SCHOOL OF VETERINARY MEDICINE

ADDIS ABABA UNIVERSITY

HEMATOLOGICAL AND SEROLOGICAL RESPONSE OF SOME CHICKEN TYPES AT ON STATION TO NEWCASTLE AND MAREK'S DISEASE VACCINATION

BY

EDILU JORGA

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Epidemiology

JUNE, 2011

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LIST OF ABBREVIATIONS

$	Dolar
4HAU	4 Hemaglutination unit
AACMC	Australian Agricultural Consulting and Management Company
AGID	AgarGel Immunodifusion
ANOVA	Analysis of variance
CD8 /CD4	Cluster of Differentiation 4/8
CEF	Chicken Embryo Fibroblast
CMI	Cell Mediated Immunity
CRBC	Chicken Red Blood Cell
CTLS	Cytotoxic Lymphocytes
dsDNA	double strand Deoxyribonucleic Acid
ESR	Erythrocyte Sedimentation Rate
ELISA	Enzyme Linked ImmunoSorbent Assay
FAO	Food and Agriculture Organization
FEE	Feather follicle epithelium
GHV-2	Gallid Herpes Virus-2
GLM	General Limited Model
Hb	Hemoglobin
HVT	Herpes Virus Turkey
IFN	Interferon
IgG	Immunoglobulin G
IgM	ImmunoglobulinM
IL	Interleukin
ILCA	International Livestock Center for Africa
MD	Marek's Disease
MDV	Marek's Disease Virus
mMDV	mild Marek's Disease Virus
NO	Nitrogen Oxides
NVI	National Veterinary Institute

PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
qPCR	Real time quantitative PCR
RBC	Red Blood Cell
RIR	Rhode Island Red
SD	Standard Deviation
UK	United Kingdom
vMDV	virulent MDV
VN antibody	Virus neutralizing antibody
vvMD	very virulent Marek's Disease virus
vvMDV	very virulent Marek's disease virus
WBC	White Blood Cell
WHL	White Leg Horn

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ABSTRACT

An experimental study on five Ethiopian chicken ecotypes namely Jarso, Tepi, Chefe, Arsi and Horro and other two chicken breeds (Fayoumi and Bovans) was conducted from December 2010 to June 2011. Total of 260 chickens among which 70 Chefe, 40 Jarso, 21 Horro, 9 Arsi, 30 Tepi, 20 Bovans and 70 Fayoumi chickens were included in the study. The objective of the study was to evaluate the responses of different chicken types to Marek's and Newcastle disease vaccination through hematological profile, serum protein profile and antibody titer. Day old chicks of each ecotype were randomized in to two groups one for Marek's disease (MD) vaccination and the other remain unvaccinated while all of them vaccinated against Newcastle disease vaccine (LaSota strain) on 21st, 50th and 120th day after hatching. Three round blood sample was collected at age of day 35, 65 and 105. For serum protein analysis and hemaglutination inhibition (HI) antibody titer test three round serum sample was collected on 35th, 65th and 135th day each following fifteen days after vaccination with Newcastle disease vaccine. According to the current study the total leukocyte, lymphocyte and monocyte count was found to be higher for Fayoumi and minimum count was observed for Horro chicken. Heterophil, eosinophil and basophil count was higher in Arsi chicken. Highest packed cell volume (PCV) value (36±.3.9) was observed in Fayoumi chicken whereas maximum value for hemoglobin concentration (9.002+.97) was in Arsi chicken. The comparison of MD vaccinated to none vaccinated chicken reveled higher values of all counts and PCV for vaccinated but lower hemoglobin concentration. Lymphocyte count was found to increase with time (age) while others either decrease or no clear pattern. On the other hand, the HI titer for Newcastle was compared and Jarso and Fayoumi were found to be superior over the other chicken types whereas Bovans, Tepi and Horro were with lower titer. The trend for total protein was increasing for Jarso, Tepi and Arsi and the values in these ecotypes were higher. This study generally found that the chicken types involved were of a wide diversity in their response to Marek's and Newcastle vaccination which was reflected by the difference in hematological, serological and total protein values in responses to vaccination.

Key words: ***Key words***: *Chicken types, Hemagglutination Inhibition, Hematological profile, Immune response, Marek's disease, Newcastle disease, protein profile, vaccination*

1. INTRODUCTION

Population growth, urbanization and rising income in many parts of the developing world have caused a growing demand for food of animal origin. Poultry meat and egg production account for more than 28% of the total animal protein produced in the world in 1997. The proportional contribution of poultry by the year 2020 is believed to increase to 40%, the major increase being in the developing world (Delgado *et al.*, 1999). According to Klober *et al.* (1991) chickens, ducks and turkey are the most common of all farm poultry throughout Africa, Asia and Latin America. He further noted that in most tropical countries every family-settled or nomadic-owns some kind of poultry. In Ethiopia, the word poultry is synonymous with domestic chicken (*Gallus domesticus*) because other types of poultry are almost unknown as sources of egg and meat (Alemu, 1995).

The local chickens are known for various merits. They are cheaply reared as scavenging flocks by feeding household leftovers. They need a small house or shelter to spend their night while free ranging during the day. Their meat and egg tastes are preferred to those of exotic chickens by local community (Roberts, 1999; Dessie and Ogle, 2001). Furthermore, they are "family sized," easily killed and dressed with little or no spoilage. Most importantly, they are known for their adaptation ability in terms of their resistance to endemic diseases and harsh environmental conditions. However, local chickens are blamed for poor performance in terms of growth rate (hence meat production) and egg production. Most of them are of small adult size and lay small sized eggs when compared to improved commercial broiler or layer birds respectively (Gondwe, 2004, Fayeye *et al.*, 2005; Prabakaran, 2003; Daghir, 2001))

The overall chicken population of Ethiopia is estimated to be 55 million heads (FAO, 2000). Of which indigenous chickens comprise about 99%. They constitute by far the largest proportion of the poultry population in Ethiopia and represent a pool of heterogeneous and unimproved individuals that largely differ in production performance and morphological and genetic characteristics (Duguma, 2009). No single ecotype meets the attributes of good egg traits, high growth rate and heavy weight at slaughter. However, it contributes 98.5% and 99.2% of the national egg and poultry meat production respectively with an annual output of

72,300 metric tons of meat and 78,000 metric tons of egg (Solomon, 2003; Dessie and Ogle, 1996).

Poultry diseases are the standing constraints incriminated for reduction of total numbers and impairment of productivity (Ashenafi, 2000). According to the work done by Ashenafi, (2000) some farmers have given up rearing poultry because of disease problems. Many diseases have been documented in smallholder poultry among which infectious diseases such as Newcastle disease (ND), Salmonellosis, coccidiosis, Marek's disease (MD) and fowl pox are considered to be the most important causes of mortality (which could reach as high as 80–90%) in local chicken. These diseases are easily contracted under free-range conditions due to poultry's scavenging habits. Furthermore, disease control is very difficult to carry out under free roaming management and is therefore rarely practiced by the poultry producers (Eshetu, *et al.*, 2001).

The diseases have already become endemic in village poultry population and thus it recurs every year inflicting heavy losses although, the epidemiology of ND and MD in village birds in Ethiopia is not clearly understood (Dessie and Jobre, 2004). Vaccination is the most important method of disease control which results in a quite significant increase in chick survival (Al-Garib, 2003). Although ND represents the most severe poultry disease responsible for huge economic losses, few studies on ND have been done on chickens of Ethiopian ecotype. Furthermore, the actual effect of vaccination in protecting from high mortality and the difference in the level of antibody titer at different stage of vaccination has yet to be identified. Moreover, difference in antibody response to ND vaccine between families and lines of Ethiopia indigenous chickens ecotypes is yet not studied. Such a wide knowledge gap on response of local chicken ecotypes to ND vaccine hinders effective ND control and the attempt to increase the productivity of local chicken. Currently MD was found to be a major cause of failure in intensively managed Ethiopian chicken ecotypes due to high mortalities and morbidities in various geographical areas of the country (Forssido, 1986; Brannang and Pearson, 1990; Solomon, 2003; Hassen, 1992; Demeke, 2003). It was characterized by visceral tumor lesions and nerve paralysis of the wing and leg (Duguma *et al.*, 2005).

Diagnosis and control of these varies diseases need understanding of the immune function of chicken. Lymphoid and nonlymphoid systems constitute two broad structural categories of the avian (and mammalian) immune system. Among lymphoid components, the bursa of Fabricius, a site of B-lymphocyte development and differentiation, and the thymus, a site of T lymphocyte development and differentiation, are considered to be primary lymphoid organs, whereas the spleen is usually termed a secondary lymphoid organ. In addition, lymphoid structures distributed throughout the intestinal tract represent the "intestinal arm" of the immune system (Jeurissen *et al.*, 1994). Humoral immunity is the adaptive function of the immune system through which antibodies are produced in response to an antigenic challenge. Cell-mediated immunity involves immune mechanism(s) by which cells infected with a foreign agent, such as a virus, are destroyed, and is accomplished via a direct effector (e.g. an activated T cell) and target cell contact (Weinstock *et al.*, 1989).

The nonlymphoid components of the immune system include cells that provide a nonspecific immunological defense to the host. Being the first line of immunological defense, the cells of the mononuclear phagocytic system are the primary players in this category (Glick, 1995). Most vertebrates have five types of WBCs: lymphocytes, neutrophils, eosinophils, basophils and monocytes. The immunological function of each of the WBC types has been reviewed extensively elsewhere (Thrall, 2004). Briefly, neutrophils/heterophils and lymphocytes make up the majority (i.e. nearly 80% combined) of WBCs in birds (Jain, 1993). Heterophils are the primary phagocytic leukocyte and proliferate in circulation in response to infections, inflammation and stress. Lymphocytes are involved in a variety of immunological functions such as immunoglobulin production and modulation of immune defence (Campbell, 1996). The remaining 20% of the leukocytes represent a combination of eosinophils, which play a role in the inflammation process (Jain, 1993) and are associated with defence against parasites (Kiesecker, 2002). Blood monocytes and tissue macrophages are unique because of their wide distribution throughout the body fluids, organs, and cavities. Macrophages phagocytize and degrade foreign antigens, such as bacteria, without any lag period after encounter.

In general, blood examination is performed for several reasons as a screening procedure to assess general health (Jain, 1993). Managing abnormalities in birds requires an understanding

of how diseases change the biochemical function and electrolyte of the bodies. Because the clinical signs of illness in birds are frequently subtle, clinical chemistry is necessary to evaluate cellular changes. Evaluation of the avian hematological profile (hemogram) involves counting the various blood cells per microliter of blood, packed cell volume (PCV), hemoglobin concentration as well as cytologic evaluation of the cells. Most workers have studied the avian blood and found a great variation for R.B.C. and also Hb, erythrocyte sedimentation rate (ESR) and others influenced by factors which include breed, sex and the nutrition supplied to the bird. According to Latimer and Bienzle (2000) the leukogram is particularly useful because blood leukocyte numbers and morphology are fairly stable in health but may change drastically in disease, providing access to avian immunological responses. In addition serum protein which consists of many different individual protein molecules is also important in avian immunology (Eckersall, 2008). Blood proteins are important complementary constituents in the diagnosis of gastrointestinal, hepatic, renal or infectious diseases. Determination of these proteins seldom leads to a specific diagnosis but will help to evaluate the nature, severity and progress of a disease (Lumeij, 2008). Otherwise, the progress of clinical pathology in birds is slow because there is a lack of appropriate information concerned to reference values for different avian species. In Ethiopia no study has been conducted on hematological and serological profile of chicken either for assessing disease condition, determine physiological status or for immunological evaluation. Moreover, there were no experimental studies that interpret bird's proteins responses after antigen exposition and immune stimulation and literature on hematological and serological values of Ethiopian indigenous birds. Thus, specific experimental studies are necessary to interpret birds' hemogram and serum protein profile in addition to the antibody titer responses after antigen exposition and immune stimulation. Therefore the objectives of this thesis were:

- ✓ To investigate the effect of MD vaccination on the hematological and serum protein profile in different chicken type of indigenous chickens under confined management;
- ✓ To evaluate the difference in immune response between chicken type vaccinated against ND on station and
- ✓ To determine the impact of MD vaccination on immune response (antibody titer) to ND vaccination

2. LITRATURE REVIEW

2.1. Etiology and nomenclature of Marek's disease

Marek's disease (MD) is a devastating disorder affecting chickens worldwide. It is a highly contagious viral neoplastic disease in chickens caused by an alphaherpesvirus known as *Marek's disease virus* (MDV) or *gallid herpesvirus 2* (GaHV-2) an oncogenic (tumor-inducing) herpes virus classified under genus mardivirus, subfamily *Alphaherpesvirinae*, family *herpesviridae* of Group I dsDNA. .The disease is characterized by T cell lymphomas as well as infiltration of nerves and organs by lymphocytes, polyneuritis, immunosuppression and, rarely, atherosclerosis. The seminal description by Jozef Marek in 1907 identified the disease as *polyneuritis*. Other common synonyms included *neuritis*, *neurolymphomatosis gallinarum*, and *range paralysis*. Jungherr and colleagues proposed that the term *lymphomatosis* be subdivided into *visceral*, *neural*, and *ocular* forms. Lymphoproliferative diseases (also called *leukosis*) of chickens include MD and lymphoid leukosis (LL). In retrospect, neural and ocular lymphomatosis were probably MD; whereas visceral lymphomatosis included both MD and LL. MD virus can also induce other clinically distinct disease syndromes such as *transient paralysis*, *early mortality syndrome*, *cytolytic infection*, *atherosclerosis*, and *persistent neurological disease* (Hirai, 2001).

