

ADDIS ABABA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE

**ONSTATION EVALUATION OF DIFFERENT GRAINS AS CARRIER FOR
THERMOSTABLE (I2) NEWCASTLE DISEASE VACCINE VIRUS,
DEBREZEIT,ETHIOPIA**

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

BY

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DEBRE-ZEIT, ETHIOPIA

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HN	Haemagglutinin Neuraminidase
I-2	Thermostable, virulent strain of Newcastle disease virus
ICPI	Intracerebral Pathogenicity Index
IgA	Immunoglobulin A
ILCA	International Livestock Center for Africa
IUPI	Intracerebral Pathogenicity Index
MDT	Mean Death Time
ND	Newcastle Disease
NDV	Newcastle Disease Virus
OIE	Office International des Epizooties
PANVAC	Pan African Veterinary Vaccine Center
PBS	Phosphate Buffer Saline
PMV-1	Pachivirus-1
RBCs	Red Blood Cell
RNA	Ribonucleic Acid
SPF	Specific-Pathogen-Free
UNDP	United Nations Development Programme
EID	Embryo Infection Dose

LIST OF ABBREVIATIONS

ACIAR	Australian Centre for International Agricultural Research
APMV	Avian Paramixovirus
CFSPH	Center for Food Security and Public Health
CSA	Central Statistical Authority
ELISA	Enzyme-Linked Immunosorbent Assay
FOA	Food and Agricultural Organazation
GM	Geometric Mean
HA	Haemagglutination
HI	Haemagglutination Inhibition
HN	Haemagglutinin Neuraminidase
I-2	Thermostable, avirulent strain of Newcastle disease virus
ICPI	Intracerebral Pathogenicity Index
IgA	Immunoglobulin A
ILCA	International Livestock Center for Africa
IVPI	Intravenous Pathogenicity Index
MDT	Mean Death Time
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OIE	Office International des Epizooties
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ABSTRACT

In the current experimental study the suitability of different local grains as a carrier for thermostable Newcastle disease vaccine I2 for oral immunization of chickens was assessed by haemagglutination inhibition test and challenge experiment. The study was conducted at school of veterinary medicine, Debrezeit, Ethiopia from December 2010 until June 2011. A total of 225 chicks were divided into 15 treatment groups and each treatment group had 15 chickens kept in separate pens. The chickens were vaccinated with I₂ vaccine by conventional methods (drinking water), cracked maize and wheat, millet, barely and sorghum which were used in untreated, parboiled and cracked form. Chickens were vaccinated three times at the age of 2, 5, 15 weeks. Following the primary and the second vaccination the result showed that only chickens vaccinated with conventional methods and vaccine coated cracked maize and parboiled barely gave GM antibody titer of $\geq \log_2^3$ but at third dose of vaccine except for chickens vaccinated with untreated and cracked form of sorghum, barely, millet all vaccinated birds showed GM antibody titer of $\geq \log_2^{3.2}$. At this time chicken vaccinated by conventional, cracked maize and parboiled barely were found to produce the highest GM titer of $\log_2^{7.1}$, $\log_2^{6.8}$ and $\log_2^{5.1}$ respectively but the least response ($\log_2^{1.9}$) was observed in chicken vaccinated by vaccine coated cracked sorghum. The experimental challenge showed that 80% mortality rate in the unvaccinated control group. The vaccine applied by drinking-water, cracked maize, parboiled barely was found 100% protective against the challenge. High protection capacity was also observed with I2 delivered by parboiled wheat (85%). Similarly 80% protection rate was found with vaccine coated untreated wheat, and parboiled sorghum. Limited survival rate (60%) was observed in the chicken under, untreated millet and cracked millet treatment group. The least survival rate with high mortality was observed in chicken treated with vaccine coated sorghum. Generally in the current experimental study cracked maize, parboiled barley, parboiled wheat, parboiled sorghum and untreated wheat found to be good candidate carrier for I2 vaccine virus.

Key word: *Grains, Challenge experiment, Chickens, Debrezeit, Ethiopia haemagglutination inhibition, Newcastle disease, Survival rate*

1. INTRODUCTION

Chicken production play important socioeconomic roles in developing countries (Galea, 2005). Nearly all rural and peri-urban families in developing countries keep a small flock of free range local chickens (Jain et al., 2009). Village chicken is also an integrated component of many all-kind, many-poll-burn and many other household (Branchett and Gueye, 1999). The small chicken population accounts for more than 90% of the total national chicken population in most African countries. According to Ntuiya (2005) small farming families, land-less farmers and people with incomes below the poverty line were able to raise chickens with low inputs and harvest the benefits of eggs and meat via scavenging feed resources.

Most of the 826 million people are still suffering from malnutrition. Nearly 1.2 billion people living on less than one US\$ a day are found largely in the arid zones of Africa and Asia (UNDP, 2007). Village chicken represents an appropriate system for supplying the fast-growing human population with high quality protein and providing additional income to resource-poor small farmers, especially women (Alders, 1998).

Village chickens need low levels of inputs (i.e. housing, cages, feeds, breeds, vaccines, drugs, equipment and infrastructure). They contribute significantly to food security, poverty alleviation and the ecologically sound management of natural resources. Moreover, village chickens constitute an important component of the agricultural and household economy in the developing world. Such chickens go beyond direct food production as well as employment and income generation for small farmers, especially women (Gueye, 2000). They also serve as a means of capital accumulation and as a barrier against livestock theft in areas where there is no circulation of currency. These birds, which make up around 80% of poultry stocks in many developing countries of Africa and Asia (Gueye, 1998; Goodger et al., 2002), are also valued in religious and socio-cultural lives of local communities (Gueye, 1998; Goodger et al., 2002).

The overall chicken population of Ethiopia is estimated to be 32 million heads (CSA, 2005). Every family in rural Ethiopia practices individual chicken raising (Gueye, 2004). Small poultry production in Ethiopia represents a significant part of the national economy in general

1. INTRODUCTION

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The overall chicken population of Ethiopia is estimated to be 32 million heads (CSA, 2005). Every family in rural Ethiopia practices traditional chicken rearing (Demeke, 2004). Rural poultry production in Ethiopia represents a significant part of the national economy in general

and the rural economy in particular. It contributes 98.5% and 99.2% of the national egg and poultry meat production respectively (Demeke, 2004). It amounts to an annual output of 72,300 metric tons of meat and 78,000 metric tons of egg (ILCA, 1993).

Unfortunately, village chickens have been marginalized from research and development efforts by decision makers, which is certainly true in Ethiopia as well. Subsequently, the sector is constrained by many factors. High mortality of chicks due to diseases, malnutrition due to poor quantity and quality of feed, predation, poor housing and insufficient water supply are some of the diverse constraints on village chicken production in Ethiopia (Tadelle and Ogle, 2001; Duguma, 2009). These factors affected the village chicken production and productivity as well as contributed in a gradual decline in the Ethiopian poultry population. In this line the Ethiopian poultry population was estimated at 85 million in 1954 (Burley, 1957) and 32 million in 2005 (CSA, 2005). The estimated the average number of chickens per household was 6.5 in 1984 whereas the average number of chickens per household is estimated at 4.1 in 2003. These figures show that the country's poultry population has declined by 64% over the last 50 years, while the average number of chickens per household has declined by 37% over the last 20 years (Jenny, 2008).

