondent's age group: (1) up to 30 years (2) >30 – 60 years (3) over 60 years
6. Occupation of the respondent: (1) farmer, (2) trader, (3) employee, (4) others (specify)……..
7. What is the relationship of respondent to the household head? (1) self (2) spouse (3) son (4) daughter (5) employee (6) relative, specify ----------
8. Poultry kept in the homestead?

Poultry	**Number**	**Reason for raising (tick from 1-7 below)**
1. Chicken		
2. Ducks		
3. Pigeon		
4. Guinea fowls		
5. Turkeys		

Key: 1=family food, 2=selling to earn money, 3=for manure, 4=for ceremonies, 5=as wealth

6=social culture, 7=others

9. Which other animals are kept in the homestead (indicate type and numbers)?.....

.....

10. Which crops do you grow?

.....

.....

B) MANAGEMENT OF POULTRY

11. What type of birds do you keep? (a) Local breeds (b) Cross breeds

12. What type of management system do you practice? (a) Free-range system (b) backyard system

13. Who does the day to day management of the animals? (1) Husband (2) wife (3) children (4) Employee (5) others, specify ----------

14. Where do you source your chicken stock? (1) purchase from the market (2) gift from neighbours (3) gift from the home (4) hatched from own flock (5) others……..….

15. Number of chicken kept. (1) Adult male…… (2) Adult female…….. (3) Growers…….. (4) Chicks……

16. a) Do you house your birds at night? (a) Yes (b) No

b) If yes, what type of house do you use to keep your birds? (a) mud-walled (b) grass thatched (c) stone house (d) wooden house (e) others.....

17. How are the birds housed? (a) all of them together (b) adults different from growers and chicks (c) different birds at different areas.

18. a. Do the birds have laying nests in a separate enclosure? (1) Yes (2) No

b. Are the laying nests are in the same house where other birds also sleep (1) Yes (2) No.

19. (i) Do you confine your birds? (1) Yes (2) No

(ii) What is the reason for confinement……

(iii) When do you confine your birds: (1) planting season (2) harvesting season (3) wet season (4) dry season (3) others……..

20. (i) Do you give feed supplement to your chicken? (1) Yes (2) No

(ii) If yes, do you give the supplementary feeds during;

a. Confinement period only (1) Yes (2) No.

b. During confinement and other periods (1) Yes (2) No

c. For other reasons (Specify)……

(iii) What type of feeds do you supplement with? (1) Commercial chicken feeds (2) kitchen leftovers (3) cereal grains (4) bran (5) others……

21. Rank the problems you face in poultry keeping

i. Diseases ……..…..

ii. Predation ……

iii. Accidents

iv. Lack of feed

v. Lack of water

vi. Lack of market

vii. Lack of medication/vaccines

viii. Parasites

ix. Others, specify ----

22. What diseases do you commonly encounter (put them starting with the most common):

i. ……

ii. ……..…….

iii. ……..…….

23. (i) Do you vaccinate your chicken? (1) Yes (2) No

(ii) Which diseases do you vaccinate against?

……

……

……

……

(iii) At what age do you vaccinate your chicken against the above mentioned diseases

……

……..…

……

(iv) Who vaccinates the birds? (1) owner (2) veterinary doctor (3) animal health assistant (4) other, specify----------

24. Are newly introduced chicken initially isolated from the other birds?

C) NEWCASTLE DISEASE AND ITS CONTROL

39. Are you aware of a disease known as Newcastle disease? (1) Yes (2) No

If yes, answer the following questions.

40. What is the local name for it? ----------

41. What are the signs seen in the sick birds?----------

---

42. What period (s) of the year is it common? (a) January to March (b) April to June (c) July to September (d) October to December

43. Which birds are mostly affected? (1) adults (2) growers (3) chicks (4) weak (5) with parasites

44. How many of the chicken get sick? (1) all (2) most (3) few (4) none

45. How many of the sick chicken die? (1) all (2) most (3) few (4) none

46. How do chicken get Newcastle disease? ----------

---

47. What do you do with the sick birds? (1) Kill and consumed by the family (2) kill and give to dogs / cats (3) sold in the market (4) give to other people (5) others specify ------

48. What do you do with the dead birds? (1) Burry carcass (2) burn carcass (3) home consumption (4) give to dogs (5) others, specify ----------

49. (i) Do you vaccinate your chicken against Newcastle disease? (1) Yes (2) No

(ii) At what age do you vaccinate your chicken?.....