2.2. Etiology of ND

Newcastle disease is caused by avian paramyxovirus; a negative single stranded, non-segmented RNA virus belonging to the genus *Avulavirus* of the family *Paramyxoviridae*. So far nine serotypes of avian paramyxoviruses have been found, APMV-1 to APMV-9. Five of these serotypes can cause disease in poultry; APMV-1, APMV-2, APMV-3, APMV-6 and APMV-7. Of these APMV-1 is the most pathogenic serotype and is also referred to as Newcastle disease virus (NDV) (Caupa and Alexander 2009, Kahn, 2005).

2.2.1. ND virus classification

The importance and impact of a NDV isolate is directly related to its virulence. One of the most characteristic properties of different strains of NDV has been their great variation in pathogenicity for chickens. Strains of NDV have been grouped into five pathotypes on the basis of the clinical signs seen in infected chickens (OIE, 2008). These are:

1. Viscerotropic velogenic: a highly pathogenic form in which haemorrhagic intestinal lesions are frequently seen;
2. Neurotropic velogenic: a form that presents with high mortality, usually following respiratory and nervous signs;
3. Mesogenic: a form that presents with respiratory signs, occasional nervous signs, but low mortality;
4. Lentogenic or respiratory: a form that presents with mild or subclinical respiratory infection;
5. Asymptomatic enteric: a form that usually consists of a subclinical enteric infection.

Pathotype groupings are rarely clear-cut (Alexander, 2003) and even in infections of specific pathogen free (SPF) birds, considerable overlapping may be seen. In addition, exacerbation of the clinical signs induced by the milder strains may occur when infections by other organisms are superimposed or when adverse environmental conditions are present.

2.2.2. MD virus strain classification

Serotypes: Previously MDV herpesvirus has been classified into 3 distinct virus groups that correlated with biologic properties however, McGeoch and coworkers (McGeoch *et al*, 2000) recently, proposed to place all MDV serotypes in a separate subgroup, a3, of the alphaherpesvirinae. Serotype 1 MDV is the prototype virus for this group of avian viruses, and except where otherwise indicated MDV refers to serotype 1 virus (Witter, 1997). Two additional groups of nononcogenic herpesviruses isolated from chickens (serotype 2) and turkeys serotype 3, respectively. The serotypic classification for MDV and HVT strains, originally based on the recognition of common and distinct antigenic epitopes for each

serotype, type-specific monoclonal antibodies and recently has been justified based on the complete sequence for serotype 2 HPRS-24 and serotype 3 FC126 virus strains (Afonso *et al.* 2001, Kingham *et al*, 2001). Although distinguishable by serologic tests, the 3 serotypes also share many common antigens. Thus, sera against a serotype usually will react with antigens of other serotypes, although somewhat less vigorously than with homologous antigens.

Pathotypes: Virulence or oncogenicity is associated with only serotype 1 MDVs. Within this group, however, a wide variation in pathogenic potential is recognized and undoubtedly represents a continuum from nearly avirulent to maximally virulent. Current classification schemes recognize 4 groups of viruses which is designated as mild (mMDV), virulent (vMDV), very virulent (vvMDV), and very virulent plus (vv_MDV) (Witter, 1997). Pathotyping of virus isolates involves comparative pathogenicity tests in vaccinated and unvaccinated maternal antibody positive chickens with prototype viruses as controls (Witter, 1988).

2.3. Epidemiology and economic significance

2.3.1 Occurrence and distribution of MD

MD exists in poultry-producing countries throughout the world. Probably every flock of chickens raised in areas where poultry is prevalent becomes infected, and many experience some loss. Reporting systems vary, however, and it is difficult to determine the true incidence. The incidence of infection is surely much higher than the incidence of disease. Even in susceptible chickens, infection does not always induce clinical disease and, in genetically resistant or vaccinated chickens, infection may rarely cause overt disease. Sporadic outbreaks of MD occur on individual farms or regions (Witter *et al,* 1980).

The incidence varies and it depends mainly on the oncogenicity of MDV, genetic resistance, environments, age; maternal immunity of host, management and stress. In the serotype **1** group of MDV, a large differentiation of oncogenecity exists within some isolates. The incidence of MD on a farm with the same genetic stock and same age differs between houses

and even between parts of houses. A flock from a single house, or a part of it, could be infected with high pathogenic MDV and chicks from other houses with viruses of low pathogenicity or even with apathogenic viruses. Infection with apathogenic MDV prior to infection with pathogenic strains may induce natural immunity and reduce incidence of MD (Witter *et al*, 2005).

2.3.2. Spread of the infection with MD

MDV is transmitted readily by direct or indirect contact between chickens, apparently by the airborne route, thus exposure occurs by horizontal infection. The virus is resistant to environmental exposure and can remain viable for long periods in houses especially if units are not decontaminated between cycles. MDV spreads rapidly throughout flocks by contact with infected chickens, premises, litter, dust and chopped feathers. Sheddings of the virus from the skin through desquamated dander, start before the clinical signs of disease, and may continue throughout the whole life. MDV in danders and feather follicules survive more than one year in dust at 4°C and several months at 20—25°C, walls, fans and probably in litter depending on outside temperature, and are the main source of infection. Dust with danders and moulted feathers can be readily transported passively from infected houses over long distances by air or with clothing, infected eggs, utensils, trucks and other ways Chicks can usually be infected very early in life, from the first day after hatching, although it may be some weeks or months later. With age the possibility of infection is reduced because of increased resistance. Up to now vertical transmission of the virus has not been proven (Joslpovlc, 1990).

2.3.3. Economic importance of MD

Marek's disease has a worldwide distribution where ever poultry is reared. The disease has a tremendous economic impact, firstly because of cost of vaccination and secondly because of continuing losses due to the disease. The annual losses caused by MD prior to introduction of the vaccination in 1970 were estimated to be $150 million in U.S. and $ 40 million in U.K. The annual losses due to this disease world over have been estimated at more than 1 billion

US dollars. The vaccination can prevent formation of tumor but generation of infectious virus is not prevented. The virus is shed in the environment as dander dust after maturation in the feather follicle epithelium. For this reason, this disease remains one of the most economically important diseases of poultry in all countries of the world where intensive rearing of poultry is undertaken (Nair, 2005).

2.3.4. Epidemiology and clinical feature of Newcastle disease

It has been showed that many different species can be infected with NDV. It is believed that all bird species are most likely at risk to be infected, but the effects of the disease varies very much with different species i.e. chickens are most sensitive whereas ducks and geese are least sensitive (Caupa and Alexander 2009). ND is a highly infectious viral disease caused by a paramyxovirus which mainly affects poultry (Alders and Spradbrow 2001). The ND virus can infect through the respiratory tract, the ocular mucous membranes and the digestive tract. The incubation period usually ranges from 2 to 15 days depending on the strain of virus. The clinical signs in birds infected with ND virus vary greatly from very high morbidity and mortality (up to 100 %) to asymptomatic carriers. The severity of an infection is dependent on factors like the virulence and tropism of the virus, host species, age of host, immune status, other diseases and environmental conditions (Kahn, 2005). Young birds are usually more susceptible for infection but the disease causes heavy losses in birds of all ages. Depending on where the virus has its predilection site symptoms from the respiratory tract, digestive system or nervous system can be observed.

Under village conditions, the virus is unlikely to survive outside the host for more than a month. The virulence of the disease depends on the particular strain of the virus. Of the highly virulent strains, which are particularly common in South-East Asia and Africa, some grow in the gut (viscerotropic strains), while others grow mainly in the central nervous system (neurotropic strains). The most common indication of a serious outbreak of a neurotropic strain of the disease is seen in a nervous symptom exhibited in infected birds where tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms, sometimes complete paralysis and the chickens simply fall over and die. Symptoms from the respiratory tract are

gasping, coughing, sneezing and rales. Less virulent strains affect only the respiratory system, with varying degrees of severity. General symptoms that can be seen are greenish diarrhoea, depression and inappetence, partial or complete drop in egg production and an increased production of deformed eggs, increased respiration, and coughing, gasping and even rapid death without any exhibition of other symptoms (Kahn, 2005; Alders and Spradbrow 2001). Most avian species, domestic or feral, can be infected by strains of ND virus; however, the consequences of these infections will vary with the strain of virus and the species of the host infected. Chickens are the most susceptible host (Alders and Spradbrow, 2001). Ducks and geese are also susceptible to infection, but these species rarely succumb to the disease.

ND is highly transmissible: birds are normally infected through direct contact with diseased or carrier birds, but the virus can also be carried on contaminated objects such as chicken or egg crates, feed, vehicles, dust and clothing. The virus is usually inactivated by direct sunlight, but in cool weather the virus can survive in feces and contaminated housing for up to 21 days. The virus can also persist in poultry products (meat and eggs) and can be carried by migrating wild birds. Human activity was found to influence the occurrence of ND. Seasonal high sales of chickens and population movements are some of the factors that lead to outbreaks (Spradbrow, 1999).

2.3.5. The importance of Newcastle disease in village chickens in developing countries

The major constraint to production of village chickens in many developing countries is Newcastle disease (ND). In these countries circulating strains of ND virus are capable of causing 100 % mortality in unprotected flocks. Outbreaks of ND are unpredictable and discourage villagers from paying proper attention to the husbandry and welfare of their chickens. There are many constraints to village chicken production including a range of bacterial and other viral diseases, internal and external parasites poor nutrition and predation. However, in areas where ND is endemic, ND control through vaccination is generally a very cost-effective intervention and given a high priority by farmers. Village chicken farmers are disheartened by the loss of large numbers of their birds to ND outbreaks that often occur on an annual basis. Once the dramatic losses caused by ND can be controlled, farmers will be

more receptive to other messages concerning improved poultry husbandry (Alexander 2003; Leslie 2000; Capua *et al.* 2002).

2.3.6. Ethiopian condition of MD

In Ethiopia studies have been conducted based on clinical sign and pathological lesion on MD in commercial exotic chicken breeds revealed high morbidity and mortality (46%) in non-vaccinated birds (Lobago and Weldemeskel, 2004) and about 5% in MD vaccinated chickens (Lobago *et al*, 2003). In study conducted on backyard chicken under confinement of different ecotype the overall mortality was 66.2%. The highest mortality was observed within the Jarso ecotypes, 87.5% and the least within Tilili ecotypes, 58.3%. Comparison of mortality among age groups showed highest mortality at 14 weeks age 14.1% and 15 weeks age 16.1% and then mortality pattern continued at a decreasing rate. An overall fatality rate was high (97.9%), indicating that almost all diseased (sick) chickens ultimately died (Duguma *et al* 2005).

2.4 Immunity

In birds as well as in mammals the lymphoid tissues can be divided into primary immune tissue and secondary lymphoid tissue. In birds the primary includes Bursa Fabricius (BF) and thymus while the secondary consists of the spleen, Peyer's patches, cecal tonsils, Meckel's diverticulum, lymph nodes, bone marrow, glandula pineale and the Harderian gland. Although they have a lot in common, some anatomically differences do exist between the lymphoid tissues in mammals and birds. Thymus, the central organ for differentiation of T cells, is in birds divided into 12 lobi and BF, the central organ for B cell differentiation in birds does not even exist in mammals where the same process takes place in the bone marrow (Sharma, 1999).

The immune mechanisms can be divided into the innate and the adaptive responses. The innate immune response mainly consists of Natural Killer cells (NK cells) and phagocytizing cells such as macrophages and heterophil granulocytes (the avian counterpart to the neutrophil

granulocyte in mammals). In birds macrophages play an important role being the first line of cellular defense towards invading pathogens. In the lungs and air sacs macrophages do not exist and here the first line of cellular defense consists of heterophils. NK cells are a population of non-adhering and non-phagocytizing lymphocytes capable of inducing lysis of target cells. Most of the research concerning the function of NK cells in birds has focused solely on their role in neoplastic diseases and therefore their role in non-neoplastic diseases is not very well described (Alexander, 2003).

2.4.1. Immunity in NDV

Several experiments have been made in the attempt to determine the immunological mechanisms that become activated when birds are infected with NDV. Many have tried to clarify the role of the humoral immune response and the cell mediated immune response (CMI) in protection of the bird. Using a live ND-vaccine as a substitute for a true infection it has been shown that the first response was a CMI raising only a few days post infection. This response was followed by a humoral immune response after seven days.