The most striking problem in relation to the reduction in the village chicken population is high mortality rate of birds. This amounts as high as 80-90% within the first few weeks after hatching, due to diseases and predation (Wilson *et al.*, 1987). Among the infectious diseases, Newcastle disease (NCD) is the major cause of mortality irrespective of age and sex, which occurs almost any time of the year (Serkalem *et al.*, 2005; Nwanta *et al.*, 2008). NCD highly infectious and causes more losses than any other diseases in the tropics. It spreads rapidly through the flock and mortality can reach up to 100%. NCD is believed to be the most devastating chicken disease in free-range systems.

Studies among intensely reared poultry have shown that protection of chickens against ND or control of the disease can only be achieved practically by vaccination (Echeonwu *et al.*, 2007).

Village chickens are reservoirs for different strains of ND virus. Thus, village chicken serves as the source of ND virus for many ND outbreaks in the nearby commercial farms (Awan, *et al.*,

1994) It is widely speculated that vaccination of the free roaming village chicken flock not only protect them but also form a useful intervention against transmission of NDV to intensely reared nearby commercial poultry (Alders and Sparadbrow,. 2001).

Indeed, a successful village chicken vaccination programme would improve the confidence of the rural farmers regarding the profitability of village chicken farming. It heralds a realizable poverty alleviation strategy. However, village chicken vaccination efforts would meet with a number of obstacles. Firstly, their free-range life style renders them not amenable to the conventional vaccine delivery methods, namely, aerosol/sprays or drinking water methods. These are only practiced in enclosures for mass vaccination. Secondly, the eye-drop and injection methods applied individually and demand catching of each chick for vaccination. Thirdly, these modes of vaccine administration demand cold chain. In general, they are designed for intensely reared commercial poultry but are not feasible for village chicken flock in their feral nature (Echeonwu *et al.*, 2007).

Hope for village chicken vaccination came with the advent of heat stable strains of I2 and V4 ND virus vaccines. Subsequently, it introduced an innovative mode of ND vaccination, namely, oral delivery through chicken feeds. This presented a feasible method for vaccination of large scattered population of free roaming village chickens as convenient means of protecting them and other poultry in the locality against ND (Spradbrow, 1993/4).

In Ethiopia the heat stable I-2 master seeds have been produced at PANVAC. Only a minority of feed grains examined in sub-Saharan Africa offered sufficient potential for carrying I2 vaccines(Spradbrow, 1993/4). This indicate that I2 vaccines are highly sensitive to the grain characteristics they are delivered on and as the result no single grain type can be identified as suitable. grain characteristics vary between countries, in larger countries we might also expect them to vary from region to region due to soil characteristics (Oakeley,2000). This is certainly true in Ethiopia where different agro-ecological zone and soil types exist that vary from region to region. Consequently, development of a feed-borne vaccine strategy necessitates comprehensive examination of all potential grains in any country.

- ❖ To evaluate the antibody titer for immune response among chickens vaccinated with NDV-I2 vaccine delivered using different carrier crop grains,
- ❖ To determine the protection level against ND challenge achieved in vaccinated chickens depending on the type of carrier grain in which the vaccine is administered

in the developing country for a long time, now a day's many researchers and development agents realized that the village chicken production plays a major role in poverty alleviation and food security at household level. The local chicken sector makes a significant contribution to human livelihood and contributes significantly to food security of poor households and can be considered an initiative enterprise owing to its low cost (Gondar, 2004; Abeyegedra, 2007). Family chicken is nearly the sole source of livelihood for the family but is one of a number of integrated and complementary farming activities contributing to the overall well being of the household. Village chickens were regarded as a walking bank by many families and were often sold in times of emergency cash needs (Morris, 2001).

Rising income and urbanization in many parts of the developing world caused a growing demand for high quality food items like animal proteins. There are only few alternative animal protein sources available in the sector including chicken and chicken products (Daniel, 2003). The per capita chicken meat consumption in the Ethiopia is reported to be 2.55kg per annum. Chicken meat was relatively cheap, available and affordable source of animal protein in the country (Akong and Tadele, 1997; Kassa et al., 2003).

Chicken provide major opportunities for increased protein production in countries for farmers because of the presence of short generation interval, high rate of productivity, the ease with which its products can be supplied to different areas, the ease with which its products can be sold due to their relatively low economic values, its minimal association of with religious taboos and its complementary role in relation to other crop livestock activities (Jais et al., 2004). Generally village chicken keeping has a symbolic importance with the context of many economic, social and cultural activities and/or religious ceremonies (Gaye, 2000). Village chicken production for its role in the supply of chicken and eggs and source of income for resource poor women, the

2. LITREATURE REVIEW

2.1. Importance of Village Chicken

Chicken production is an important agricultural activity of among all rural communities in Africa, which makes the best use of locally available resource (Guèye,2002a). Though neglected in the development themes for a long time, now a day's many researchers and development agents realized that the village chicken production plays a major role in poverty alleviation and food security at household level. The local chicken sector makes a significant contribution to human livelihood and contributes significantly to food security of poor households and can be considered an initiative enterprise owing to its low cost (Gondwe, 2004; Abdelqader, 2007). Family chicken is rarely the sole means of livelihood for the family but is one of a number of integrated and complementary farming activities contributing to the overall well-being of the household. Village chickens were regarded as a walking bank by many families and were often sold to meet emergency cash needs (Moreki, 2001).

Rising income and urbanization in many parts of the developing world caused a growing demand for high quality food items like animal proteins. There are only few alternative animal protein sources available in the tropics including chicken and chicken products (Odunsi, 2003). The per capita chicken meat consumption in the Ethiopia is reported to be 2.85kg per annum. Chicken meat was relatively cheap, available and affordable source of animal protein in the country (Alemu and Tadelle, 1997; Kenea et al., 2003).

Chicken provide major opportunities for increased protein production an incomes for farmers because of the presence of small generation interval, high rate of productivity, the ease with which its products can be supplied to different areas, the ease with which its products can be sold due to their relatively low economic values, its minimal association of with religious taboos and its complementary role in relation to other crop livestock activities (Jens et al., 2004). Generally village chicken keeping has a symbolic importance with the context of many economic, social and cultural activities and/or religious ceremonies (Gueye, 2000). Village chicken production for its role in the supply of chicken and eggs and source of income for resource poor women, the

smallholder rural poultry production in the country cannot be neglect. Therefore, providing the necessary support to the development of poultry production in rural areas is essential in order to increase the household income and achieve food security (Gueye, 2002b).

2.2. Newcastle Disease

2. 2.1. History of Newcastle Disease

According to Alexander (1988), Newcastle Disease was the name given to a highly pathogenic disease observed in chickens in England in 1926 by Doyle, who reported the first outbreak to have occurred in the spring of 1926 on a farm near Newcastle-upon- Tyne. The disease had also emerged in March 1926 in the island of Java, Indonesia. According to Alexander (1988), wherever the first outbreak occurred, highly virulent disease of poultry appeared within a very short time in England, Java, Philippines, India, Srilanca, Korea and within a few years had spread throughout the world. This disease was sufficiently different from other highly virulent diseases at the time to be recognized as a new disease.