(iii) How frequent do you vaccinate? (a) Every 3 weeks (b) after 6 months (b) yearly (c) >1 year (d) others

50. Do you treat chicken infected with Newcastle disease? (a) Yes (b) No

51. If yes, what types of medicine do you use? .....

…….....

52. Do you use herbal medicine to treat Newcastle disease? (a) Yes (b) No

53. If yes, which ones?.....

54. How is the medicine / herbs administered to the chicken?.....

E) PRODUCTIVITY AND MARKETING

55. At what age do your birds start laying (in months) (1) 6 to 7months (2) >7 months (3) others……..……

56. How many eggs are produced per hen per clutch?…….……

57. What is the size of the eggs (1) small (2) medium (3) large

58. For how long do the birds lay before they sit on the eggs? .....

59. How many clutches per bird per year? ……

60. How many eggs do you set per hen for incubation? .....

61. How many of these eggs hatch? (1) all (2) most (3) few (4) none

62. How many hens are currently sitting on eggs?.....

63. How many chicks are weaned?.....……

64. How long does it take before chicks are weaned…….

65. What proportions of your chicks die before weaning? …….

66. Is there any change in egg production with season? (1) Yes (2) No

67. In which season is egg production lowest? (a) January to March (b) April to June (c) July to September (d) October to December

68. What are the reasons? (1) Availability of food (2) disease outbreak (3) other, specify

……

69. (i) Rank the uses of eggs that you produce?

(i) Home consumption

(ii) Sell

(iii) Hatching

(iv) Gifts

(v) Others ……..……..

70. If sold, where do you sell the eggs? (1) Market (2) homes (3) hotels and restaurants (4) eatery (5) others, specify…

71. Is the market for eggs reliable (1) Yes (2) No

72. If No explain……

(vi) What is the cost of an egg? .....

73. Where do you sell your birds? (1) Market (2) homes (3) hotels and restaurants (4) others, specify …….

74. How do you determine which birds to sell? (a) health (b) weight (c) sex (d) sick (e) Age (f) others…….

75. Which birds do you sell frequently? (a) Cocks (b) old hens (c) growers (d) chicks.

76. Record the price of the chicken

Birds	**Average Price (Kshs)**
Cock	
Hen	
Pullet	
Cockerel	
Chick	

E) IMPACTS OF THE DISEASE ON SOCIAL AND CULTURAL ACTIVITIES

77. Are there socio – cultural activities where chicken are utilized? ----------

---

---

78. Which of these activities are affected by outbreaks of Newcastle disease ? ----------

---

---

79. What other factors may be associated with the occurrence of Newcastle disease? ----------

---

Appendix 2: Formulae for reagents used

(i) Hanks balanced salt solution

Solution A stock

Sodium chloride (NaCl)	40 g
Potassium chloride (KCl)	2.0 g
Magnesium sulphate ($MgSo_4.7H_2O$)	0.5 g

Dissolve in 200ml de-ionised water. Dissolve 0.7 g Calcium chloride ($Cacl_2$) in 30 ml de – ionized water. Mix and make up to 250 ml with de- ionized water. Add 0.5 ml chloroform. Store at $+4^0$C. Solution is stable for at least 1 year.

Solution B stock

Sodium hydrogen phosphate ($Na_2HPO_4.12H_2O$)	0.76 g
Potassium hydrogen phosphate (KH_2PO_4)	0.30 g
Dextrose	5.60 g

Dissolve in 200 ml de – ionised water. Make up to 250 ml with de – ionised water. Add 0.5 ml chloroform stored at $+4^0$C. Solution is stable for at least 1 year.

Working solution

Solution A	50.0 ml
Solution B	50.0 ml
Water	870.0 ml
0.4% Phenol red	2.0 ml

Mix solution A and B with 870 ml de – ionised water. Add 2.0 ml phenol red

distributed into bottles in desired volumes and autoclave at 10 lbs for 15 minutes. Before use, adjust PH as desired with 7.5 % sodium bicarbonate ($NaHCO_3$).

(ii) Phosphate buffered saline (PBS)

Solution A

Sodium chloride (NaCl)	8.00 g
Potassium chloride (KCl)	0.20 g
Sodium phosphate ($NaPO_4$)	1.15 g
Potassium hydrogen phosphate (KH_2PO_4)	0.20 g
0.4% Phenol red	2.00 ml

Dissolve in de – ionised water. Add 2 ml of 0.4% phenol red. Make up to 800 ml and autoclave at 10 lbs for 15 minutes.

Solution B

Magnesium chloride ($MgCl_2.6H_2O$) 0.1 g

Dissolve in 100 ml de – ionised water. Autoclave at 10 1bs for 15 minutes.