A rise was seen in the CMI until the second week and of the antibodies until the third week. The CMI declined after approximately four weeks. The CMI induced by the live ND vaccine could not be induced again using a virulent NDV infection but only by revaccination. The age of the chicken, type of vaccine and the number of vaccinations before challenge also has a great influence on the degree of the CMI. Chickens passively immunized with NDV specific antibodies are protected against clinical disease when exposed to a virulent NDV (Sharma, 1999) and furthermore chickens with an antibody titer lower than the protective titer has shown to be able to survive infection with virulent NDV. On the other hand a CMI alone does not deliver adequate protection against ND. Al-Garib, (2003) made an experiment using chickens with chemically suppressed T and B cell populations. After exposing the birds to virus in form of a ND-vaccination there was an increase in the number of T cells with a relatively higher increase of CD8+ cells than CD4+ cells indicating a Tc cell dominated response.

2.4.2. Immunity to MDV

Innate defense mechanisms such as macrophages and natural killer (NK) cells, as well as adaptive immune responses including the production of antibodies and elicitation of effector CD4_ T and cytotoxic T lymphocytes (CTLs), play a role in immunity against MDV (Davison and Kaiser, 2002). In this regard, cytokines produced by different cell types play a key role in the induction of the immune response to MDV (Kaiser *et al*, 2004).

NK cells are the first line of defense because these cells can lyse virus-infected and tumor cells without prior exposure to the pathogen. NK cells are also potent inducers of IFN. In order to lyse target cells, NK cells must recognize the target cells as foreign (e.g., the MHC class I has been altered, or the expression is downregulated). NK cells are also activated early after vaccination with SB-1 and HVT. The enhanced NK cell activity could be beneficial when chicks become infected shortly after vaccination perhaps by providing a source of IFN- or lysing virus infected cells (Heller and Schat. 1997).

2.4.2.1. Humoral immunity

Chickens infected with MDV develop precipitating and VN antibodies within 1-2 weeks; a transient immunoglobulin M (IgM) response is replaced by IgG. These antibodies are made against a large number of proteins. Most of these are not relevant for protective immune response, because they detect either nonstructural proteins or proteins that are not expressed in the virus envelope or on the surface of virus-infected cells. Due to the cell-associated nature of MDV antibodies are of limited importance in MD immunity. VN antibodies are important only when cell-free virus infects chickens or when MDV proteins are expressed on the surface of cells (Van Zaane, *et al* 1992).

2.4.2.2 Cell-mediated immunity

Cytotoxic T lymphocytes (CTL) recognize small peptide fragments of 8-12 amino acids in the context of self-MHC class I antigens. These peptides are generated from *de novo* synthesized proteins through a complex process involving the proteasome and transporters associated with antigen-processing (TAP) 1 and 2. *In vitro* demonstration of antigen-specific CTL requires effector and target cells expressing the same MHC class I antigens. Schat and coworkers

stabily transfected and expressed MDV genes in REV-transformed cell lines with known MHC antigens. These cell lines were used to show that CTL from infected or vaccinated chickens recognize peptides derived from pp38, Meq, ICP4, ICP27, gB, gC, gH, gI, and gE (Omar and Schat, 1997, Schat, and Xing, 2000). The effector cells developed around 7 days PI and were characterized as typical CTL expressing CD3, CD8, and TCR__1 but not CD4. Chickens vaccinated with a rFPV vaccine expressing gB developed CTL specific for gB in addition to VN antibodies (Nazerian, *et al*, 1992).

Cytokine responses associated with cell-mediated immune response to MDV infection have been characterized, particularly in the spleen (Kaiser *et al*., 2003) and peripheral blood lymphocytes (Quere *et al*., 2005). MDV infection is associated with a significant increase in the expression of interferon (IFN) - α, IFN-γ, interleukin (IL)-1β, IL-6, IL-8, IL-10 and IL-18 (Abdul-Careem *et al*., 2007; Jarosinski *et al*., 2005 and Quere *et al*., 2005).

2.4.2.3 Vaccinal immunity

HVT, attenuated MDV, and serotype 2 MDV protect against early replication of virulent viruses in the lymphoid organs of challenged birds and reduce the level of latent infection. Based on current knowledge, the following sequence of events is proposed to explain vaccine-induced immunity with challenge occurring within 3 days after hatch as is typical in the field. NK cells are activated as early as 3 days post vaccination, probably producing IFN-_ and killing limited numbers of virus-infected B cells when they develop between 1—3 days after challenge. IFN-_ is perhaps produced by other cells at this early stage (e.g., macrophages). IFN-_ can reduce virus replication and stimulate macrophages to initiate the production of iNOS. This enzyme will produce NO between 3 and 7 days post vaccination, thus limiting the replication of challenge virus. Antigen-specific CTL develop starting at 7 days post vaccination and may eliminate additional cells infected with challenge virus. The combination of these effector mechanisms will push the challenge virus into latency. Memory CTL will be able to quickly eliminate reactivated virus-infected cells unless altered by immunoevasive properties of MDV (Calnek, 2001).

Host genetic resistance and vaccination prevent lymphoma formation accompanied by a significant reduction in viral infectious particle as well as viral genome load in lymphoid organs (Kaiser *et al.*, 2003) however, host responses appear unable to control viral replication in the FFE (Islam *et al.*, 2004; Abdul-Careem *et al.*, 2007). For instance, viral genome load in the feather follicle is several folds higher than in the spleen. The apparent differential host response in lymphoid tissues and the feather follicles may be due to the lack of development of protective immune response against MDV in the feather (Baigent *et al.*, 2005).

2.5. Diagnosis

2.5.1. Diagnosis of MD

The gross appearance of neural lesions is generally diagnostic. Histological examination of nerve and visceral lesions will show characteristic lymphocytic proliferation. The condition should be differentiated from botulism and from "transient paralysis", an emerging condition of unknown etiology, but suspected to be an autoimmune response to vaccination in specific strains of commercial laying hens. The causal virus may be isolated and identified by submitting tissues to a suitably equipped laboratory using specific tissue culture techniques.

2.5.1.1. Identification of the agent

Virus isolation: Infection by MDV in a flock may be detected by isolating the virus from the tissues of infected chickens. However, the ubiquitous nature of MDV must be taken into consideration and the diagnosis of MD should be based on a combination of MDV isolation or detection of the genome by polymerase chain reaction (PCR) and clinical disease. Commonly used sources are buffy coat cells from heparinised blood samples, or suspensions of lymphoma cells or spleen cells. When these samples are collected in the field, it is suggested that they be transported to the laboratory under chilled conditions. As MDV is highly cell associated, it is essential that these cell suspensions contain viable cells. The cell suspensions are inoculated into monolayer cultures of chicken kidney cells or duck embryo fibroblasts (chicken embryo fibroblasts (CEF) are less sensitive for primary virus isolation). Areas of cytopathic effects, termed plaques, appear within 3–5 days and can be enumerated at about 7–

10 days. Another, less commonly used source of MDV for diagnostic purposes is feather tips, from which cell-free MDV can be extracted (OIE, 2010).

With experience, plaques caused by the different virus serotypes can be differentiated fairly accurately on the basis of time of appearance, rate of development, and plaque morphology. HVT plaques appear earlier and are larger than serotype 1 plaques, whereas serotype 2 plaques appear later and are smaller than serotype 1 plaques. MDV and HVT plaques may be identified as such using specific antibodies raised in chickens. Monoclonal antibodies may be used to differentiate serotypes (Lee *et al.*, 1983).

Polymerase chain reaction (PCR): Genomes of all three serotypes have been completely sequenced (Afonso *et al.*, 2001). PCR tests have been developed for the diagnosis MD. Real-time quantitative PCR (qPCR) to quantify MDV genome copies has also been described (Abdul-Careem *et al.*, 2006; Baigent *et al.*, 2005; Islam *et al.*, 2004). In addition, PCR tests that enable differentiation of oncogenic and non-oncogenic strains of serotype 1 MDV, and of MDV vaccine strains of serotypes 2 and 3 (Bumstead *et al.*, 1997; Handberg *et al.*, 2001) have been described. PCR may also be used to quantitate virus load in tissues (Baigent *et al.*, 2005 and Reddy *et al.*, 2000) or differentially detect MDV and HVT in the blood or feather tips (Baigent *et al.*, 2005; Davison and Kaiser, 2002).

2.5.1.2 Serological tests

The presence of antibodies to MDV in non-vaccinated chickens from about 4 weeks of age is an indication of infection. Before that age, such antibodies may represent maternal transmission of antibody via the yolk and are not evidence of active infection.

Agar gel immunodiffusion: There is no prescribed test for trade, but the agar gel immunodiffusion (AGID) test is employed most commonly to detect antibody. The test is conducted using glass slides coated with 1% agar in phosphate buffered saline containing 8% sodium chloride. Adjacent wells are filled with antigen or serum and these are incubated in a humid atmosphere at 37°C for 24 hours for diffusion to take place; positive sera show reactions of identity with known positive serum and antigen. The antigen used in this test is

either disrupted MDV-infected tissue culture cells or an extract of feather tips, or skin containing feather tracts obtained from MDV-infected chickens (OIE, 2010).

Other tests for MDV antibody include the direct and indirect fluorescent antibody test. These demonstrate the ability of a test serum to stain MDV plaques in cell cultures (Silva *et al.*, 1997). These tests are group specific and more sensitive than the AGID test. A virus neutralisation test for the ability of a serum to neutralise the plaque-forming property of cell-free MDV can also be employed. However, this test is more suitable for research purposes than for routine diagnostic use. Enzyme-linked immunosorbent assays (ELISA) for detecting MDV antibodies are available (Sharma, 1998; Zelnik *et al.*, 2004).

2.5.2. Diagnosis of ND

2.5.2.1 Identification of the virus

When investigations of ND as a result of severe disease and high mortality in poultry flocks is needed, it is usual to attempt virus isolation from recently dead birds or moribund birds that have been killed humanely. Suspensions in an antibiotic solution prepared from tracheal and cloacal swabs (or faeces) obtained from live birds, or of faeces and pooled organ samples taken from dead birds, are inoculated into the allantoic cavity of 9–11-day-old embryonating fowl eggs. The eggs are incubated at 37°C for 4–7 days. The allantoic fluid of any egg containing dead or dying embryos, as they arise, and all eggs at the end of the incubation period are tested for haemagglutinating activity. Any haemagglutinating agents should be tested for specific inhibition with a monospecific antiserum to NDV. NDV (APMV-1) may show some antigenic cross-relationship with some of the other avian paramyxovirus serotypes, particularly APMV-3 and APMV-7. The pathogenicity of any newly isolated virus can be assessed by determining the intracerebral pathogenicity index. The pathogenicity of isolates can also be evaluated using molecular techniques, i.e. reverse-transcription polymerase chain reaction and sequencing. NDV is subject to official control in most countries and the virus has a high risk of spread from the laboratory; consequently, appropriate laboratory biosafety and biosecurity must be maintained; a risk assessment should be carried out to determine the level needed (Hanson, 1980).

2.5.2.2. Serological tests

NDV may be employed as an antigen in a wide range of serological tests, enabling neutralisation or enzymelinked immunosorbent assays (ELISA) and HI to be used for assessing antibody levels in birds. At present, haemagglutination and haemagglutination inhibition test (HI) test are widely used for detecting antibodies to NDV in birds, although many poultry producers are using commercial ELISA kits to assess post-vaccination antibody levels and serological evidence of infection.

The value of serology in diagnosis is clearly related to the expected immune status of the affected birds. HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16, 2^4 or log2 ^{4} when expressed as the reciprocal or more against 4 hemaglutination unit (HAU) of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titre is 1/8 (23 or log2 3) or more. Back titration of antigen should be included in all tests to verify the number of HAU used. HI titres may be used to assess the immune status of a flock. In vaccinated flocks that are being monitored serologically, it may be possible to identify anamnestic responses as the result of a challenge infection with field virus, but great care should be exercised as variations may occur from other causes. For example, it has been demonstrated that APMV-3 virus infections of ND-virus-vaccinated turkeys will result in substantially increased titres to NDV (Allexander, *et al.* 1983; OIE, 2010)

There are a variety of commercial ELISA kits available and these are based on several different strategies for the detection of NDV antibodies, including indirect, sandwich and blocking or competitive ELISAs using MAbs. At least one kit uses a subunit antigen. Usually such tests have been evaluated and validated by the manufacturer, and it is therefore important that the instructions specified for their use be followed carefully. The HI test and ELISA may measure antibodies to different antigens; depending on the system used. ELISAs may detect antibodies to more than one antigen while the HI test is probably restricted to those directed against the HN protein. However, comparative studies have demonstrated that the ELISAs are

reproducible and have high sensitivity and specificity; they have been found to correlate well with the HI test (Adair, *et al*. 1989; OIE, 2010).

2.6. Prevention and control

2.6.1. Prevention and control of MD

Hygiene and management: Two basic factors regarding Marek's disease virus infection needed to be remembered when considering MD control are 1) MD vaccine only prevent rumor development from Marek's disease and not infection and 2) full immunity from vaccination takes at least 5 days. This means that all flocks become infected with the virus during their lives and usually shed the virus in the environment starting at about 5 weeks of age. Therefore flock older than 5 weeks of age serve as a source of early exposure to chick before the vaccine has achieved to immunize them. Also, the previous flock in a house has contaminated that house with fully pathogenic virus. The cleaning a disinfection used between flocks must estimate or dramatically reduce this source of infection. With bad management and hygiene it is impossible to achieve good protection even using all kinds of available vaccines. MDV can survive in infected houses for more than one year. Rearing chickens in isolators with controlled management is impossible for commercial poultry production. The three basic management practices need to be addressed for successful control of MD include, reducing early exposure, reducing immunosuppresants and placing chick with well-vaccinated chicks (Sharma, 1999).