It is not clear when the first outbreak of ND occurred in Africa. In South Africa, ND was first diagnosed in 1944. According to Bamhare (2001) Namibia confirmed its first outbreak of ND in 1950. Thereafter, the disease was recorded again in 1962 in rural flocks, and in the early 1970s and 1989, was diagnosed in commercial chickens in Namibia. In Zambia, ND was first reported in 1952 and the spread to commercial farms was experienced in 1957. The first documented evidence of ND in Ethiopia dates back to 1978, when an outbreak occurred in Eritrea in the northern part of the country (Serkalem et al., 2005).

2.2.2. Etiology

According to Jordan and Pattison (1996), ND is caused by a group of closely related viruses that form the avian Paramyxovirus type (PMV-1). Nine serogroups of avian paramyxoviruses have been recognized: APMV-1 to APMV-9. APMV-1 remains the most important pathogen for poultry while others are known to cause disease in poultry and other types of birds (Alexander, 2003). APMV-1 strains are further classified into three pathotypes based on their virulence in chickens. Lentogenic strains are the least virulent, mesogenic strains are moderately virulent, and velogenic strains are the most virulent. Most strains cluster toward the two extremes of virulence, and are either lentogenic or velogenic. Velogenic viruses can be subdivided into a neurotropic form, which is typically associated with respiratory and neurologic signs, and a viscerotropic form with hemorrhagic intestinal lesions. These clinical forms overlap and are rarely clear-cut, even in specific pathogen free (SPF) chickens (Jordan and Pattison, 1996).

Susceptibility to physical and chemical agents

The infectivity of NDV and other avian paramyxoviruses may be destroyed by physical and chemical treatments such as heat, irradiation (including light and ultraviolet rays), oxidation processes, pH effects, and various chemical compounds. The rate at which infectivity is destroyed depends on the strain of virus, the length of time of exposure, the quantity of virus, the nature of the suspending medium, and the interactions between treatments. No single treatment can guarantee destruction of all viruses but may result in a low probability of infective virus remaining (Alexander, 1997).

Biologic properties and virus replication

The main biological properties of paramyxoviruses, which characterize the groups include: Neuraminidase Activity, Cell Fusion and Hemolysis. Hemagglutination activity is the ability of NDV and other avian Paramyxoviruses to agglutinate red blood cells (RBCs). This is due to the binding of the hemagglutinin neuraminidase (HN) protein to receptors on the surface of the RBCs. This property and the specific inhibition of agglutination by anti-sera have proven to be

powerful tools in the diagnosis of the disease. Chicken RBCs usually are used in hemagglutination (HA) tests but NDV will cause agglutination of all amphibian, reptilian, and avian cells (King, 1999). Human, mouse, and guinea pig RBCs can be agglutinated by all NDV strains tested, but the ability to agglutinate cattle, goat, sheep, swine, and horse cells varied with the strain of NDV. Other avian Paramyxoviruses also appear to be able to agglutinate a wide range of RBCs, but the exact range may vary with the isolate as well as the serotype. Paramyxoviruses will agglutinate cells other than RBCs if they possess the correct receptors (King, 1999).

Neuraminidase activity of Paramyxoviruses is due to the presence of the enzyme neuraminidase which is also part of the HN molecule and present in all members of the *Rubulavirus* genus. An obvious consequence of the possession of this enzyme is the gradual elution of agglutinated RBCs. The exact function of the neuraminidase in virus replication is unknown, but it seems likely that neuraminidase removes virus receptors from the host cell which prevents the reattachment of released virus particles and virus clumping (Alexander, 2003).

The initial step of virus replication is attachment of the virus to cell receptors, mediated by the HN polypeptide. Fusion of the viral and cell membranes is brought about by action of the fusion (F) protein, and, thus, the nucleocapsid complex enters the cell. Intracellular virus replication takes place entirely within the cytoplasm. Because the virus RNA has negative sense, the viral RNA-directed RNA-polymerase (transcriptase) must produce complementary transcripts of positive sense that may act as messenger RNA and use the cell's mechanisms, enabling the translation into proteins and virus genomes. The F protein is synthesized as a nonfunctional precursor, F₀, that requires cleavage to F₁ and F₂ by host proteases. The HN of some strains of NDV may also require posttranslational cleavage. The viral proteins synthesized in an infected cell are transported to the cell membrane, which becomes modified by their incorporation. Following the alignment of the nucleocapsid close to modified regions of the cell membrane, virus particles are budded from the cell surface (Alexander, 2003).

2.2.3. Epidemiology of Newcastle disease

Occurrence and distribution

The widespread use of NDV vaccines in commercial poultry throughout the world makes the true geographical distribution of ND difficult to assess. It is usually considered that virulent NDV is either enzootic or a cause of regular epizootics in poultry throughout most of Africa, Asia, Central America and parts of South America. In more developed areas, such as Western Europe, sporadic epizootics occur on a fairly regular basis despite the wide spread use of vaccination. The OIE (2007) lists only five countries where the disease has never occurred (French Guiana, New Caledonia, Samoa and Vanuatu).

Host range

Newcastle disease primarily affects birds. Some avian species become ill, while others carry these viruses asymptotically. Infections also occur in humans, but have not been reported in other species of mammals (Spradbrow, 2001). APMV-1 viruses are known to infect more than 250 species of birds in 27 orders; other avian species may also be susceptible. Wild birds, particularly waterfowl, tend to carry these viruses asymptotically. Most of the viruses found in wild birds are lentogenic, however, virulent APMV-1 has become established in some cormorant populations and causes disease in juvenile birds (CFSPH, 2008). Susceptibility to illness varies widely among poultry and pet birds. Chickens, are highly susceptible to disease. Turkeys are less likely to develop severe symptoms, and the susceptibility of game birds (pheasants, partridges, quail and guinea fowl) varies with the species. Outbreaks have been reported in ostriches, Pigeons are susceptible to disease, and lentogenic or mesogenic APMV-1 viruses (PPMV-1) are endemic in pigeon populations. Susceptibility to disease varies widely in psittacine birds; cockatiels often die or develop neurological signs, but some species tend to carry velogenic viruses subclinically (CFSPH,2008).

Transmission

ND is highly contagious; it can spread from one chicken to another very easily. Infected birds pass ND virus out in their droppings and in their breath (Alexander, 2003). Infection can take place by either inhalation or ingestion and that spread from one bird to another depends on the availability of the virus in an infectious form. It is tempting to assume that NDV is primarily transmitted by fine aerosols or large droplets that are inhaled by susceptible birds. It is clear that infectious virus may be present in aerosols and that birds placed in an atmosphere containing such aerosols become infected. This is the basis for mass application of live vaccines by spray and aerosol generators (Spradbrow, 2001).