Solution C

Calcium chloride ($CaCl_2$) 0.1 g

Dissolve in 100 ml de – ionised water. Autoclave at 15 lbs for 15 minutes.

Working solution of PBS

Add 8 parts of solution A to 1 part of solution B and 1 part of C.

(iii) 7.5% Sodium bicarbonate

7.5 g Sodium bicarbonate

100 ml distilled water

0.4% phenol red 0.2 ml

Saturate with CO_2 till orange in colour

Dispense in tightly stoppered bottles and autoclave at 10 lbs for 15 minutes.

(vi) Tincture of iodine

96% Alcohol	76 ml
Distilled water	2 ml
Potassium iodide	2.2 g
Iodine	2.0 g

Appendix 3: Individual birds Hemagglutination inhibition results

ID	Group	4/4/12	26/4/12	3/5/12	10/5/12	17/5/12	24/5/12	2/6/12
401	G7	1:256	1:256	1:256	1:512	1:1024	1:1024	1:2048
403	G7	1:64	1:256	1:512	1:1024	1:1024	1:1024	1:512
404	G7	1:64	1:256	1:256	1:2048	1:1024	1:1024	1:1024
406	G7	1:16	1:512	1:512	1:1024	1:1024	1:2048	1:1024
407	G7	1:16	1:128	1:1024	1:1024	1:1024	1:512	1:2048
408	G7	1:128	1:128	1:512	1:512	1:512	1:1024	1:1024
409	G5	1:64	1:128	1:512	1:1024	1:1024	1:2048	1:2048
411	G5	1:16	1:512	1:512	1:512	1:512	1:512	1:512
413	G5	1:16	1:256	1:512	1:512	1:1024	1:512	1:1024
414	G5	1:16	1:512	1:512	1:1024	1:1024	1:1024	1:1024
416	G5	1:128	1:128	1:512	1:1024	1:1024	1:1024	1:1024
412	G6	1:32	1:128	1:512	1:1024	1:1024	1:1024	1:1024
418	G6	1:64	1:512	1:1024	1:1024	1:1024	1:1024	1:2048
420	G6	1:64	1:256	1:512	1:512	1:1024	1:1024	1:1024
421	G6	1:64	1:256	1:256	1:512	1:1024	1:512	1:1024
422	G6	1:128	1:256	1:512	1:512	1:512	1:1024	1:512
426	G4	1:256	1:256	1:256	1:128	1:128	1:128	1:64
427	G4	1:64	1:64	1:16	1:16	1:16	1:16	1:16
428	G4	1:128	1:128	1:64	1:128	1:64	1:32	1:32

430	G4	1:128	1:128	1:64	1:64	1:64	1:64	1:64
432	G4	1:64	1:64	1:32	1:64	1:64	1:32	1:64
435	G3	1:64	1:64	1:64	1:32	1:32	1:32	1:32
437	G3	1:128	1:128	1:32	1:64	1:64	1:64	1:32
438	G3	1:64	1:32	1:32	1:32	1:32	1:32	1:32
442	G2	1:128	1:128	1:128	1:128	1:128	1:128	1:64
444	G2	1:128	1:128	1:128	1:128	1:128	1:128	1:64
445	G2	1:64	1:64	1:32	1:32	1:64	1:64	1:32
446	G2	1:256	1:128	1:32	1:32	1:32	1:32	1:32
448	G2	1:128	1:128	1:128	1:128	1:128	1:64	1:64
450	G8	1:64	1:64	1:64	1:32	1:16	1:16	1:4
480	G8	1:128	1:128	1:128	1:128	1:128	1:128	1:128
481	G8	1:128	1:128	1:64	1:128	1:64	1:64	1:64
483	G8	1:128	1:128	1:128	1:128	1:64	1:64	1:64
484	G8	1:16	1:16	1:16	1:16	1:16	1:16	1:16
449	G1	1:512	1:512	1:128	1:256	1:256	1:256	1:256
476	G1	1:128	1:64	1:64	1:64	1:64	1:64	1:64
477	G1	1:64	1:64	1:64	1:32	1:16	1:32	1:16
479	G1	1:64	1:64	1:64	1:64	1:32	1:64	1:64
433	G1	1:64	1:128	1:128	1:128	1:128	1:64	1:64
434	G1	1:64	1:64	1:32	1:64	1:64	1:64	1:32

441	G1	1:16	1:16	1:16	1:8	1:8	1:8	1:8
482	G1	1:256	1:256	1:32	1:128	1:128	1:64	1:64

Appendix 4:

Seasonal Newcastle disease antibody titer dynamics in village chickens of Mbeere District, Eastern Province, Kenya

D C Kemboi, H W Chegeh, L C Bebora, N Maingi, P N Nyaga, P G Mbuthia, L W Njagi and J M Githinji*

University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya
kemboidc@gmail.com
*** Ministry of Livestock, Central veterinary laboratories, Private Bag-00625, Nairobi, Kenya**

Abstract
This study was conducted in Mbeere district, Eastern Province Kenya, to establish Newcastle (ND) antibody titre levels in healthy, non-vaccinated village chicken chicks, growers and adults, in wet and dry seasons.
In wet season, all ages, many birds had higher titers in comparison to the dry season. There was, thus, a statistically significant difference (p=0.05) between the two seasons with a decrease on the number of birds with protective titer from 100% (wet season) to 83% (dry season). These results show continued endemicity of the disease in the area. Reason for not being able to isolate the virus from swabs and tissues of the birds may be presence of the high ND antibody titers; reducing the viral titers to levels not easily detectable by the serological test used. (Meaning that a more sensitive test was needed for detection). From the serological results, therefore, it is advisable to target vaccination at the beginning of the dry season, so as to boost immunity in these birds.

Key words: *hemagglutination inhibition, indigenous chicken, seasonality, seroprevalance*

Introduction
Free-range poultry keeping is the most common type of poultry production system in Kenya. These birds, however, have low production levels, compared to their exotic counterparts. Diseases are reported to be the main constraint to poultry production, especially Newcastle disease (ND) which causes mortalities as high as 100% (Njagi et al 2010a). Studies by (Kasiiti 2000) and (Njagi et al 2010a) showed that the ND virus (NDV) is present in healthy village chicken and that hens that survive outbreaks or have antibodies from previous exposure to Newcastle disease may maintain the virus endemicity in the village chicken. Thus, carrier chicken, village poultry population dynamics, other poultry species, wild birds and heterogenecity of the virus are some of the risk factors that have been associated with the maintenance of NDV (Awan et al 1994, Njagi et al 2010b). Management practices, including confinement, mode of disposal of poultry waste and carcasses and recovery rates of chicken from disease outbreaks also favour maintenance of virus in village populations (Njagi et al 2010b). Nyaga et al (1985) indicated that Newcastle disease outbreaks are reported during the cold and dry periods of the year with peaks in April, June-July and September-November periods meaning that antibody titers to NDV virus can be found in

birds all year round. The aim of the study was therefore to determine the prevalence of antibodies to NDV in naturally exposed, non- vaccinated multi-age village chickens in the wet and dry seasons in Mbeere District as an indicator of Newcastle disease endemicity. It was based on the hypothesis that season does not affect the immune response to NDV in village chicken in Mbeere District, Kenya.

Objective

- To determine antibody titers to Newcastle disease in chicken and recover Newcastle disease virus in dry and wet seasons.

Materials and Methods

Study area

Mbeere district has a human population of 219,220 and a large population of free-range chicken of 202,410 (KNBS 2009). These birds are kept for income, food and socio-cultural purposes. Other agricultural activities practised in the district include cattle, sheep and goats keeping; and millet, green grams, sorghum and cotton production. The district lies between latitude 0^o 20' and 0^o 50' South and longitude 37^o 16' and 37^o 56' East, at altitude 500 to 1200 metres above sea level. Long rains fall between mid-March and June while short rains occur October to December. Dry periods are between January and early March; and between August and September. The daily temperature ranges from 20 - 30 ^{o}C (Onduru et al 2002).

Experimental design

The birds were purchased from farms in Mbeere district in Eastern province. The study was cross-sectional and sampling was purposive and convenient (based on reachable willing owners, regardless of the number of chicken kept; so long as the birds were kept on free-range system, had no history of ND vaccination, and no parasite control/treatment was exercised).

Forty eight chicken (24 birds of both sexes each in dry and wet season) consisting of 7 chicks, 8 growers and 9 adults (wet season) and 9 chicks, 8 growers and 7 adults (dry season) with no previous history of Newcastle disease vaccination or parasite control. The wet season was in November while the dry season was in March. The chicks were less than 2 months old; growers were between 2 to 8 months; and adults, above 8 months of age (Sabuni 2009). All birds were labelled and transported in cages to Kabete, University of Nairobi campus for sampling. Collected serum samples were tested for NDV specific antibody by hemagglutination inhibition (HI) test while cloacal and oro-pharyngeal swabs were processed for NDV isolation (OIE 2000).