Genetic selection for MD resistance together with other control methods, should be most important and of great value for poultry breeders and poultry production. For more than 50 years it is known that in many breeds genetically controlled differences exist in susceptibility to MD (Joslpovlc, 1990).

Vaccination: Control of MD is essentially achieved by the widespread use of live attenuated vaccines. Commercial biological products used in the control of MD are the cell-associated or cell-free (lyophilised) live virus or HVT, respectively. Marek's disease vaccines are injected *in ovo* at the 17th or 18th day of embryonation (Sharma, 1999) or subcutaneously at hatch.

Three types of vaccine are available: Type 1: attenuated chicken strain (e.g. Rispen's); Type 2: apathogenic chicken strain (e.g. SB1) and Type 3: apathogenic turkey, strain (e.g. HVT)

Due to the interfering effect of maternal antibody on HVT it is advisable to alternate vaccine types in successive generations. In countries where highly pathogenic MDV occurs, parents should be vaccinated with Rispen's strain, allowing commercial progeny to be immunized using the less expensive HVT strain alone or in combination with the potentiating SB1 strain. Cell-associated, frozen vaccines require special storage in a liquid nitrogen canister. Careful reconstitution using the diluents supplied by the vaccine manufacturer is necessary to maintain viability of the vaccine virus. Improper vaccination technique may lead to defective immunization with resulting "breaks". It is essential to place day old chicks in houses which have been thoroughly decontaminated to allow vaccinated flocks to develop immunity. Rearing farms and broiler growing units should be operated as single-age units with all-in-all-out cycles (Nair, 2004).

Currently vaccines based on biotechnology are available and have their own advantages. Although genetically engineered recombinant vaccines have been developed and tested in laboratory and field trials (Lee *et al.*, 2010), they are still not in commercial use.

2.6.2. Prevention and control of ND

The non specific control of Newcastle disease includes only the technological principles that prevent the spread of Newcastle disease and almost all other poultry diseases. These principles are :.all in - all out, one age on a farm, one production purpose, one genetic basis and a long-enough pause between cycles so a radical break in transmitting biological contamination from the processing cycle of previous poultry generationis possible. The procedures which prevent the spreading of ND are also non-specific but their purpose is to control the disease (Hanson, 1984). However these are less practical in backyard poultry production.

Eradication of ND is unlikely and there are few poultry species which are resistant to the disease. Continual vaccination programs currently offer the only effective way of controlling

ND (Udo *et al.* 2006; Alders and Spradbrow 2001). Thus, chicken vaccination is one of the most important technical possibilities to improve village chicken production. ND control was found to have positive effects on bird off-take, egg production, egg offtake and flock size (Udo *et al.* 2006; Woolcock *et al.* 2004). Different types of live vaccines for control of ND are prevalent because they allow mass application techniques. ND vaccines currently in use in many countries include: La Sota (live vaccine, thermolabile); Hitchner B1 (live vaccine, thermolabile), ITA-NEW/NEW COVER (inactivated vaccine, thermostable); NDV4-HR (live vaccine, thermostable); and I-2 (live vaccine, thermostable). Lentogenic strains like La Sota are immunogenic enough and widely used all over the world, but still have variable residual pathogenicity. Vaccination via drinking water and aerosol vaccination are efficient, though aerosol vaccination causing high protection in a shorter period of time, and is less affected by maternal antibodies (Al-Garib, 2003, Toth, 2000).

All vaccinal and many other strains of ND virus induce the production of specific HI antibodies in poultry. Their quality is in a significant positive correlation with the immunity rate of poultry, so it is possible to ascertain the specific immunity of vaccinated poultry by relatively simple laboratory trials (Woolcock *et al.* 2004).

2.7. Variability of the immune response

The immune reaction of very young poultry is much poorer than that of matured poultry or that several weeks old. Unfortunately, the ND virus infects very young animals too, so protection is needed even then. There are differences between particular genetic lines and their hybrids in the immune response ability. It seems that these differences are related to the general resistance to micro-organisms and only in a minor way to the specific agent. The morphological basis of the resistance of an animal includes immunocompetent organs or a complete immune system. The differences between particular hybrids or poultry lines can already be noticed by simple weighing the bursa of Fabricius. Diseases like Marek's disease or lymphoid leucosis are significant indicators not only of the specific resistance, but also of the resistance to all possible noxious elements in the environment (Toth, 2000).

Effect of viruses: The immune system of poultry can be destroyed by different viruses. Among them, two are prominent: the viruses of Marek's and Gumboro diseases. In 1972 the high incidence of Gumboro disease in Yugoslavia was followed by the drop in the immune response to a specific vaccine (Mazija *et al.*, 1984). The introduction of twofold vaccination at the age of 4 or 25 days by spraying has highly improved the immune response of chickens, but it still remains that in case of infection with pathogenic virus; this technique cannot completely prevent mortality.

There are many examples of immunosuppressive effects of Marek's disease virus. This infection complicates the clinical picture and the course of coccidiosis. The discovery that B cells of all three immunocompetent organs - tymus, spleen and bursa of Fabricius are acceptable to early cytolitic infection with the virus of Marek's disease Hirai, (2001) completely explains the fact mentioned. This is especially so considering the fact that in the continuation process; the degeneration even of T cells of the same organs shows up. The pathogenic virus of Marek's disease reduces, by its activity, the common resistance to diseases even before causing the clinical signs of the disease. These inconvenient effects could be recognized most clearly in the comparative investigations of production abilities of chickens of the control group and the experimental group vaccinated with Marek's disease vaccine.

3. MATERIALS AND METHODS

3. 1. Experimental animal and site description

The study was conducted at the School of Veterinary Medicine Addis Ababa University Debre Zeit. The School is located 45km southeast of Addis Ababa. The site is located at an altitude of 1900 meters above sea level. It receives an average annual rainfall of 851mm and a minimum and maximum temperature of 8.9°C and 26.2°C, respectively. The average humidity level of the site is 58.6%.

The experimental animals were chicken of various Ethiopian ecotypes originated from different ecological areas of the country as indicated on Fig. 1. The ecotypes include Horro, Tepi, Arsi, Chefe and Jarso located in the west, southwest, south east, central and east of the country, respectively. The rest experimental chickens were Fayoumi and Bovans breeds obtained from Debre Zeit Agricultural Research Center and Genesis farm, respectively.

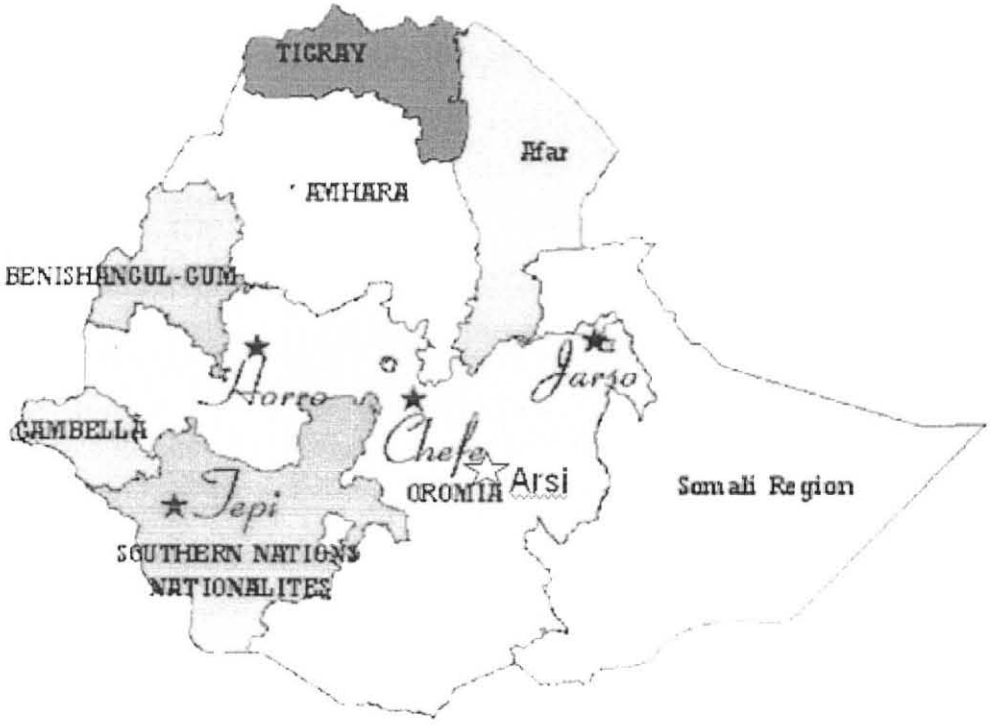


Fig. 1. Map of Ethiopia showing the origins of the chicken ecotypes used in the current study.

3. 1. 1. Acquisition of study animals

Eggs of indigenous chickens were purchased from markets of the respective areas (Horro, Tepi, Arsi, Chefe and Jarso) and transported to be hatched at Genesis farm Ethiopia, Debrezeit. The eggs of Fayoumi and Bovans were obtained from Debre Zeit Agricultural Research Center and Genesis Farms. All eggs for incubation were sorted against cracks, morphological deformities, and dirt (soiled) and fit eggs were disinfected with 70% ethanol. The eggs of all chicken types were incubated and hatched simultaneously but in separate compartments in one hatchery. Total of 260 chickens among which 70 Chefe, 40 Jarso, 21 Horro, 9 Arsi 20, Bovans, 70 Fayoumi, and 30 Tepi chickens were included in the study.

3. 1. 2. Management, housing, and diet of study animal during the experiment

House of 25m x 8m was constructed in the campus and equipped with infra-red bulbs, cleaned, disinfected and *teff* straw bedded. Hatched chicks were raised on electrically heated brooder using infra-red bulbs. The birds were kept intensively and stocked at 10 birds/$2m^2$. They were fed on starter commercial layer ration (0-8 weeks), grower commercial ration (9-20 weeks) and layer ration (21 weeks onwards) with standard formulated ration. Clean potable water was supplied to all stage of birds on adlibtum. During the experiment, no attempt was made to clean and disinfect except addition of *teff* straw bedding every 3 week. Antibiotics, anticoccidial and vitamins were supplied for all chick flocks under study when disease is suspected in a pen. Individual chicks in every group of ecotype were managed identically and the similar management and follow up was carried out.

3. 2. Experimental design

All the studied in each of chicken types were grown strating from day-old chickens and each vaccinated against ND at 21, 50 and 120 days of age. Then the day old chicks of each chicken type were classified into Marek's disesase vaccinated and non-vaccinated group by simple random technique. Only MD vaccinated chicken were included for Arsi and Bovans brown chickens due to inadequate and low number of hatching in this study. Therefore, there were two populations for each chicken type, namely those that received MD and ND vaccine as well as those that received only ND vaccine. Bleeding from brachial vein for analysis of

antibody titer against ND and total serum protein was conducted on 35th, 65th and 135th day age following ND vaccination at 21, 50 and 120 days of age. On other hand, for hematology (differential WBC, PCV and hemoglobin) analysis chicken bleeding was conducted at age of 35, 65 and 105 days.

3. 3. Vaccines used

MD vaccine prepared from herpes virus of Turkey strain (HVT) (a vial of 1000 doses produced by Lohmann animal health Gmbh and Co. Kg Germany With batch No. 9450021) was obtained from Alema farm. It was injected (0.2 ml) on the neck subcutaneously at day-old in the hatchery room according to the treatment design indicated above for each chicken type. A commercial ND vaccine (*LaSota* strain) was obtained from National Veterinary Institute (NVI, batch number 2-10) vaccine production laboratory. A vial of lyophilized vaccine (500 doses /vial) was reconstituted with 4ml sterile phosphate buffered saline (PBS) and stored at -70°C in 0.2 ml aliquots. The vaccine was suspended in distilled water and given by drinking water to all experimental chicken according to the recommendations of the producer company.

3. 4. Data Collection and Laboratory Analysis

3. 4. 1. Blood sample for hematological analysis

Blood sample was collected at age of 35, 65 and 105 days. Thin smears for total and differential leukocyte count were prepared immediately after brachial (wing) vein puncture and heparinised capillary tubes were used for determination of PCV. Blood sample for hemoglobin estimation was collected using 3ml syringe. Total white blood cells (WBC) count, differential WBC count (lymphocytes, eosinophils, hetrophil, monocytes and basophil), packed cell volume (PCV), and haemoglobin concentration (Hb) were evaluated as described by Campbell (1995). Thus, hematological data were collected and evaluated at different ages of 35, 65 and 105 days.