In naturally occurring infections, large and small droplets containing virus will be liberated from infected birds as a result of replication in the respiratory tract. The virus-laden particles may be inhaled or impinge upon the mucous membranes, resulting in infection. The ability of such aerosols to form and to support infectious virus for a sufficient period for transmission, however, depends on humidity and other environmental factors, as well as the concentration of infected poultry (Alexander, 2003). During the course of infection of most birds with NDV, large amounts of virus are excreted in the feces. Ingestion of feces results in infection; this is likely to be the main method of bird-to-bird spread for a virulent enteric NDV and the pigeon variant virus neither of which normally produces respiratory signs in infected birds (Alexander *et al.*, 2006).

Vertical transmission (i.e., passing of virus from parent to progeny via the embryo) remains controversial. The true significance of such transmission in epizootics of ND is not clear. Experimental assessment using virulent viruses is usually hampered by cessation of egg laying in infected birds. Infected embryos have been reported during naturally occurring infections of laying hens with virulent virus (King and Seal, 1997), but this generally results in the death of the infected embryo during incubation. Cracked or broken infected eggs may serve as a source of virus for newly hatched chicks, as may virus-laden feces contaminating the outside of eggs. Virus may also penetrate the shell after laying, further complicating the assessment of true vertical or transovarian transmission. Infected chicks may be hatched from eggs infected with vaccinal or other lentogenic viruses that do not necessarily cause death of the embryo (Chen and Wang,

2002). In naturally occurring infections, it is not clear how the embryos become infected, although La Sota vaccine has been shown to be present in most of the reproductive organs after vaccination (Chen and Wang, 2002).

Morbidity and mortality

Morbidity and mortality rates vary greatly depending on the virulence of the strain and susceptibility of the host. Lentogenic and mesogenic viruses usually kill few birds; in poultry, the mortality rate is approximately 10% for mesogenic strains and negligible with lentogenic strains. Concurrent infections may increase the severity of illness and result in a higher death rate. In contrast, velogenic isolates have morbidity and mortality rates up to 100% in un-vaccinated chickens (Alexander, 2003).

Incubation period and clinical signs

The incubation period of ND after natural exposure has been reported to vary from 2—15 days (average 5 to 6 days). In chickens infected with velogenic isolates, an incubation period of 2 to 6 days is common. Incubation periods up to 25 days have been reported in some avian species (Alexander , 2003).

The clinical signs of ND vary considerably according to the type of the virus involved, the type of bird, the age and health of the bird and environmental conditions. According to Jordan and Pattison (1996), the most common signs recorded during outbreaks of the disease are the following: respiratory signs such as difficult breathing, gasping, nasal-discharge and / or coughing. Gastro intestinal sickness like green diarrhea nervous signs which includes prostration, frenzy, paralysis (legs and neck), and inability to swallow are also common. Poor condition and general weakness is normally noticed in chickens when ND has affected them (CFSPH, 2008).

The speed with which signs appear variable depending on the infecting virus, the host species and its age and immune status, infection with other organisms, environmental conditions, the route of exposure, and the dose (McFerran, and McCracken, 1988). In chickens, lentogenic strains usually

cause subclinical infections or mild respiratory disease with coughing, gasping, sneezing and rales. Mesogenic strains can cause acute respiratory disease and neurologic signs in some chickens, but the mortality rate is usually low. Lentogenic or mesogenic strains can produce more severe symptoms if the flock is co-infected with other pathogens (Alexander , 2003).

Velogenic strains cause severe, often fatal, disease in chickens. The clinical signs are highly variable. Most birds are lethargic and inappetent, and the feathers may be ruffled. Conjunctival reddening and edema may be an early sign. Some birds develop watery, greenish or white diarrhea, respiratory signs (including cyanosis) or swelling of the tissues of the head and neck. Neurologic signs including tremors, clonic spasms, paresis or paralysis of the wings and/or legs, torticollis (twisted neck) and circling may also be seen. Nervous signs can occur concurrently with other symptoms but are generally seen later in the course of the disease. Egg laying often declines dramatically, and eggs may be misshapen, abnormally colored, and rough or thin-shelled, with watery albumen. Sudden death, with few or no symptoms, is also common. Birds that survive for two weeks usually live but may have permanent neurological damage and/or a permanent decrease in egg production. The symptoms may be less severe in vaccinated birds (CFSPH, 2008).

2.2.4. Economic significance

The global economic impact of ND is enormous. Until the emergence of the highly pathogenic Asian H₅N₁ influenza virus this impact was unsurpassed by any other poultry virus and probably represented a bigger drain on the world's economy than any other animal virus. In developed countries with established poultry industries, not only are outbreaks of ND extremely costly, but control measures, including vaccination, represent a continuing loss to the industry (Leslie, 2000). Even countries free of ND usually face the cost of repeated testing to maintain that status and for the purposes of trade (Alexander,1997)

In many developing countries ND is endemic and, therefore, represents an important limiting factor in the development of commercial poultry production and the establishment of trade links. Many countries rely on village chickens to supply a significant portion of dietary protein in the

form of eggs and meat, especially for women and children. The constant losses from ND (Sen *et al.*, 1998) severely affect the quantity and quality of the food of people on marginal diets. Therefore, the economic impact of ND should not only be measured in direct losses in commerce but in some countries, the effect on human health and loss of potential socioeconomic growth should also be considered (Sen *et al.*, 1998).

2.2.5. Diagnosis

Clinical signs and lesions

Virus isolation and laboratory characterization are important for a definitive diagnosis of ND. Nevertheless, if the disease is known to be present in a given area, signs and lesions may be considered highly suggestive, especially for village chickens. Other typical characteristics of the disease include: rapid spread; death within 2-3 days; a mortality rate of over 50 percent in naïve populations; and an incubation period of 3-6 days or, on rare occasions, 2-15 days (Alexander *et al.*, 2006) are also supportive. On necropsy, typical lesions are mucus in the trachea, and usually hemorrhages in the intestine, particularly in the proventriculus. It should be borne in mind that all the preceding signs and lesions can be caused by other diseases.

Serological diagnosis

In the absence of vaccination, the presence of specific antibodies against the ND virus indicates that the bird has been infected by the virus at some time, but not necessarily that it was suffering from the disease at the time of sampling. In practice, a high antibody titre is indicative of a recent infection. Two methods are used to measure antibody titres: the haemagglutination inhibition (HI) test, and the enzyme-linked immunosorbent assay (ELISA) (Alexander *et al.*, 2004).

Haemagglutination inhibition test : The HI test is based on the principle that the haemagglutinin on the viral envelope can bring about the agglutination of chicken red blood cells and that this can be inhibited by specific antibodies. The serum samples are diluted in serial twofold dilutions

in phosphate buffered saline and then a fixed quantity of viral antigen is added to each well. Following incubation, a suspension of red blood cells is added to each well and the plate is incubated again. In the absence of any antibody against the virus, haemagglutination occurs, appearing as a diffuse red color at the bottom of the well. In wells where the antibody against the virus is of a sufficient level haemagglutination is inhibited and the red blood cells sediment and appear as a small pellet at the bottom of the well. The presence or absence of agglutination is accurately assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing RBCs and PBS only) should be considered to show inhibition (Alexander *et al.*, 2004).