Collection and processing of blood and swabs

Blood was collected from the jugular vein at post-mortem by severing the neck and collecting the blood into universal bottles, without anticoagulant. Serum was separated from respective clotted blood samples by centrifugation at 3000 rpm for 10 minutes, decanting the serum into vials and keeping the vials frozen at -20^{o}C until hemagglutination –inhibition (HI) test was performed.

Swabs were taken from the oro-pharynx and cloaca using sterile cotton swabs and placed in 2ml viral transport medium comprising minimum essential medium, with penicillin (2000 international units/ml) and streptomycin (2000µg/ml). The swabs were expressed, centrifuged at 3500 rpm for 10 minutes and the supernatant transferred to a sterile bijoux bottle. All samples were stored at -20^{o}C until virus isolation was done.

Serology

Presence of NDV antibody was detected by hemagglutination inhibition test as described by OIE (2000). A cut off titer of 1:8 was considered specific indicating that the birds had been previously exposed to the virus, while titers less that this value were considered non specific. The validity of the results was

assessed against a negative control serum included in the test. The HI titers were determined in all chicken, and the geometric mean titter (GMT) of each group calculated.

Virus isolation
The processed swabs and tissues were inoculated into embryonated eggs, incubated and harvested as previously described by Nyaga et al (1985) and the ND virus presence from the swabs and tissues was checked using hemagglutination test. The samples were passaged only once.

Statistical analysis
Data on antibody titers from hemagglutination inhibition test results per group was analysed using Genstat Discovery edition 3 for descriptive statistics. The mean geometric titer (GMT) per group were calculated and used in the analysis. The titers were compared across the various age groups and seasons. A critical probability of $P< 0.05$ was adopted as cut off point for statistical significance.

Results and discussion

Seasonality of antibody titers against Newcastle disease

Wet season
A total of 24 indigenous village chickens (7 chicks, 8 growers and 9 adults) of all sexes were examined for antibodies against Newcastle disease virus in their serum using hemagglutination inhibition. All of the 24 birds tested positive for antibodies against Newcastle disease with the titers ranging from 1:16 (2^4) to 1:256 (2^8) (Table 1).

Table 1: Hemagglutination Inhibition titers of different age group of chicken during the wet season

Age groups	No. of samples	Antibody Titer								
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	GMT
Chicks	7	-	-	-	1	-	4	1	1	70.7
Growers	8	-	-	-	1	2	4	-	1	53.8
Adults	9	-	-	-	-	1	5	3	-	74.7
TOTAL	24	0	0	0	2	3	13	4	2	65.8

Key: GMT- Geometric mean titer (GMT = $\sqrt[n]{x_1x_2x_3...x_n}$*)*

All of the serum samples were found to be positive for antibody against Newcastle disease virus. The chicks had a titer ranging from 1:16 to 1:256 while growers had titer ranging from 1:16 to 1:256 with majority having 1:64. Adults had a titer ranging from 1:32 to 1:128 with majority (5) having titers of 1:64.
There was a significant difference ($P<0.05$) between the lower geometric mean antibody titers in growers (53.8), than in chicks (70.7) and adults (74.7) but no significant difference between chicks and adults ($P>0.05$).
All the birds showed serological evidence of specific immunity (Table 2). that is the level of antibody titer that show the bird has been in contact with ND virus a titer of 1:8 (2^3) and above (Allan and Gough 1974). A titer of between 1:16 to1:128 is considered protective whereby the bird is protected from developing Newcastle disease. Using this criterion, 100% of the birds in the wet season had protective levels of antibodies (Table 5).

Table 2: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition during the wet season.

Age groups	Total Samples	Specific immunity	Non specific immunity	Percentage specific immunity
Chick	7	7	-	100
Growers	8	8	-	100
Adults	9	9	-	100
Total	24	24	-	100

Key: No. – Number of serum samples; GMT – Geometric mean titers

Dry season

A total of 24 indigenous village chicken (9 chicks, 8 growers and 7 adults) of all sexes were examined for antibodies against Newcastle disease virus using hemagglutination inhibition test. All the 24 birds tested positive for antibodies against Newcastle disease with the titer ranging from 1:4 (2^2) to 1:128 (2^7) (Table 3).

Table 3: Hemagglutination inhibition titers of different age group of chicken during the dry season.