3. 4. 2. Serum sample collection for protein analysis and HI test

Serum sample for protein analysis: a 3ml syringe was used to collect blood sample by brachial vein puncture. Then the blood was left at room temperature over night to separate the serum. The serum was then collected into ependorf tube and stored at -20°C until the date of analysis. The serum samples were collected three times specifically at age of 35, 65 and 135 days. Serum protein response to ND and MD vaccination of each chicken type and treatment group was analyzed using spectrophotometer (HUMA STAR @ 80). 1000µl of burette reagent obtained from LiNEAR chemicals.S.L. (Germany) were added to 10µl of serum sample. The absorbance was red at 540 nm against the standard (7g/dl)

Hemagglutination inhibition test (HI): serum antibody response to ND vaccination of each chicken type was analyzed using HI test as described by Alexander (1988) using Phosphate buffered saline, formalinized LaSota virus (4 HAU) as a source of antigen and 1% chicken erythrocytes (CRBC) as an indicator system.

3. 5. Data management and Analysis

Data was entered in to excel spread sheet and cleaned. SPSS version 15.0 software was used for the data analysis. Geometric mean for HI titer and arithmetic mean for blood cell count and standard deviation/error (SD or SE) were determined and classified by chicken type, age and MD vaccination status. The statistical significance differences between various treatments was determined using the ANOVA and GLM for hematology, protein profile and serology data. Where results were significant Post Hoc multiple comparisons test were made to establish differences between treatment groups. Difference among means was considered significant when p-value is less than 0.05 (95% confidence level).

4. RESULTS

4. 1. Results of Hematological Analysis

4. 1. 1. Differential white blood cell count across different chicken types

Mean ± SD of total and differential leukocyte count, across the different chicken types is presented in Table 1. The total leukocyte count observed was lowest in Horro (23194.4±6766.5) and highest in Fayoumi (33850.1±11856.3) and intermediate for Tepi (7861.5±8776.8), Jarso (28863.6±7148) and Chefe (30739.5±9870.6) and Arsi (33347.7±15998.8) *(P=0.000)*. The lymphocyte count was indicated in Table 1 and higher in Fayoumi chicken followed by Jarso chicken. The count for Chefe and Arsi was medium whereas Tepi and Horro had relatively lower lymphocyte number (p = 0.08). The trend for lymphocyte count across the ecotypes was generally higher than the other leukocyte types. The monocyte count was also higher for Fayoumi followed by Tepi and it was medium for Jarso, Chefe and Arsi and lower for Horro chicken ecotype. Heterophil response was significantly higher for Arsi which was very far from other ecotypes. Tepi, Fayoumi, Jarso and Chefe had similar values and lowest for Horro (p = 0.000). Eosinophil and Basophil count was also higher for Arsi chicken and lower in Chefe chicken. Generally there was a variation in the counts of mainly total leukocyte, lymphocyte and heterophil in all chicken types; however, Fayoumi and Arsi chickens showed uniformly higher counts as summarized in Table 1 below. The differential leukocyte count by age indicated that total WBC and lymphocytes increased progressively with increasing age but the other WBC were fluctuating (Table 2).

The differential leukocyte counts at 35, 65 and 105 days of age were shown in Table 2. Total leukocyte count was higher at age 65 day (33386.6±9568.7) followed by age 105 day (29948.7±10156.3), lymphocyte had an increasing trend with the lowest at 35 day and highest at 105 day (14918.5 ±12612.9 and 18374.6 ±8071.9), respectively. Monocyte had a decreasing trend with maximum observed at 35 day (6388.5 ±9473.1, 6386.3 ±3502.6 and 4689.8 ±3001.7), respectively. Heterophil count was highest at 65 day (10058.1 ±14850.9)

with lowest value seen at the 105 day. Eosinophil and basophil had highest value at 35 day.

Table 1. Mean ± SD of total and differential leukocyte count of the different chicken ecotypes.

Ecotype	No. chicken	Total WBC	Differential leukocyte count				
			Lymphocyte	Monocyte	Heterophil	Eosinophil	Basophil
Arsi	11	33347.7± 15998.8	15667.3 ±7411.6	5039.2 ±2225.8	25042.5 ±42704.7	1394.3 ±1811.4	239.5 ±297.6
Horro	27	23194.4± 6766.5	12717.4 ±4771.7	3294.4 ±1521.9	6532.8 ±3546.5	908.7 ±877.3	129.8 ±156.1
Chefe	96	30739.5± 9870.6	16995.8 ±7964.6	5635.2 ±3333.2	7134.6 ±3796.0	1000.3 ±2462.3	137.9 ±288.8
Fayoumi	90	33850.1± 11856.3	18665.3 ±8519.7	7384.3 ±10828.8	7214.8 ±3913.5	1037.1 ±1083.0	136.9 ±233.9
Tepi	38	27861.5± 8776.8	13014.1 ±5920.7	5761.6 ±2981.5	7910.4 ±4717.7	673.5 ±570.4	82.8 ±164.7
Jarso	55	28863.6± 7148.5	18466.7 ±16235.5	5469.3 ±2677.4	7182.0 ±4624.6	732.6 ±641.8	110.9 ±200.5

P value =0.000 for Total WBC and heterophil, P= 0.008 for lymphocyte

Table 2. Mean± SD of total and differential leukocyte count at age of 35, 65 and 105 day.

Age in days	No. chicken	Total WBC	Differential count				
			Lymphocyte	monocyte	Heterophil	Eosinophil	Basophil
35	117	28077.2± 10594.1	14918.5 ±12612.9	6388.5 ±9473.1	7250.5 ±4467.7	1025.4 ±1061.9	172.8±297.5
65	104	33386.6± 9568.7	17669.6 ±7116.8	6386.3 ±3502.6	10058.1 ±14850.9	991.4 ±2362.6	61.4±146.6
105	101	29948.7± 10156.3	18374.6 ±8071.9	4689.8 ±3001.7	6057.5 ±2878.3	746.9 ±826.5	144.8±214.9

P value <0.05 for total WBC, total lymphocyte, heterophil and total basophil counts

4. 1. 2. Differential white blood cell counts among vaccinated to only ND vs. MD and ND

Hematological values between MD and ND vaccinated versus only ND vaccinated chicken in all ecotypes showed that the count was higher for total leukocyte, lymphocyte, monocyte, heterophil in MD and ND vaccinated versus only ND vaccinated chicken. The difference was significant in total leukocyte, lymphocyte and basophil counts ($P < 0.05$).

The lymphocyte count was compared across ages between different vaccination status among chicken types as shown in Table 3 below. The age specific lymphocyte count indicated that at age 35 day only ND vaccinated Horro and Tepi showed higher lymphocyte count than the MD and ND vaccinated group. The reverse was observed for Chefe, Fayoumi and Jarso. Significant difference was observed only in Fayoumi chicken ($p < 0.05$). At age of 65 day in all chicken types MD and ND vaccinated chickens showed superior count than only ND vaccinated ones, however significant difference was not observed. At age of 105 day lymphocyte count was higher in only ND vaccinated than MD and ND vaccinated except in Fayoumi and Jarso which showed higher count in the MD and ND vaccinated group. MD vaccinated Fayoumi and Jarso responded positively to lymphocyte count at all ages whereas Horro and Tepi at the age of 65 day and Chefe at age of 35 day.

Monocyte count in chicken types between MD and ND vaccinated versus only ND vaccinated chickens was compared as shown Table 4. At age of 35 day MD and ND vaccinated were superior in monocyte count than the only ND vaccinated for Chefe, and Jarso. Whereas in case of Horro and Tepi only ND vaccinated chicken were superior however difference was not significant ($p > 0.05$). At the age of 65 day monocyte count was higher in MD and ND vaccinated for Horro and Jarso but lower for Chefe, Fayoumi and Tepi with no significance difference. At the age of 105 day monocyte number was higher in only ND vaccinated Chefe, Tepi and Jarso whereas it was higher for Horro and Fayoumi in MD and ND vaccinated ones.
The comparison of heterophil count between MD and ND vaccinated versus only ND vaccinated was presented in Table 5. Only ND vaccinated Horro, Chefe and Jarso had higher heterophil count but MD and ND vaccinated Fayoumi and Tepi were higher in heterophil count at age of 35 day. At age of 65 day except for Fayoumi counts were higher in only ND

vaccinated than MD and ND vaccinated chicken but the mean difference was not statistically significant ($p > 0.05$). At age of 105 day except for Chefe only ND vaccinated chicken had higher heterophil count; however, the difference was not significant.

Table 3. Comparative mean ± SD of lymphocyte count between MD and ND vaccinated versus only ND vaccinated chickens of each ecotype at age of 35, 65 and 105 day.

Age in day	MD and ND Vaccination status	Arsi	Horro	Chefe	Fayoumi	Tepi	Jarso	Total
35	ND vaccinated	0	9707 ±1722.4	10589.1 ±5061.5	11708.47 ±5684.338	9442.444 ±4111.71	18616.27 ±26501.94	12172.72 ±12111.6
	MD & ND Vaccinated	25398.33 ±4455.63	7472.6 ±3628.632	18951.06 ±3488.427	20774.88 ±7180.063	7478.333 ±2734.63	21103.33 ±27355.83	17909.36 ±12571.1
	Total	25398.33 ±4455.63	8310.5 ±3115.913	14537.81 ±6054.978	15853.11 ±7800.589	8656. ±3650.147	19735.45 ±26198.51	14918.46 ±12612.9
65	ND vaccinated	0	15221.8 ±5105.838	15071.82 ±4491.51	19704.6 ±8320.139	15785.33 ±5414.31	14678.88 ±3020.4	16471.41 ±6054.87
	MD & ND Vaccinated	14949.5 ±3448.2	16454.5 ±3497.8	17889.44 ±10346.22	23009.77 ±8453.819	20711.25 ±2912.98	16981.25 ±5219.81	18822.53 ±7893.59
	Total	14949.5 ±3448.24	15769.67 ±4247.94	16437.94 ±7893.58	21239.14 ±8394.94	17755.7 ±5058.16	16060.3 ±4524.87	17669.58 ±7116.78
105	ND vaccinated	0	16117.4 ±2387.24	22709.93 ±10211.98	19039.85 ±8221.378	16548.25 ±4242.72	17447.43 ±3611.32	19160.89 ±7649.72
	MD & ND Vaccinated	9086.75 ±2205.77	10874.4 ±3273.959	19065.46 ±7376.808	20200.21 ±9528.43	10948 ±5553.067	22203 ±8096.828	17423.2 ±8540.196
	Total	9086.75 ±2205.77	13495.9 ±3864.273	20955.19 ±8983.398	19641.52 ±8771.57	14394.31 ±5368.51	19983.73 ±6663.28	18273.95 ±8120.90
Total	ND vaccinated	0	14293.62 ±4253.01	15507.06 ±8242.136	16288.26 ±8121.175	13568.7 ±5483.716	17090.08 ±17016.07	15617.23 ±9638.17
	MD & ND Vaccinated	15667.27 ±7411.66	11253.79 ±4904.03	18614.13 ±7403.24	21263.44 ±8267.086	12163.67 ±6641.55	19701 ±15700.2	18064.91 ±9931.21
	Total	15667.27 ±7411.66^{abdf}	12717.41 ±4771.67^{abdg}	16995.86 ±7964.63^{acef}	18665.29 ±8519.75^{acef}	13014.08 ±5920.717^{acdg}	18466.75 ±16235.55^{abef}	16837.21 ±9846.426

Means represented with similar letter do not differ significantly ($p < 0.05$)

Table 4. Mean ± SD of monocyte count between MD and ND vaccinated versus only ND vaccinated chickens of each ecotype at age of 35, 65 and 105 day.

Age in day	Vaccination status	Arsi	Horro	Chefe	Fayoumi	Tepi	Jarso	Total
35	ND vaccinated	0	2502.333 ±1202.08	4507.842 ±2638.25	4716.632 ±2264.51	7634 ±4443.33	5052.636 ±2255.04	5033.721 ±2934.07
	MD & ND Vaccinated	6393.3 ±1052.52	2467.4 ±866.6855	6119.882 ±1771.731	13590.69 ±24198.69	5811.667 ±1791.169	5835.222 ±1291.47	7864.161 ±13252.3
	Total	6393.333 ±1052.525	2480.5 ±917.82	5269.08 ±2383.4	8773.343 ±16768.2	6905.067 ±3644.42	5404.8 ±1881.02	6388.462 ±9473.086
65	ND vaccinated	0	3888.4 ±1809.01	7835.471 ±5430.84	7432.467 ±3856.28	6929.833 ±1532.82	5637.125 ±3346.1	6878.588 ±4148.56
	MD & ND Vaccinated	5287.25 ±3252.37	4375 ±1601.1	5412.438 ±1881.89	7135.538 ±3128.80	6766 ±485.448	5691.25 ±3440.1	5912.623 ±2699.26
	Total	5287.25 ±3252.37	4104.667 ±1631.98	6660.667 ±4233.13	7294.607 ±3476.27	6864.3 ±1179.41	5669.6 ±3313.19	6386.317 ±3502.64
105	ND vaccinated	0	1998.4 ±496.18	5137.429 ±3599.00	5537.923 ±2522.39	3622.375 ±1674.84	6147.429 ±3334.44	4806.809 ±2962.76
	MD & ND Vaccinated	3775.5 ±1074.09	4434.4 ±1217.98	4582.308 ±2187.91	5805.571 ±5014.83	3548.8 ±2117.85	4536.75 ±2189.1	4737.959 ±3139.42
	Total	3775.5 ±1074.095	3216.4 ±1554.70	4870.148 ±2960.68	5676.704 ±3940.73	3594.077 ±1769.97	5288.4 ±2802.29	4771.667 ±3038.33
Total	ND vaccinated	0	2841.615 ±1481.669	5815.52 ±4223.042	5810.553 ±3092.803	6054.957 ±3462.166	5527.231 ±2836.92	5558.396 ±3479.665
	MD & ND Vaccinated	5039.182 ±2225.877	3714.929 ±1486.91	5439.283 ±1990.11	9104.442 ±15238.4	5311.867 ±2073.44	5417.448 ±2575.58	6240.013 ±8284.45
	Total	5039.182 ±2225.87	3294.444 ±1521.97	5635.24 ±3333.224	7384.3 10828.84	5761.632 ±2981.505	5469.345 ±2677.44	5898.129 ±6345.82

Table 5. Mean ± SD of heterophil count between MD and ND vaccinated versus only ND vaccinated chickens of each ecotype at age of 35, 65 and 105 day.