The HI titre is the reciprocal of the highest dilution of serum which completely inhibits haemagglutination and is usually and most conveniently expressed as the logarithm to the base 2. Although the test is difficult to standardize between laboratories, the HI titre gives an indication of the immune status of the bird. A titre of $\log_2 2^3$ is indicative of protection. If no vaccination has taken place, diagnosis of the infection can be made on this basis, although it cannot be determined exactly when it took place. Sequential samples taken at different times can indicate whether the titre is rising indicative of a recent infection or declining (Alexander *et al.*, 2004).

Enzyme-linked Immunosorbent Assay: The ELISA works on the principle of recognition of anti-NDV antibodies, attached to a plate coated with viral antigen, by antibodies produced in another species against chicken antibodies. This anti-chicken antibody is conjugated to an enzyme that catalyses a reaction, causing change of color which can then be read quantitatively on a spectrophotometer designed to read microtitration plates (Alexander *et al.*, 2004).

Virus isolation

The definitive diagnosis of Newcastle disease is done through isolation and identification of the virus. Tracheal and cloacal swabs are good sources of virus for isolation from living birds without having to kill them. A cotton-covered stick is inserted into the trachea or cloaca, and then put into a vial containing phosphate buffered saline plus penicillin and streptomycin. It is important to ensure that cloacal swabs are coated with faeces. These samples must be kept cool

during transport to the laboratory where they should be stored at 4°C if they are to be processed within 48 hours or frozen at least at 20°C until the isolation attempt. Although cloacal swabs or faeces should always be sampled, virus can also be isolated from homogenised organs from dead birds, chosen to reflect the clinical signs (Alexander *et al.*, 2004).

Nine-day-old embryonated eggs are injected with 0.1mL of the suspension into the allantoic cavity and incubated. The eggs are candled twice daily. As dead embryos occur, they are chilled, together with all eggs after 5 to 7 days of incubation, they are chilled at 4°C at which point the allantoic fluid is then harvested and tested for its ability to haemagglutinate chicken red blood cells. Diagnosis is based on the inhibition of haemagglutination by specific anti-NDV serum. This proves infection of the bird by the virus, but does not indicate whether the virus is a pathogenic or a virulent strain. The full diagnosis of ND requires an assessment of the virulence of the virus using the intracerebral pathogenicity index (ICPI) test in day-old chicks or the mean death time (MDT) in eggs (Alexander *et al.*, 2004).

Intracerebral pathogenicity index (ICPI) test involves the inoculation of virus derived from fresh infective allantoic fluid into the brain of ten day-old chicks from specific pathogen-free parents. Each bird is examined at 24-hour intervals for eight days and graded zero if normal, one if sick and two if dead. The index is the mean score per bird per observation over the 8-day period. The most virulent viruses give ICPI values approaching the maximum score of 2.0, while lentogenic viruses give values of, or close to, 0.0. Where day-old chicks are not available, the mean death time (MDT) in eggs (i.e. the mean time in hours for the minimum lethal dose to kill all the inoculated embryos), can be used as a guide to virulence. The MDT has been used to classify ND virus strains into velogenic (taking under 60 hours to kill); mesogenic (taking 60 to 90 hours to kill); and lentogenic (taking more than 90 hours to kill) (Joseph and Bernard, 2009)

2.2.6. Newcastle disease control in village chickens

Chickens are susceptible to many infectious diseases. One of the most important of these is the viral disease known as Newcastle disease, which causes devastating losses in both commercial and village chickens. Reducing losses of large numbers of village chickens to virulent Newcastle

disease is an essential first step to improving their productivity (FAO, 2002). Effective control of ND requires good sanitation, management, quarantine, an appropriate vaccination program, and monitoring and reporting system.

Vaccination against Newcastle disease

Vaccination has been considered as the safeguard against endemic ND (Alders and spradbrow, 2001). The selection of a ND vaccine for use in rural chicken will depend on the local conditions in each country. Immunogenicity, type of vaccine (inactivated or live) and safety are the main factors governing the choice of vaccine (Alexander, 2000). Several different vaccines of appropriate immunogenicity and safety are available for the control of ND in village chickens. Factors which need to be considered when choosing a vaccine include storage and handling condition, cost, prior experience with the use of vaccines, structures of existing veterinary services.

Veterinary authorities wishing to use vaccines to control ND in village chickens must consider available resources, the demand for the vaccine and the willingness of farmers to pay for the vaccine. They must then decide on the option best suited to these circumstances. In most cases where farmers are to contribute wholly or partially to the cost of the vaccine will be a major factor. The lower price of the vaccine, the greater the number of farmers who will be able to afford to pay for it and, consequently, the greater the vaccination coverage (Young *et al.*, 2002). Vaccination against Newcastle disease can be performed using live or inactivated vaccines.

Inactivated vaccines

Inactivated vaccines are produced by growing a ND virus in eggs, and then treating the infective allantoic fluid with an inactivating agent, such as formalin or betapropiolactone. An adjuvant, such as mineral oil, is usually then added to make the inactivated virus more immunogenic. Since the vaccine is no longer capable of replication or spread, it has to be injected individually into every bird needing vaccination. It is normally injected into the back of the thigh muscle (sometimes the breast muscle is used), using 0.3 or 0.5 ml per bird. This requires some training,

and cannot be done by every keeper of chickens without prior demonstration. Inactivated vaccines produce very high levels of antibodies against NDV, and provide good protection against the virulent virus. Although inactivated vaccine gives good protection, it is relatively expensive to produce. It also carries a slight risk to the user of accidental self-injection. (Alexander *et al.*, 2004)

Live vaccines

Live vaccines differ from inactivated vaccines in that they can replicate in the host. This is both an advantage and a disadvantage. It is an advantage in that it is not necessary to vaccinate every bird individually; the vaccinal virus can spread on its own from one bird to another. It is, however, a disadvantage in that, since an infection with a live virus is involved, this may result in clinical signs because of the innate virulence of the vaccine virus or by exacerbating other organisms that may be present, especially in the respiratory tract. The severity of this reaction depends therefore on the particular vaccinal strain used (Joseph and Bernard, 2009) and the presence or otherwise of concurrent infection with other pathogens.

Another advantage of live vaccines compared to inactivated vaccines, is their ease of application as they can be applied to the drinking water or with an eye-dropper. The majority of live vaccines are derived from asymptomatic enteric or lentogenic strains, although some vaccines derived from mesogenic strains are still in use.

A). Live lentogenic vaccines: The best known strains of live lentogenic vaccines are HB1, F, and LaSota (Alexander *et al.*, 2004). The HB1 and F strains are mild and can be used safely and effectively in all classes of chickens. LaSota produces moderate vaccinal reactions, especially in immunologically naive birds and is not usually recommended for primary vaccination. In theory, LaSota would also be unsuitable for vaccinating a multi-age population, including young chicks which is inevitably seen in the village situation. This is because the virus spreads and it is not practical to isolate the adults from the chicks. All conventional live vaccines have disadvantage of needing to be kept at low temperatures to maintain their efficacy. This is not a problem for intensive poultry production in an industrial setting, but the maintenance of the “cold chain”

during distribution can be very difficult in village settings, particularly where there is high ambient temperature (Alexander. 1997).