Age groups	No. of Samples	Antibody Titer								
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	GMT
Chicks	9	-	1	1	2	4	1	-	-	20.2
Growers	8	-	-	-	2	-	3	3	-	58.7
Adults	7	-	-	2	2	-	2	1	-	26.3
TOTAL	**24**	**0**	**1**	**3**	**6**	**4**	**6**	**4**	**-**	**31.1**

The chicks had antibody titer ranging from 1:4(2^2) to 1:64 (2^6) while growers had titers ranging from 1:16 (2^4) to 1:128 (2^7). Adults had titers ranging from 1:8 (2^3) to 1:128 (2^7).

The geometric mean antibody titer of the different age groups during dry season was 31.1.

There was a significant difference between the higher geometric mean antibody titers in growers (53.7), and that in chicks (20.2) and adults (26.3) ($P<0.05$) but no significant difference between chicks and adults ($P>0.05$).

95.8 % of the birds showed serological evidence of specific immunity with titers of 1:8 (2^3) and above (Allan and Gough 1974), with 100% of the growers and adults and 88.8% of the chicks (Table 4). A titer range of 2^4-2^7 is considered protective and using this criterion 83% of the birds had protective levels of antibodies (Table 5).

Table 4: Serum samples of chicken showing immune response to Newcastle disease virus using hemagglutination inhibition during the dry season.

Age groups	Total Samples	Specific immunity	Non specific immunity	Percentage specific immunity
Chick	9	8	1	88.8
Growers	8	8	-	100
Adults	7	7	-	100
Total	24	23	1	95.8

There was a significant difference ($P<0.05$) in the mean antibody titers between the two seasons. Adults and chicks had significant higher antibody titers in the wet season than dry season ($P<0.05$) while growers had no significant difference in levels of antibody titers between the two seasons.

Table 5: Protective Newcastle disease antibody levels for the dry and wet season

Age groups	Wet season		Dry season	
	Protective NDV Ab titer ($2^4$to 2^7)	Non protective NDV Ab titer	Protective NDV Ab titer ($2^4$to 2^7)	Non protective NDV Ab titer
Chicks	100%	-	77.8%	21.2%
Growers	100%	-	100%	-
Adults	100%	-	71.4%	28.6%
Total	100%	-	83%	17%

Key: NDV-Newcastle disease, Ab-Antibody

Isolation of NDV

No virus was isolated from swabs and tissues from the birds from one passage in embryonated eggs. Overall the birds had a higher levels of antibody titer that is unexpected in unvaccinated birds during both the wet and the dry season yet the owners did not vaccinate there birds confirming the endemicity of the virus in village chicken in Mbeere as previously reported by Njagi et al (2010a) in the same region, Otim et al (2004) in Uganda and Zeleke et al (2005) in Southern and Rift Valley districts in Ethiopia. Using Allan and Gough (1974) criterion that states that a titer of 1:8 and above is generally accepted as indicative of specific immunity, most birds had specific immunity meaning they had come in contact with the NDV. This wide range of NDV titer may be due to natural infection which is known to produce higher antibody titers than vaccination (Luc et al 1992). The continued hatching of chicks and the presence of birds that survived previous ND outbreaks mean there will always be susceptible chicken in free range chicken to which infected birds can transmit the disease (Martin 1992). This may have been the case during the wet season where the farmers reported an outbreak of a disease similar to Newcastle and serum samples from the birds showed high ND antibody titer. This implies that chicken in the village get infected at different times producing a near cyclic pattern of the disease hence maintaining an endemic situation throughout the year (Otim et al 2004, Njagi et al 2010a). Free range management system that allows the uninterrupted cycle of infection as the virus passes from one age to another may also be a cause of this endemicity as suggested by Zeleke et al (2005). The chicken are also prone to acquire infections from wild birds and in some instances ducks that some farmers kept together with chicken that have been shown to harbor and shed the NDV without showing any clinical signs of the disease (Njagi 2008).