Age in days	Vaccination status	Arsi	Horro	Chefe	Fayoumi	Tepi	Jarso	Total
35	ND vaccinated	0	8767.333 ±4684.171	6040.474 ±2742.972	5832.8 ±3416.3	8888.8 ±6990.8	8402 ±4158.61	6956 ±4202.507
	MD & ND Vaccinated	18474 ±9840.	4276.8 ±724.9908	5965.706 ±2658.94	7852 ±4149.8	9982.3 ±3479.7	6694.556 ±3860.65	7571.429 ±4757.25
	Total	18474 ±9840.2	5960.75 ±3459.862	6005.167 ±2665.19	6755.886 ±3849.41	9326.2 ±5705.983	7633.65 ±4017.126	7250.564 ±4467.75
65	ND vaccinated	0	8767.333 ±4684.17	9684.647 ±3733.85	9100.733 ±4391.71	7953.833 ±4550.01	8281.875 ±4109.22	8802.49 ±4115.56
	MD & ND Vaccinated	45671 ±71159.8	4276.8 ±724.9908	8262.25 ±5336.07	9172 ±4731.403	6737.5 ±2295.192	8086.75 ±7313.244	11266.3 ±20433.7
	Total	45671 ±71159.8	5960.75 ±3459.86	8995 ±4564.98	9133.821 ±4466.70	7467.3 ±3694.851	8164.8 ±6098.755	10058.09 ±14850.9
105	ND vaccinated	0	6089.8 ±1885.633	5993.643 ±1919.02	6111.385 ±2568.29	7317 ±4832.17	5682.429 ±2271.30	6215.34 ±2751.51
	MD & ND Vaccinated	9340.5 ±3384.84	5089 ±1650.501	6768.385 ±4335.16	5549.143 ±2422.13	5498.4 ±2087.43	4908.625 ±2076.34	6025.408 ±3111.56
	Total	9340.5 ±3384.84	5589.4 ±1751.922	6366.667 ±3266.63	5819.852 ±2461.64	6617.538 ±3990.14	5269.733 ±2127.48	6118.396 ±2926.91
Total	ND vaccinated	0	6965.385 ±3675.01	7266.38 ±3371.61	6952.83 ±3792.148	8098.174 ±5512.64	7632.846 ±3787.34	7329.333 ±3923.57
	MD & ND Vaccinated	25042.55 ±42704.7	6131.143 ±3511.11	6991.348 ±4242.96	7501.302 ±4067.35	7622.4 ±3304.54	6777.966 ±5299.28	8331.386± 12407.82
	Total	25042.55 ±42704.7[a]	6532.815 ±3546.52[b]	7134.594 ±3796.05[b]	7214.878 ±3913.497[b]	7910.368 ±4717.701[b]	7182.091 ±4624.664[b]	7828.779 ±9189.063

Means represented with similar letter do not differ significantly ($p < 0.05$)

4. 1. 3. PCV and hemoglobin concentration among chicken types

PCV had increased with age whereas hemoglobin value decreased as shown in Table 6. The PCV value during at age of 35 day was higher in only ND vaccinated Horro, Fayoumi and Tepi whereas it was higher in MD and ND vaccinated Chefe and Jarso. The average PCV for MD and ND vaccinated in all chicken at first bleeding was 32.38 ±4.43 but it was 31.89 ±4.22 for only ND vaccinated group. The average PCV was significantly higher for Fayoumi and lowest for Arsi at this age ($p < 0.05$). At age of 65 day only ND vaccinated Horro, Fayoumi and Tepi showed higher PCV value but Chefe and Jarso it was the MD and ND vaccinated group that showed higher values. The average value at age of 65 and 100 day for MD and ND vaccinated versus only ND vaccinated group was summarized in Table 6 below. In general, at the age of 35 and 65 day PCV value was higher in only ND vaccinated group but it was higher in MD and ND vaccinated chicken at the age of 100 day. The PCV value among chicken types was significantly ($p < 0.05$) different while between MD and ND vaccinated versus only ND vaccinated was not ($p > 0.05$). Mean PCV and hemoglobin concentration were determined at different age of the chicken. PCV had increased with age whereas hemoglobin value decreased.

The hemoglobin concentration in MD and ND vaccinated was higher only for Fayoumi at age of 35 day of the chickens. However, at age of 65 day Fayoumi, Chefe and Tepi chicken showed higher concentration in MD and ND vaccinated groups. The hemoglobin value was highest for Tepi chicken (9.520±.6450) and all the other ecotypes had hemoglobin values between 9.469±.6077g/dl for Chefe chicken (8.820±.7702). The trend for both PCV and hemoglobin concentration was increasing in all indigenous chicken ecotypes and Fayoumi as indicated in Table 6 below.

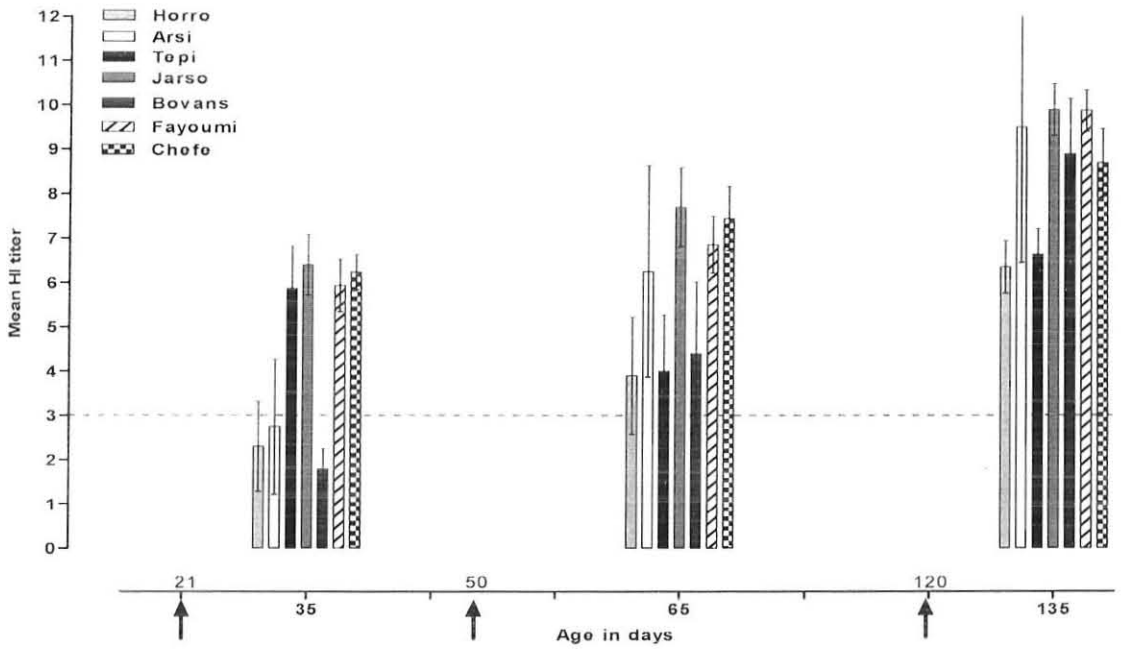
Table 6. Comparative mean ± standard deviation of PCV and hemoglobin (Hb) concentration between MD and ND vaccinated versus only ND vaccinated chickens at age of 35, 65 and 100 days.

Age	Vaccination status	Arsi	Horro	Chefe	Fayoumi	Tepi	Jarso	**Total**
PCV at 35 day	ND vaccinated	0	32.8 ±2.77	32.53 ±4.96	34.24 ±4.71	30.13 ±2.75	30.33 ±3.54	**32.38 ±4.43**
	MD and ND vaccinated	29.3 ±2.06	29.6 ±0.89	33.53 ±5.30	33.50 ±3.71	29.71 ±3.25	30.09 ±3.30	**31.89 ±4.22**
	Total	**29.3 ±2.06** acdg	**31.2 ±2.57** acdg	**33.00 ±5.08** acdh	**33.86 ±4.18** bcdh	**29.93 ±2.89** acfg	**30.20 ±3.32** acfg	**32.13 ±4.31**
PCV at 65 day	ND vaccinated	0	32±1	31.95 ±2.91	36.94 ±2.59	33.63 ±3.29	30.67 ±2.55	**33.45 ±3.56**
	MD and ND vaccinated	33± 2.94	31.± 1.67	32.4± 3.06	35.78 ±2.05	33.00 ±3.21	30.91 ±1.58	**33.18 ±2.99**
	Total	**33± 2.94** acd	**31.± 1.32** acd	**32.1± 2.96** acd	**36.34± 2.36** abe	**33.33 ±3.15** ace	**30.80 ±2.02** acd	**33.31 ±3.27**
PCV at 100 day	ND vaccinated	0	39.± 1.64	35.4± 3.27	38.18± 3.86	36.2± 5.31	37.0± 3.50	**36.9± 3.83**
	MD and ND vaccinated	37± 2.45	35.± 0.84	35.24± 2.17	38.94± 2.82	36.0± 3.92	38.73± 1.74	**37.18± 2.92**
	Total	**37± 2.45** abef	**37.± 2.17** abef	**35.3± 2.77** abeg	**38.57± 3.34** acdf	**36.1± 4.55** abeg	**37.9± 2.74** acef	**37.05± 3.38**
Hb at 65 days	ND vaccinated	0	8.7± 0.46	8.25± 0.88	8.53± 0.65	8.03± 0.45	7.96± 0.40	**8.30± 0.70**
	MD and ND vaccinated	8.7± 1.33	8.2± 1.39	8.21± 1.11	8.56± 0.95	7.97± 0.55	7.92± 0.44	**8.27± 0.96**
	Total	**8.7± 1.33** abd	**8.4± 1.02** abd	**8.23± 0.98** abd	**8.54± 0.81** abe	**8.00± 0.48** acd	**7.94± 0.41** acd	**8.28± 0.84**
Hb at 100 day	MD Non vaccinated	0	8.9± 0.77	9.13± 0.61	9.33± 0.67	9.75± 0.52	8.36± 0.41	**9.14± 0.71**
	MD vaccinated	9.3± 0.44	9.3± 0.67	9.08± 0.78	9.60± 0.52	9.26± 0.71	9.20± 0.80	**9.31± 0.69**
	Total	**9.3± 0.44** abd	**9.1± 0.71** abd	**9.11± 0.69** abe	**9.47± 0.61** acd	**9.52± 0.64** acd	**8.82± 0.77** abe	**9.23± 0.70**

Means represented with similar letter do not differ significantly ($p < 0.05$)

4. 2. Response of antibody titer against NDV Virus Vaccine

Antibody (HI) titer to NDV in the different chicken ecotypes (in log2) expressed in geometric mean ± SD presented in Table 7 and Fig. 2. The overall serum antibody titer during 3 times vaccination i.e. 1st (day 21), 2nd (day 50) and 3rd (day 120) was evaluated. Analysis of sera collected at age of 35, 65 and 135 day indicated a significant (p = 0.000) increasing trend in antibody titer as from 4.83±1.98, 6.07±1.93 to 8.31±2.42, respectively.



Arrows indicated age of chicken vaccination in day

Fig. 2. The trend of antibody titer against ND vaccine among chicken types

Fifteen days (at age 35 day) after the first vaccination the antibody titer of Arsi, Horro, and Bovans was less than 3 and the lowest titer was observed in Bovans (1.800). Fayoumi, Chefe, Tepi and Jarso had mean antibody titer above 5 with highest for Jarso (6.505). The antibody titer again fifteen days (age at 65 day) after the first booster (at age 50 day) showed still lowest titer for Horro (3.900) followed by Tepi (3.917) and Bovans (4.400). At age 65 day the Jarso chickens maintained its titer at the highest titer (7.802) followed by Chefe (7.181) and Fayoumi (6.995). Arsi ecotype showed better improvement (6.250) whereas Tepi (3.917)

showed a fall in antibody titer at age of 135 day after booster vaccination (age 120 days) as shown on Table 7 and Fig. 2.