B) Thermostable vaccines : Conventional methods for controlling Newcastle disease rely upon regular vaccination of Individual birds using live vaccine delivered by the intra-nasal, intra-ocular, or intramuscular routes. Live vaccines have traditionally been heat-labile (requiring storage at or below 48C° until application). Consequently, there has been need for an effective cold chain. This factor has been a primary constraint to Newcastle disease control amongst small and dispersed backyard flocks. Complete and effective cold chains are expensive and difficult to maintain particularly where vaccine must be transported over large distances on poor roads. Any break in cold chain removes any guarantee of effective protection against Newcastle disease (Oakeley, 2000).

Additional constraints to conventional control strategies focus on the logistical demands of vaccinating backyard flocks. Extensively raised birds can be difficult to catch and that vaccination procedures are 'laborious, time consuming and not always successful'. In particular, the use of heat-labile vaccines has meant that vaccination campaigns remain the responsibility of veterinary professionals and technicians with the inevitable high associated labour costs. This represents the second major constraint because the majority of the developing countries do not have the resources to implement vaccination campaigns on the scale and frequency necessary to cover their backyard poultry flock (Sonaiya *et al.*, 1999).

One of the more-promising developments in recent years has been the work on the production of vaccines that are tolerant to heat and thus do not require a cold chain (Bell,2001). Based on research in Australia dating back to the 1970s, two heat-resistant vaccine strains have been developed: V4 (NDV4-HR) and I₂ (Grimes, 1999; Ideris *et al.*, 1999). Two potential advantages for backyard producers are that such vaccines remove the need for a complete cold chain and offer the flexibility of delivery via alternative routes than the nares, eye or muscle (Spradbrow, 1993/4).

NDV4-HR vaccine: The heat resistant V4 (NDV4-HR) vaccine against ND has yielded encouraging results in many countries in Africa (Alders and Spradbrow, 2001) and Southeast Asia (Spradbrow, 1993/4). NDV4-HR vaccine is a live vaccine with the following characteristics it is thermostable, retaining its activity for 12 weeks at a temperature of 28°C in freeze dried form (Adwar and Lukesova, 2008); it can be administered via eye-drop (intraocular), nose drop (intranasal), oral drench, or drinking water; mixed with certain feeds or by injection (Spradbrow, 1992). Its ease of administration makes it suitable for use by village farmers; the vaccine strain can be transmitted by contact from vaccinated to non-vaccinated birds (Alders *et al.*, 1994); The chickens will be tested for the acceptability of the grain before the beginning of vaccination experiment).

I-2 ND vaccine : I₂ ND vaccine is similar to NDV4-HR but is free of commercial ownership, and the master seed virus is available to laboratories in developing countries wishing to produce the vaccine locally. Strain I₂, an avirulent Australian ND virus isolate, was identified after testing of forty five isolates of avirulent ND viruses. These isolates were examined for antigenicity, safety and ability to spread (Spradbrow, *et al.*, 1995). Eighteen selected isolates were then tested for thermostability, and isolates showing enhanced heat resistance were selected. I₂ gave the best results and was grown in eggs from a minimum disease flock to form a master seed. The master seed was then tested for safety and for freedom from bacterial contamination (Bensink and Spradbrow 1999). Subsequent testing confirmed that the master seed is free from extraneous viruses, and bacterial and fungal contamination.

I₂ ND vaccine has undergone testing in several countries including Bhutan, Cambodia, Ghana (Amakye-Anim *et al.*, 2000), Myanmar (Hlaing *et al.*, 2000), Senegal, Tanzania and Vietnam. In laboratory trials I-2 ND vaccine has proven to be protective against local virulent strains of ND virus (Tu *et al.*, 1998;). In Mozambique I₂ ND vaccine provided approximately 80% protection in the field, in the face of an outbreak, when given every 18 months via eye drop (Dias *et al.*, 2001). I₂ ND vaccine produced in non-SPF eggs is recommended for the control of ND in village chickens only. It is not recommended for use in commercial, small-scale commercial or peri-urban chicken flocks unless more rigorous quality assurance procedures are implemented to ensure the absence of vertically transmitted pathogens of importance in intensively raised flocks.

ND vaccine of acceptable standard can be produced from strain I-2 in central laboratories or even regional laboratories in developing countries. The vaccine can be produced in eggs which are not specifically pathogen free, but which come from a flock that is regularly screened for key poultry diseases. It can be produced and stored in liquid form, and suitably diluted in a protective solution such as 1 percent gelatin in which the vaccine will maintain its activity for at least twelve weeks at 22°C; before use (Bensink and Spradbrow, 1999).

2.2.7. Administration of vaccine

Vaccines are provided in a standard dose and are not formulated to account for body weight or age (Tizard, 2000). Vaccines can be administered using either individual or mass vaccination methods. Eye-drop and injection are individual methods used in the commercial poultry industry. Individual methods of application produce more consistent protection in more birds than mass vaccination methods. Mass application makes it possible to vaccinate a large number of chickens in a minimum period of time. Mass application methods include, spray, drinking water and in-feed administration (FAO, 2002).

Administration of ND vaccine via drinking water and spray

Drinking water administration is one of the mass application methods of vaccination in poultry industry and is probably the most common and simplest method of ND vaccine administrations. Drinking water vaccination elicits minimal respiratory reaction compared to vaccination by aerosol when mild to moderate respiratory signs are observed four to five days post vaccination. Spray is also a popular and effective mass application method used to vaccinate large flocks in the poultry industry (Meulemans, 1988).

Administration of ND vaccine via feed

An ideal vaccine carrier feed should be cheap, readily available in the target locality and should not contain substances that would inactivate the vaccine virus (Spradbrow, 1993/4). Although the carrier feed need not be nutritious for it to be effective in conveying the vaccine virus, it ought to be palatable and desirable to the chickens (Iroegbu and Nchinda, 1999). Preliminary work with feed-coated V4 virus vaccine administered to chickens showed that it was possible to deliver the vaccine virus to chickens on feed (Ibrahim *et al.*, 1992; Spradbrow, 1992). Subsequent trials with different types of feed carriers have produced variable results due to variations in the characteristics of feed types, including their constituents (Spradbrow, 1995). Several workers have suggested various treatments to be given the carrier foods so as to enhance the viability of the vaccine. These include prior washing before coating or soaking overnight followed by washing (Cumming, 1992). These treatments are expected to render foods, erstwhile observed to be “unfriendly” to vaccine virus, satisfactory as vaccine carriers (Darminto and Daniels 1992,)

The role of the carrier would be simply to adsorb the virus from an aqueous suspension and to release it in a viable form in the digestive tract of a chicken (Spradbrow, 1993/4). Feed vaccines can be tested by feeding chickens and thereafter by measuring both the antibody response and resistance to challenge (Oakeley, 1998). A feed delivered system of vaccination was proposed as it potentially solves two of the problems of a conventional village chicken vaccination program, namely the need for trained staff to administer injectable products and the difficulty of catching individual birds for administration of vaccine (Ibrahim *et al.*, 1992).