Both cold and hot seasons have been associated with ND outbreaks in Kenya (Nyaga et al 1985) but start of wet season has been associated more with outbreaks (Jintana, 1987); although in Vietnam (Nguyen 1992) and Uganda (Mukiibi 1992) higher seasonal incidence and severity of ND is reported in dry season. This may be the reason behind the higher antibody titers that were demonstrated during the wet as compared to the dry season in this study; a ND outbreak may have occurred during the wet season. This is in line with Martin (1992)'s suggestion that outbreaks are often associated with change in season especially between wet and cold weather. It is also supported by Awan et al (1994)'s conclusion that ND is associated with periods of stress, which could be due to change in climate and lowered resistance at the beginning of wet season, due to inadequate feed. The decrease in antibody titers observed in the dry season in the current study may have been an indication of lowered resistance. Moreover, concentration of antibodies has been reported to decline within 3-4 months of non-stimulation (Otim et al 2005). This may then lead to outbreak of the disease in the susceptible birds. From this study, the maintenance of the cyclicity of the disease in Mbeere chicken can, therefore, be linked to two factors; availability of susceptible population of chicken and lowered immunity, as manifested by low antibody titres. Part of this cyclicity was observed during the study period where in the wet season (November 2011), the number of birds per homestead was low, with some homesteads having as few as two birds; most of them being adults. This low number could be attributed to an outbreak of Newcastle disease that had caused high mortalities, all the 24 birds that were screened for Newcastle disease antibodies, within this season, turned positive with high antibody titers. Contrary to this, during the dry season (March 2012), the number of birds had increased significantly, the flock composition constituted mostly growers and chicks and most of the hens were either brooding or incubating. This could be explained by the fact that dry season was the harvesting time; there was, therefore, abundant grain harvest. With improved nutrition, there was increased egg laying and hatching; enabling the farmers to restock their flocks that had gone down during the wet season. Restocking resulted in increased number of chicks which ended up being susceptible to the disease, as observed during the study in November 2011. This fuelled ND outbreaks and maintained the virus within the recovered chicken; which became a source of infection for the next cycle. The maternal antibodies, if any were passed to the chicks, waned off within 3-4 months (Otim et al 2005). Village chicken scavenge for their feed with little supplementation and this scavenging behaviour encourages the spread of ND. Otim et al (2005) associated socio-cultural activities in rural households

with ND outbreak. The ND dynamics appear to depend on regional and community activities and control measures need to take this into account.
Adults and chicks had a significantly higher titer compared to growers during the wet season and a significantly lower titer during the dry season with the finding in dry season similar to that of Njagi et al (2010a). This is because chicks and juveniles get maternal antibodies from immunized hens through the eggs or through contact with infected discharges and excretions during feeding and drinking (Mwakapuja 2009). The low antibody levels in the two groups during the two seasons may be due to the low level of antibodies in adults that will correspond to the low levels in eggs and hence chicks.The actual cause of the apparent low levels of antibody titer seen in the grower group in comparison to the other groups during the wet season could not be identified; further study, therefore, needs to be done on this. The lack of isolation of the virus from the birds may be due to neutralization of the virus by the high levels of protective antibodies (Alexander 2003). Healthy looking birds may harbour virulent NDV but if they have high antibody titer this may prevent them from having clinical disease (Njagi et al 2010a). Njagi et al (2012) suggested that ducks with protective levels of antibodies ($2^4$to 2^7) may not develop clinical disease but instead remain virus carriers and when immunosupressed they have been shown to shed the virus. This may also happen in chickens and the birds with antibodies may shed and act as source of infections to other susceptible birds during periods of stress.

Conclusions

Based on the results of this research it is concluded that:

- In all seasons birds have high antibody titers, and the antibodies tend to wane off during the dry season making the birds susceptible to introduction of velogenic strain of NDV.
- That vaccination is recommended during the start of dry season to maintain high levels of antibodies and prevent outbreaks and especially in chicks and adults;
- That age has influence on seropositivity of Newcastle disease with adults and chicks having a higher titer during the wet season than growers while in the dry season growers had a higher antibody titer than adults and chicks.
- Flock owners need to be educated on disease transmission and prevention and they be discouraged from restocking their farms with chicken from the market since these birds, though healthy looking, maybe harbouring the Newcastle disease virus.

Acknowledgements

The authors would like to thank Julius Kibe, Mary Mutune, Ezekiel Weda and Richard Otieno for their technical assistance, the farmers of Mbeere for their corporation and RUFORUM for their sponsorship and funding of the project.

References

Alexander D J 2003 Newcastle disease, other Paramyxoviridae and Pneumovirus Infections. In; Diseases of Poultry 11th Edition. Saif, Y.M., Barnes, H.J., Glossons, G.R., Fadly, M.A McDougald, D.J., and Swayne, D.E (Eds), Iowa State press, Ames. (pp. 63-100),

Allan W H and Gough R H 1974 A standard hemagglutination inhibition test for Newcastle disease; a comparison of macro and micro methods, Veterinary Records. (95): 120 – 123.

Awan M A, Otte M J and James A D 1994 The epidemiology of Newcastle disease in rural poultry: A review, Avian Pathology 23: 405-423.