The antibody titer against NDV following the 2nd booster vaccination was high for Jarso (10.709) followed by Fayoumi (9.837). It remained higher after each vaccination as compared to the other chicken ecotypes. Bovans, Chefe and Arsi chicken showed medium titer (8.400), (8.578) and (9.500), respectively. Tepi (6.889) and Horro (6.100) showed the lowest titer with Horro remaining at the bottom even after the 3rd vaccination. On the other hand, Bovans showed better improvement after the 2nd booster vaccination. Generally the response of all the Ethiopian chicken ecotypes, Fayoumi and Bovans to NDV (LaSota) vaccination had an increasing trend, however Jarso and Fayoumi responded well in all the vaccinations. The response was lowest for Horro chicken ecotype.

Table 7. Geometric ± SD antibody (HI) titer to NDV in different chicken types at age of 35, 65 and 135 days.

Age	Ecotype	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
HI at 35th day	Arsi [b]	2.750	.682	1.399	4.101
	Horro [b]	2.300	.431	1.446	3.154
	Chefe [a]	6.284	.231	5.827	6.741
	Fayoumi [a]	6.083	.231	5.626	6.540
	Tepi [a]	5.861	.360	5.149	6.573
	Jarso [a]	6.505	.320	5.872	7.139
	Bovans [b]	1.800	.431	.946	2.654
HI at 65th day	Arsi [ade]	6.250	.939	4.391	8.109
	Horro [bdf]	3.900	.594	2.724	5.076
	Chefe [ace]	7.181	.317	6.553	7.810
	Fayoumi [ace]	6.995	.317	6.366	7.624
	Tepi [bdf]	3.917	.495	2.937	4.897
	Jarso [ace]	7.802	.440	6.931	8.674
	Bovans [bde]	4.400	.594	3.224	5.576
HI at 135th day	Arsi [acehi]	9.500	.993	7.533	11.467
	Horro [bdfgj]	6.100	.628	4.856	7.344
	Chefe [acfhi]	8.578	.336	7.913	9.244
	Fayoumi [adehj]	9.837	.336	9.171	10.502
	Tepi [bdfgi]	6.889	.524	5.852	7.926
	Jarso [adehj]	10.709	.466	9.786	11.631
	Bovans [acfgi]	8.400	.628	7.156	9.644

Chicken types with the same letter are having significant difference

The comparison of antibody titer against NDV in MD and ND vaccinated versus only ND vaccinated chicken of all type was shown in Table 8. An average titer after the first vaccination in only ND vaccinated chickens was 5.356 whereas that of MD and ND vaccinated chickens was lower 4.548 but the difference was not statistically significant ($p > 0.05$). After the first booster although not statistically significant there was difference in the antibody titer 6.086 and 5.687 in only ND vaccinated and MD and ND vaccinated chicken, respectively. The antibody response after the second booster vaccination showed significant difference between only ND vaccinated (8.898) and MD and ND vaccinated 8.234 chickens with ($p < 0.05$). Within each ecotype comparison in antibody titer between MD and ND vaccinated versus only ND vaccinated was higher in latter. This difference was consistent across the three vaccinations in Tepi and Jarso whereas Chefe showed increment after the second vaccination. However, all the chicken ecotypes showed higher antibody titer in the only ND vaccinated groups after the second booster vaccination. Generally in all the cases the immune response to NDV vaccine was higher in only ND vaccinated chicken than in MD and ND vaccinated with statistically significant difference seen only in the 2nd booster vaccination. The trend in antibody response in different chicken ecotypes was depicted in Figure 2 and Table 8.

Table 8. Geometric mean (in log2) antibody (HI) titer against NDV in MD and ND vaccinated versus only ND vaccinated chickens at age of 35, 65 and 135 days.

Age in days	Vaccination status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
HI at 35th day	ND vaccinated	5.356	.206	4.948	5.765
	MD & ND vaccinated	4.548	.185	4.181	4.915
HI 65th day	ND vaccinated	6.086	.284	5.524	6.648
	MD & ND vaccinated	5.687	.255	5.182	6.193
HI135th day	ND vaccinated	8.898	.300	8.303	9.493
	MD & ND vaccinated	8.234	.270	7.699	8.768

4. 3. Result of Total Protein Profile Chicken

Total serum protein value was higher in Tepi, Jarso and Fayoumi 2.995, 2.92 and 2.81g/dl, respectively at age of 35 day as shown in Table 9. Minimum value was seen in Horro chicken 2.22 and similar to the HI titer. At age of 65 day sampling higher values were recorded in Fayoumi, Jarso, Tepi and Horro with values 4.34, 4.08, 4.001 and 4.00, respectively, and minimum value was seen in Arsi 3.60. At age of 135 sampling higher values were recorded in Arsi 6.28 followed by Jarso, Tepi and Bovans. It was lower in Fayoumi and Chefe and lowest value was recorded in Fayoumi ecotypes 3.91. Generally total serum protein value observed in this study had increasing trend for Arsi, Chefe, Tepi, Jarso and Bovans with maximum protein concentration after age of 135 day. In Fayoumi and Horro maximum values were recorded at the age of 65 day. Unlike the HI titer consistency was not observed and there was variation among chicken types between age points for total serum protein.

Table 9. Total protein profile of chicken types at age of 35, 65 and 135 days.

Age	Ecotype	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Protein at 35th day	Arsi ^{acehi}	2.475	.217	2.046	2.904
	Horro ^{acfhj}	2.220	.137	1.949	2.491
	Chefe ^{acehj}	2.553	.074	2.406	2.700
	Fayoumi ^{adfgi}	2.810	.075	2.660	2.959
	Tepi ^{bdfgi}	2.995	.112	2.773	3.217
	Jarso ^{acfgi}	2.917	.102	2.714	3.119
	Bovans ^{acehj}	2.340	.137	2.069	2.611
Protein at 65th day	Arsi ^{kln}	3.600	.429	2.750	4.450
	Horro ^{kln}	4.000	.271	3.462	4.538
	Chefe ^{klo}	3.691	.147	3.400	3.983
	Fayoumi ^{kmn}	4.343	.149	4.047	4.639
	Tepi ^{kln}	4.009	.222	3.569	4.449
	Jarso ^{kln}	4.078	.202	3.677	4.479
	Bovans ^{klo}	3.710	.271	3.172	4.248
Protein at 135th day	Arsi ^{psuvx}	6.275	.844	4.602	7.948
	Horro ^{qrtvx}	3.920	.534	2.862	4.978
	Chefe ^{qrtvx}	4.094	.290	3.520	4.668
	Fayoumi ^{qrtwx}	3.909	.294	3.326	4.491
	Tepi ^{pruvx}	5.296	.437	4.431	6.162
	Jarso ^{psuvx}	5.383	.398	4.595	6.172
	Bovans ^{prtvx}	4.880	.534	3.822	5.938

Chicken types with the same letter have significant difference

The total protein profile of MD and ND vaccinated was lower than the only ND vaccinated chicken at age 35 and 65 day while at the age of 135 day MD and ND vaccinated groups had higher value as shown in Table 10. However, the difference of the mean between MD and ND vaccinated versus only ND vaccinated was not statistically significant ($p > 0.05$).

Table 10. Protein profile of MD and ND vaccinated versus only ND vaccinated chickens at age 35, 65 and 135 days

Chicken age	MD vaccination status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Day 35	Only ND vaccinated	2.747	.065	2.619	2.875
	MD and ND vaccinated	2.581	.059	2.463	2.699
Day 65	Only ND vaccinated	4.102	.128	3.849	4.356
	MD and ND vaccinated	3.863	.118	3.630	4.096
Day 135	Only ND vaccinated	4.490	.252	3.991	4.990
	MD and ND vaccinated	4.844	.232	4.385	5.303

$p>0.05$

5. DISCUSSION

Within and between indigenous chickens of Ethiopia considerable genetic diversity was reported (Dessie, 2003). Considerable diversity in morphology within and between the ecotypes was also reported (Duguma, 2006). Remarkable diversity in their production performance was also reported (Duguma, 2009). In this study considerable diversity in hematological and serological responses to MD and ND vaccination was noticed within and between our chicken ecotypes.

Accordingly, the total leukocyte count varied significantly across the chicken types which were ranked from high to low order as Fayoumi, Arsi, Tepi, Chefe, Jarso and Horro. The lymphocyte count had varied across the chicken types which were ranked from high to low in order of Fayoumi, Arsi, Jarso, Chefe, Tepi and Horro. The monocyte response was ranked in the order of Tepi, Fayoumi, Arsi, Chefe, Jarso and Horro and similar difference was seen in heterophil, basophil and eosinophil. PCV values were higher for Fayoumi whereas hemoglobin values were higher for Arsi with minimal variation among the ecotypes. Thus response of these chicken ecotypes was generally higher in Fayoumi and Arsi which may be related to their genetic difference among chicken types in response to MD and ND vaccination in their leukocyte count. The difference was clearly significant for total WBC and lymphocyte count which may be related with the lymphoproliferetive character of the MD viruses. This is in line with the report of Duguma (2005) who reported the existence of difference in susceptibility and mortality following natural challenge of vaccinated Ethiopian chicken ecotypes that were ranked in the order of Fayoumi, Jarso, Horro, and Tepi, respectively. In addition, the survival rate of MD non-vaccinated chickens was in the order of Fayoumi, Tepi, Horro, and Jarso, respectively.

Generally the current study showed difference in the leukocyte response of chicken types to MD vaccination which is in agreement with the work of Duguma (2006) who reported the diversity in resistance to natural MD challenge within and between the indigenous chicken ecotypes as measured by mortality/survival rate. Gene diversity and genetic distance of five indigenous chickens was also measured and compared by Dessie (2003) who found largest

genetic distance of Fayoumi chickens to all indigenous chicken ecotypes. The most distant ecotypes from Fayoumi breed being Jarso, Chefe and Tilil in that order and relatively far genetic distance between Tepi and Chefe, Tepi and Jarso, Tilili and Jarso and Horro and Jarso and with smallest genetic distance recorded between Tilili and Horro ecotype (Tadele, 2003; Duguma, 2009). A high genetic distance was reported by Halima *et al.* (2009) between the two commercial breeds, Whiteleg horn (WHL) and Rhode island red (RIR), which could be due to the fact that they were selected for different production systems (egg production vs dual-purpose). The RIR chicken population has shown higher genetic distance (lower genetic similarity) with the Ethiopian chicken populations than the South African fowls. This indicated that the Ethiopian indigenous chicken populations are still not highly diluted by the RIR chicken breed. Thus the difference in the leukocyte count in the current study could be related to their genetic difference (diversity) among the studied chicken ecotypes.

Total WBC, lymphocyte and monocyte count were positively related with MD vaccination which could be indicative of the protection level of the MD vaccinated chicken to viral infection in general and MD infection in particular. In this study raise of lymphocyte was observed and statistically significantly higher in MD and ND vaccinated chickens which could be related to viral vaccination. According to Rupley (1997) under normal condition lymphocytes make up the majority of WBCs in birds. Campbell (1996) reported the raise in lymphocyte is associated with a variety of immunological functions such as immunoglobulin production, modulation of immune defense and lymphoma as in the case of MD and lymphoid leucosis.

The difference in the lymphocyte count decreased with age for example in Fayoumi chickens which was due to the raise in count in only ND vaccinated chicken that could be associated with the characteristics of the MD vaccinal virus which start to be shade from vaccinated chicken after three weeks to the external environment through the feather follicle and infect MD nonvaccinated group. Thus it shows the existence of some kind of viral infection (vaccinal) that increase with age. This finding is partially in agreement with previous report that survival of control chicken groups maintained together with the vaccinated group suggesting infection via inhalation by shedding vaccinal virus (Duguma, 2006). It has been

reported that the live MD virus can be shed from the vaccinated chicken throughout the lifespan to the environment, infecting other chickens kept nearby via inhalation (Palya, 1991).

In the current study the antibody response to ND vaccine between the five Ethiopian chicken ecotypes and reference animals (Fayoumi and Bovans) revealed statistically significant difference. This is in line with previous reports of Van der Ziipp (1983) who reported the difference in antibody response between chicken families and lines. This could be due to the difference in the immunological maturity in different breeds of chicken. Thus different breeds of chicken vary in their antibody titer following vaccination as a result of their genetic difference in their immune responsiveness to the antigen of NDV and consequently differ in their level of protection and susceptibility to virulent challenge. The current work is in line with Semina *et al.* (1992) who reported difference in antibody response (titer) between heavy and light Israel local breeds and heavy breeds were inferior in antibody titer and susceptible to NDV challenge. It is also in agreement with Barileng (2006) who reported that breed has an effect on the immune response to ND vaccination and subsequent challenge. Accordingly, commercial layers were significantly inferior in the immune response as evaluated by HI titer when compared with native breeds. In the current study Bovans has showed inferior HI titer than other breeds after the first and second vaccination and still differences exist between our native chickens.

The relationship between HI titer and consequence of challenge has been summarized by Mazija (1990) in those chickens with HI titer below 3 experience mortality due to ND virus challenge. Thus at first vaccination Jarso, Fayoumi, Chefe, and Tepi chicken can be protected from heavy mortality during challenge as they have HI titer above 3. Those chicken ecotypes with titer less than $3(2^3)$ like Arsi, Horro and Bovans were not protected as a result need second vaccination.