African feed grains examined as potential vaccine carriers include maize meal, corn chaff, mill, millet, wheat bran, barley, sorghum, rice and finger millet (rapoko). The apparent effectiveness of different grains as vaccine carriers is highly variable. Grains with potential as vaccine carriers include barley in Ethiopia (Rushton, 1995), wheat bran in Ghana (Amakye-Anim *et al.*, 2000), un-husked rice and millet in the Gambia (Rushton, 1995) and rapoko in Zimbabwe (Oakeley, 2000). Importantly grains like millet, rice and wheat bran have been effective in one country yet inappropriate elsewhere.

Only a minority of feed grains examined in sub-Saharan Africa offered sufficient potential for carrying the V4 and I2 vaccines. In similar trials conducted in Asia, most grains tested were unsuitable as carriers of the vaccines (Spradbrow, 1993/4). Implicit in these results is the fact that the V4 and I2 vaccines are highly sensitive to the grain they are delivered on and that no single grain type can be identified as suitable. Consequently, development of a feed-borne vaccine strategy necessitates comprehensive examination of all potential grains in any country (Besink and Sparadbrow 1999)

Because grain characteristics vary between countries, in larger countries we might also expect them to vary from region to region (warranting multiple trial programmes within a country). There is limited understanding of the precise impact on the vaccine of grain variety, quality and condition, and possible chemical additives. These unknown factors require clarification if the potency of vaccine (once applied) is to be reliable (Oakeley, 2000)

3.3. Experimental animal acquisition

A total of 490 fertilized eggs from homogeneous chicks breed (Bovans brown) were obtained from Delta 249 private commercial farm (Ghana) and hatched at NVI mini-hatchery room. Eggs were cleaned and disinfected and incubated at the temperature of 37 °C and finally 250 chicks were hatched and harvested between the 21st and 22nd day of incubation.

3.4. Management of study animals during the experiment

The experimental chicks were housed in clean disinfected transfer boxes equipped with infrared bulbs for heating, bedded with soft straw until fourteen days old. Then the chicks were randomly split into treatment groups. Water was given *Ad libitum*. Chicks were fed on starter commercial

3. MATERIAL AND METHODS

3.1. Experimental site

The experiment was carried out at Addis Ababa University, School of Veterinary Medicine in Debre Zeit, Ethiopia.. Debre Zeit is a town of Ethiopia, lying south east of Addis Ababa. The town is located in the East Shewa Zone of the Oromia Region, and has a latitude and longitude of 8°45'N 38°59'E with an elevation of 1920 meters. It is the administrative center of Adaa Chukala woreda (http://en.wikipedia.org/wiki/Debre_Zeit).

3.2. Preparation of experimental house

The experimental house having 16 partitions with an area of 16m × 5m was constructed at the School of Veterinary Medicine. The house was disinfected with 1% formalin starting from the ceiling, the wall and the floor. Moreover, it is also fumigated with mixture of formalin and potassium iodide. Finally, clean *teff straw* was disinfected with 1% formalin and spread over the floor. Equipments including drinker, feeder and buckets were also cleaned and disinfected and introduced to the houses and the house was closed until the arrival of the chicks.

3.3. Experimental animal acquisition

A total of 400 fertilized eggs from homogeneous chicken breed (Bovans brown) were obtained from Debre Zeit private commercial farm (Genesis Farms), and hatched at NVI mini-hatchery room. Eggs were cleaned and fumigated and incubated at the temperature of 37 °C and finally 300 chicks were hatched and harvested between the 21st and 22nd day of incubation.

3.4. Management of study animals during the experiment

The experimental chicks were brooded in clean disinfected brooder house equipped with infrared bulbs for heating, bedded with teff straw until fourteen days old. Then the chicks were randomly split into treatment groups. Water was given *Ad libitum*. Chicks were feed on starter commercial

layer ration purchase from Genesis farm for 2 months. Then feed on grower commercial ration until 5 month of age. After 5 months of age they fed on layer ration. Feed consumption and mortality were recorded daily while the average weight gain was measured weekly for each treatment group. 20% Oxytetracyclin powder and amprolium were also given when diseases were suspected and diagnosed. Vitet (mixture of antibiotics minerals and vitamins) was supplied for the first five days continuously via drinking water and for next 3 days at the time of bleeding.

3.5. Experimental design

The experimental design was complete random design to give every group of the experimental animals an equal probability of receiving treatment. The trial was conducted to assess the suitability of barely, sorghum, maize, wheat, millet as carrier for NDV-I₂. Each grain except for maize was prepared in three different forms namely, cracked, parboiled and untreated. Maize was used in cracked form. Accordingly, there were 15 treatment groups including the conventional administration methods (drinking water as positive control) and negative control. A total of 225 chickens were randomly assigned into (15) treatment groups and each treatment group had 15 chickens kept in separate pens.

3.6. Preparation of carrier grains

All carrier grains were purchased from local market, washed, sun dried and stored at room temperature until use. Then the grains were cracked and parboiled. Grains were cracked to small sizes to ease swallowing by the birds. Preparation of parboiled grains was done according to Cumming (1992) as follow. First, the water was boiled and the grain was added at ratio of 1kg per 3 liter of water and leave to boil for two minutes. It was then cooled and the water went off and cooled down further by running tape water. Finally, the water was removed maximal by straining and sun dried.

3.7. Coating of carrier grain with the virus

One ampoule (350 doses) of freeze dried I-2 ND vaccine (batch no. 1-09) was purchased from vaccine production laboratory of National Veterinary Institute (NVI) of Debre Ziet, Ethiopia. According to Wambura *et al.*, (2007) 10gram of each grain was used for each chick to deliver one dose of the vaccine and accordingly 150gram of each grain was found to be sufficient for each treatment group which was enough to deliver the vaccine for 15 chicks. The vaccine was reconstituted in 175 ml of clean sterile non-chlorinated water. The 7.5ml vaccine suspension which contained a dose sufficient for 15 chickens was further diluted in 48.5ml of clean non-chlorinated water. This final suspension of vaccine was later mixed with 150g of the carrier grain which was enough for 15 chickens in treatment group. The vaccine-grain mixture was allowed to stay for 6 hours at room temperature

3.8. Vaccination experiment

The 225 chicks were randomly split into 15 treatment groups each with 15 chicks. The chicks were vaccinated three times at 2, and 5 and 15 weeks of age. The chicks were starved overnight and in the next morning they were vaccinated with I-2 vaccine mixed wheat, millet, barely and sorghum which were prepared in three forms in addition maize was used in a cracked form. Each chicks in all groups received 10g of feed containing 1 dose (10^7 EID50) of vaccine virus. The chickens were tested for the acceptability of the grain before the beginning of vaccination experiment. The chicks in the control group were kept unvaccinated, while birds in the conventional (positive control) group were vaccinated orally via drinking water.

3.9. Judging protection level of I₂ vaccination via monitoring the chicken response

3.9.1. Serology

Blood collection for serology

Blood sample (1ml per bird) was collected using sterile syringe without anticoagulant from brachial vein according to the method described by Alders and Spradbrow (2001). Pre vaccination blood sample was collected at the age of 1, 2week and post vaccination blood 4, 7, 11, 15 and 17 weeks of age . The blood was allowed to clot over night at room temperature and serum was separated. The sera collected were then stored at -20°C until analyzed.