Jintana, D. (1987) Thailand; Poultry production. In: Cupland, J.W (Eds). Newcastle disease in poultry. A new food pelleted. ACIAR monograph. No. 5. ACIAR, Canberra, 108-109

Kasiti J L 2000 Isolation of Avian paramyxovirus from village chickens and wild birds in Kenya. Msc thesis. University of Nairobi, Kenya
Kenya National Bureau of Statistics (KNBS) 2009 Population and housing Census results, Kenya National Bureau of Statistics (publisher). www.knbs.or.ke/**Census**%20Results .
Luc P V, Hong N T and Chinh V T 1992 Levels of anti-Newcastle disease virus antibodies in industrial poultry at various ages and seasons, Agro food industry hi tech journal.348-350.
Martin P A J 1992 The epidemiology of Newcastle disease in village chickens. *In*: P.B Spadbrow (Ed). In: Proceeding of the 39th international workshop on Newcastle disease in village chickens. Control with thermostable oral vaccines, 1991 (pp 40-45). Kuala Lumpar, Malaysia: Australia centre for international Agricultural research (ACIAR).
Mukiibi M G 1992 Epidemiology of Newcastle and the need to vaccinate local chicken in Uganda. In P.B Spadbrow (Ed). *In*: Proceeding of the 39th international workshop on Newcastle disease in village chickens. Control with thermostable oral vaccines, 1991 (pp 155-158). Kuala Lumpar, Malaysia: Australia centre for international Agricultural research (ACIAR).
Mwakapuja S R 2009 Disease Surveillance on village chickens vaccinated against Newcastle disease in rural communities in Morogoro, Tanzania. Msc dissertation Sokoine University of Agriculture. Morogoro. Tanzania
Nguyen T D 1992 Poultry production and Newcastle disease in Vietnam. *In*: P.B Spadbrow (Ed). Proceeding of the 39th international workshop on Newcastle disease in village chickens. Control with thermostable oral vaccines, 1991 (pp 171-173). Kuala Lumpar, Malaysia: Australia centre for international Agricultural research (ACIAR).
Njagi L W, Nyaga P N, Bebora L C, Michieka J N, Mbuthia P G, Kibe J K and Minga U M 2010a Prevalence of Newcastle disease virus in Village indigenous chickens in varied agro-ecological zones in Kenya. Livestock Research for Rural development, 22 (5): http://www.lrrd.org/lrrd22/5/njag22095.htm
Njagi L W, Nyaga P N, Mbuthia P G , Bebora L C , Michieka J N and Minga U M 2010b A retrospective study of factors associated with Newcastle disease outbreaks in village indigenous chickens,Bulletin of Animal health and production in Africa 58: 22-33.
Njagi L W, Nyaga P N, Bebora L C, Mbuthia P Gand **Minga U M 2012** Effect of Immunosuppression on Newcastle Disease Virus Persistence in Ducks with Different Immune Status. ISRN Veterinary Science, doi:10.5402/2012/253809.
Njagi L W 2008 Endemicity of Newcastle disease virus in village indigenous chicken and the role of carrier ducks. PhD thesis, University of Nairobi, Kenya
Nyaga J M, Nyaga P N and Kariuki D P 1985 Epidemiology of Newcastle disease in Kenya, Bulletin of Animal health and production in Africa **33:** 249-251.
OIE (Office International des Epizooties) 2000 Newcastle disease. Manual of standards for diagnostic tests and vaccines, 4th edition. OIE, Paris. Pp. 221 – 232.
http://www.oie.int/eng/normes/mmanual/2008/pdf/2.03.14_NEWCASTLE_DIS.pdf, accessed 14.9.2011
Onduru D D, Gachimbi L , Maina F, Muchena F N and der Jager A 2002 Sustaining Agricultural Production in semi-arid areas of Eastern Kenya. A Case study of Mbeere District. INMASP report No. Ke-03.
Otim M O, Christensen H, Jørgensen P H, Handberg K J and Bisgaard M 2004 Molecular characterisation and phylogenetic study of Newcastle disease virus isolates from recent outbreaks in eastern Uganda. Journal of clinical microbiology. 42:28025.
Otim M O, Mukiibi G M, Christensen H, Bisgaard M 2005 Aflatoxicosis, infectious bursal disease and immune response to Newcastle disease vaccination in rural chicken. Avian Pathology, 34: 319-23.
Sabuni A Z 2009 Prevalence, intensity and pathology of ecto and hemo parasites infection in indigenous chicken in Eastern, Province of Kenya. Msc thesis, University of Nairobi, Kenya
Zeleke A, Sori T, Gelaye E and Ayelet G 2005 Newcastle Disease in village chickens in Southern and Rift Valley Districts in Ethiopia, International Journal of Poultry Science 4 (7): 507-510.

Received 1 September 2013; Accepted 18 September 2013; Published 1 October 2013