The average titer after 2nd vaccination was 6.07 ± 1.927 ($2^{6.07\pm1.927}$) (1:128) which indicate that booster vaccination around 9 week had produced high protective immunity to ND which could protect from epidemics. This is in line with the epidemic theory which suggests that if 70% of the population is immune, the disease outbreak is unlikely to occur because there is

not enough susceptible population to propagate an epidemic (Thrusfield, 1995; Young *et al.*, 2002). However, the titer for Bovans, Tepi and Horro is still less than 4 (2^4) or 1:16 which cannot fully protect chicken from mortality as result of challenge. Thus, according to this study maximum protection is expected from Jarso, Chefe and Fayoumi chickens whereas Tepi, Bovans and Horro need another booster ND vaccination for strong immunity against virulent challenge. However, this conclusion should be accepted with caution as the half-life of the anti-ND virus antibodies was not evaluated in current study; hence we did not know how long the antibodies stay in the chicken to protect them from ND.

After 2nd booster the average titer of all chicken ecotypes was 8.31±2.424 ($2^{8.31\pm2.424}$) or 1:512 which could be highly protective, thus according to Mazija (1990) all chicken are fully protected from mortality as a result of challenge with virulent NDV. Similar to the 1st booster maximum protection was expected from Jarso and Fayoumi followed by Bovans and Chefe. The response of Horro chicken was poor at all level of vaccination which could be related to their genetic difference to ND resistance. Bovans (Netherlands chicken breed) responded better after the 3rd vaccination which warrants the need for more than 3 times vaccination for better protection of this breed.

In this study chicken vaccinated with MD and ND vaccine versus only ND vaccinated were compared and in all the cases only ND vaccinated chicken responded better. Turkey herpes virus strain which was used in this study is naturally not pathogenic to chicken and not cause any tumor; however, all of the MD virus vaccinal strains can cause some kind of immune suppression. Thus the current study suggests that MD vaccine might act as immune suppression agent. This finding corresponds to the work of Hirai (2001) who reported that B cells of all three immunocompetent organs - tymus, spleen and bursa of fabricius were damaged by cytolitic infection with the virus of MD. The pathogenic virus of MD reduces, by its activity, the common resistance to diseases even before causing the clinical signs of the disease. Moreover, application of two or more different antigen simultaneously may reduce the animals' responsiveness to the antigen by production of antibody as compared to when single vaccination. The difference in the average titer between 1st, 2nd and 3rd vaccination may be related to the progressive development of the immune system as age of chickens improves

and also frequent activation of immune memory cells by booster vaccine. That, in turn, might be correlated to better responsiveness of the chicken to the antigen.

In the current study statistically significant difference in total protein values among chicken ecotypes was observed at age 35 and 135 day. There was also an increasing trend in the values of total protein in Arsi, Chefe, Tepi, Jarso and Bovans. The major components of the serum protein are albumin and globulin and their main functions are in host defenses against pathogens (immunoglobulins), in transport of metabolites, in regulation of cellular metabolism and in maintaining osmotic pressure (Eckersall, 2008). Thus increasing trend in total protein among the mentioned chicken types might suggest that immune stimulus is caused by the vaccination with MD and NDV. According to earlier report by Rivetz *et al.* (1977) protein changes occur in birds infected by NDV. Also, in acute or chronic inflammatory conditions, a rise in total protein caused by elevated globulin fractions may occur (Lumeij, 2008) since the chronic antigenic stimulation leads to production of immunoglobulins by B lymphocytes, plasma cells, or both (Campbell, 2004).

The decrease in the total protein value of Fayoumi and Horro chicken at the at age of 135 day after third vaccination was not clear but probably be a depression of the immune response observed by the decrease of globulin concentrations after the last revaccination against NDV. In this context, Schultz *et al.* (1970) observed a reduction in total protein concentrations in turkeys with blue comb. On the other hand, Rivetz *et al.* (1977) found significant differences in total protein concentrations in birds inoculated with a virulent strain of NDV, with a reduction in total protein and albumin concentrations but without any alterations in globulin concentrations. Talebi (2006) also reported significant differences in total protein and albumin concentrations in broiler chickens vaccinated against NDV. Another important possibility is a decrease of serum proteins in these chicken types may be as a result of reduction in food intake.

In summary, the hematological and serological differences among chicken types may be related to their genetic resistance and difference in immune response. As there are a number of reports on the phenotypic and genetic difference of Ethiopian chicken ecotypes, their

susceptibility to disease and response to vaccination could be related to this fact. In contrary to hematological response and HI titer total protein value was lower in Fayoumi than the indigenous chicken ecotypes. This could be related to the difference in the level of albumin (genetical) or may be the presence of globulins against other unknown infections other than haemaglutinating antibody of ND. In such case this indigenous chicken be superior; however, this needs further clarification.

6. CONCLUSION AND RECOMMONDATIONS

Rural poultry development in national economy of Ethiopia and its role in improving nutritional and financial status of small holder farmers one day might be well recognized than its present status. Thus, becomes necessary to generate information regarding the response of Ethiopian chicken to vaccinal antigen. Ethiopian chicken ecotypes, Fayoumi and Bovans have showed alterations on the total WBC, lymphocyte, monocyte, heterophil, PCV and hemoglobin value in response to MD and ND vaccines. As all the chickens were vaccinated to ND vaccine the variation among chicken ecotypes was due the effect of chicken genetic background and MD vaccination. Among the chicken types Fayoumi and Arsi were with the highest count and lowest in Horro indicating difference in the response to MD vaccination. The difference among these chicken types is considered to be the effect of their genetic diversity which implies need of knowledge of different chicken types in designing selection and breeding and also in control of diseases like MD. The control of ND in village chickens can make a vital contribution to the improvement of household food security and poverty alleviation in poor countries like Ethiopia. Differences in humoral immune function between different chicken types indicate existing difference in the levels of immune response genetically. However, according to this study a onetime vaccination of chicken with LaSota strain is not sufficient for Arsi, Horro, and Bovans chickens and that the immune response needs one booster vaccination to attain protective level of antibody titer. Higher protective antibody level was detected when chickens vaccinated three times than once or twice which indicates that booster vaccination has created significant protective antibody titer in all chicken types. The current study have not included the duration of immunity (half-life of the antibodies) after each vaccination via drinking water, hence the frequency of revaccination to maintain full immunity could vary among the chicken types involved in this study. Ethiopian chicken ecotypes revealed alterations on total serum protein concentrations even higher than Fayoumi chicken suggesting that the blood protein profile was affected by vaccination against NDV (LaSota strain) and /or MD.

According to the above conclusive remarks the following recommendations are forwarded:

- The considerable diversity in hematological and serological responses to MD and ND vaccination noticed within and between our chicken ecotypes during current study should be verified by challenging them with virulent NDV and MDV.
- An on station or field trial should be performed to evaluate the duration of immunity (half-life of the antibodies) in different Ethiopian chicken ecotypes using various ND and MD vaccine types.
- It is important that the ND vaccination strategy should consider the existing immunologigal diversity of the chicken ecotypes.

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8. ANNEXES

Annex 1. Monthly hematological data recording format

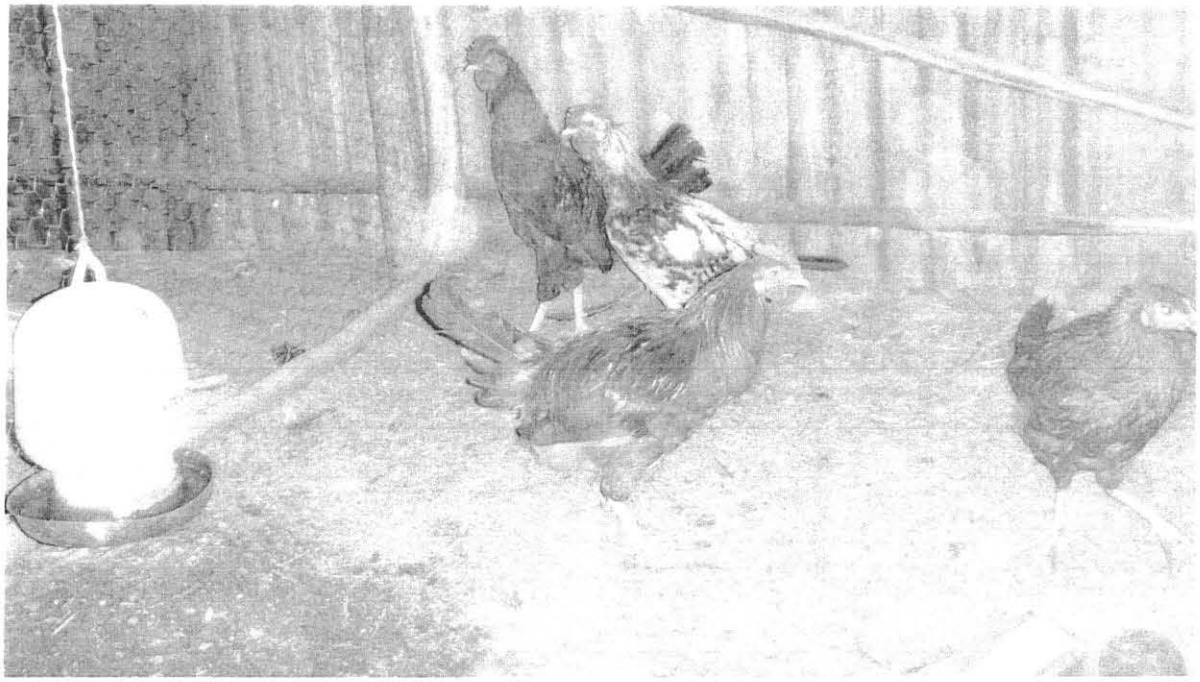
Ecotype _____ Experiment type _____

Date of entry (Age) _____ Total no of chick _____

Sample ID	Chicken Code	Total WBC	Differential count										PCV	Hb
			Lymphocyte		Eosinophil		Neutrophil		Monocyte		Basophil			
			%	total	%	total	%	total	%	total	%	Total		

Annex 2. Picture showing the studied chicken types

Jarso chicken at 6 months of age



Tepi chicken at 6 months of age



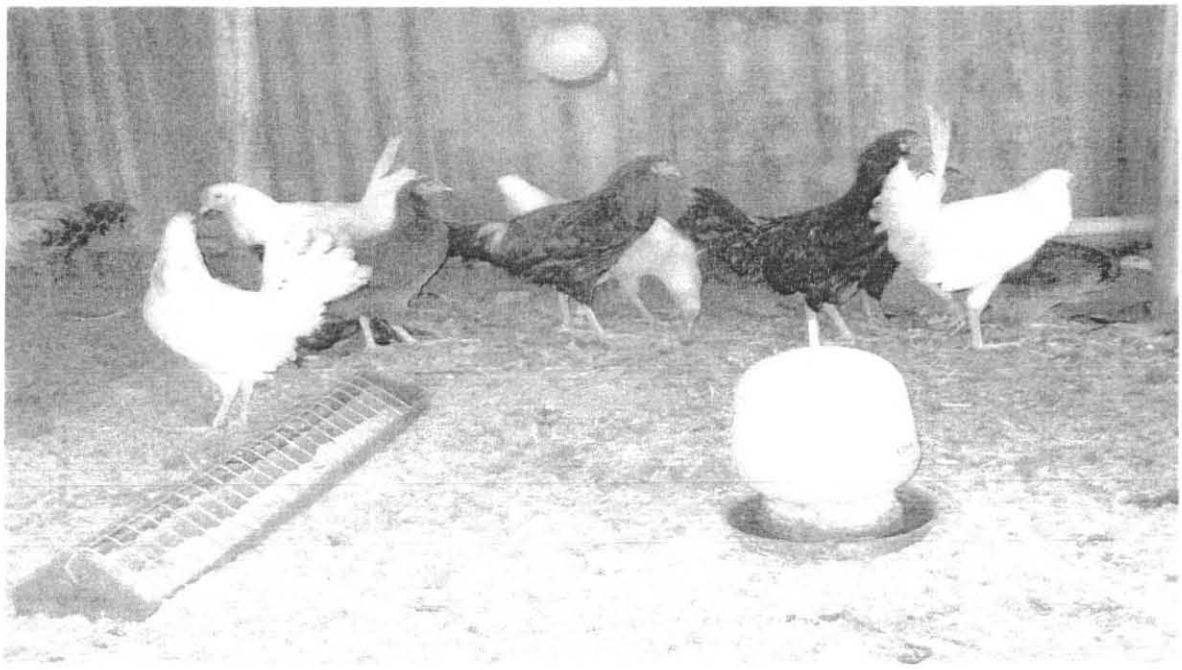
Arsi months chicken at 6 of age



Bovans at 6 months of age



Horro chicken at 4 months of age



Chefe chicken at 4 months age



Fayoumi chicken at 4 months of age



9. SIGNED DECLARATION SHEET

I the undersigned, declare the thesis in my original work and has not been presented for a degree in any university, and that all sources of material used for the thesis have been duly acknowledged

Name Edilu Jorga

Signature _____

Date of submission _____

This thesis have been submitted for examination with our approval as university advisors

Academic Advisors

Reta Duguma (DVM, MSc, Assistant professor) _____

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Yilkal Asfaw (DVM, MSc, Associate professor) _____