Haemagglutination inhibition test

Pre- and post-vaccination sera were heat inactivated at 56°C for 30 minutes. Then the serum was tested to evaluate the antibody response each chicken pre and post vaccination using the hemagglutination inhibition test. The test was performed following the method described in OIE (2000) manual. Four haemagglutination (HA) units, 1% chicken erythrocyte suspension and two-fold serial dilutions starting with dilution of 1:2 were used. The antibody level for each serum sample was recorded. That was expressed as a log base two. For convenience, the titer was recorded as just the log index. For example the titre of $\log_2 2^2$ was recorded as two. The geometric mean titers (GMTs) was calculated. The protective HI antibody titer for Newcastle disease vaccination in chickens has been reported to be the HI titer of $\geq \log_2 2^3$ (Alexnader et al, 2004; Spradbrow, 1993 / 4).

The procedure used is briefly described by (OIE, 2000) as follow. 25 μ L of phosphate buffer saline was dispensed into each well of the V-bottomed microtitre plates. Then the serum samples were shaken and 25 μ L was dispensed into the first well and the last (control) well of a microtitre plate. and a multi-channel pipette was used to made two-fold serial dilutions along the rows. After which 25 μ L of the virus dilutions containing 4HAU was added to each of the serum dilution excluding the control wells in the last column. It was uniformly mixed and incubated at

room temperature for 45 minutes; this is to enable the virus - serum mixture to react. After this 25 μ L of 1% suspension of the chicken red blood cell was added to each of the mixtures in the wells and allowed to settle for 60 minutes at + 4⁰C Finally, 25 μ L of 1 % washed red blood cells from laboratory control chickens was added to each well including the control wells in the last column.

3.9.1. The haemagglutinating antibody titer

3.9.2. Monitoring by artificial challenging

3.9.3. The haemagglutinating antibody titer was log₂⁺ at the age of 1 day. At the 14 days of age the mean

Three weeks after the third vaccination just at 18 weeks of age both vaccinated and non-vaccinated chickens were challenged by local (Alanya strain) virulent NDV intramuscular injection at the breast muscle with dose of $0.5 \times 10^{6.5}$ EID₅₀. The chickens were examined daily for four weeks for clinical signs and death due to ND.

3.10. Statistical analysis

Age of birds Number of birds HI titer (log₂)

For each variable of interest, the mean value and standard deviation (SD) was determined and classified according to treatment groups. The post vaccination mean HI antibody titres were compared by General Linear model of SPSS version (15). Where the results were significant Duncan's multiple range tests were done to establish differences in antibody response between pair wise treatments. The percentage of mortality, morbidity and survival was calculated. The presence of statistically significance difference in mortality rate between treatment groups was determined by the Chi-square tests at 95% confidence interval.

higher in chickens vaccinated by the conventional methods (log₂⁺), cracked maize and parboiled barley (log₂⁺) at four weeks of age (two weeks after vaccination). Moderate response was observed for chickens vaccinated using cracked and parboiled wheat (log₂⁺) and parboiled sorghum (log₂⁺). Chickens vaccinated using other grain cannot showed weak antibody response at this time (Table 2). There was no significant difference ($P \geq 0.05$) in mean HI antibody titre among chicks vaccinated with conventional method, cracked maize, parboiled barley and cracked Millet. However, conventional methods, cracked maize and parboiled barley significantly ($p < 0.05$) higher in GM antibody titre compared to the non grain treatment group. Similarly, and the Duncan multiple range test shows that the difference response among chickens

4. RESULT

4.1. Serological analysis

4.1.1. The base line antibody titer

The base line geometric mean titer was $\log_2 2^{3.2}$ at the age of 1 day. At the 14 days of age the titer dropped to $\log_2 2^{1.5}$. This showed that the maternal antibody had wane at the time of vaccination (Table 1).

Table 1. Pre vaccination geometric mean antibody titres of chickens.

Age of birds	Number of birds	HI titer ($\log_2$)
Day old	15	3.2 ± 0.5
Day 14 (Week 2)	15	1.5 ± 0.6

4.1.2. Monitoring the antibody titer profile following vaccination.

The result of the post vaccination serological analysis showed that the GM titer of antibody was higher in chicken vaccinated by the conventional methods ($\log_2 2^{3.2}$), cracked maize and parboiled barely ($\log_2 2^3$) at four weeks of age (two weeks after vaccination). Moderate response was observed for chickens vaccinated using cracked and parboiled wheat ($\log_2 2^{2.3}$) and parboiled sorghum ($\log_2 2^{2.1}$). Chickens vaccinated using other grain carriers showed weak antibody response at this time (Table 2). There was no significant difference ($P \geq 0.05$) in mean HI antibody titers among chicks vaccinated with conventional method, vaccine coated parboiled barely and cracked Maize. However, conventional methods, cracked maize and parboiled barely significantly ($p < 0.05$) higher in GM antibody titers compared to the rest grain treatment groups. Similarly, and the Duncan multiple range test shows that the difference response among chickens

vaccinated with vaccine coated to cracked sorghum, untreated sorghum and cracked millet was not significant ($P \geq 0.05$) when compared with the antibody level of unvaccinated controls.

At the seven week of age (49 days of age) the rise in antibody titer was recorded among all vaccinated chickens (Fig.1, 2 and 3) following the second dose of vaccine. Chickens vaccinated with conventional method, cracked maize and parboiled barely were found to induce the highest GM antibody titer of $\log_2 2^{5.7}$, $\log_2 2^{4.2}$ and $\log_2 2^{3.7}$, respectively (Table 2). The antibody responses of chicken vaccinated with the carrier grain of cracked sorghum ($\log_2 2^{1.6}$) and cracked millet ($\log_2 2^{1.5}$) remain poor even after the second vaccination (1st booster). On the other hand, a better antibody response was observed in the chickens vaccinated with untreated sorghum ($\log_2 2^{2.2}$), untreated millet ($\log_2 2^{2.4}$) and parboiled millet ($\log_2 2^{2.4}$) compared to their antibody titer at the first vaccination (Table 2). Parboiled wheat and parboiled sorghum were also found to induce moderate antibody response of $\log_2 2^{2.6}$ and $\log_2 2^{2.7}$ respectively, after the second vaccination (at the seven weeks of age). The GM antibody titer of the chicken in the control group at this time differed significantly ($p < 0.05$) with that of the chickens vaccinated using different vaccine coated carrier grains and conventional methods. Statistically higher GM titer was observed in chicken vaccinated using conventional method than in chickens vaccinated with vaccine coated carrier grains. Among chicken vaccinated using different carrier grain cracked maize and parboiled barely were shown to induce statistically higher ($p < 0.05$) GM titer than the rest of vaccine coated carrier grains.

The antibody titer in all group of vaccinated chicken drop at eleven week of age (77 days). Chickens vaccinated with conventional methods, cracked maize and parboiled barely still showed GM titer above the protective level ($\geq \log_2 2^3$) (Table 2 and Fig.1). For chicken in other treatment group the maximum GMT was $\log_2 2^{2.4}$ (parboiled sorghum and untreated barely) and the minimum GM was $\log_2 2^{1.1}$ recorded in chicken vaccinated with cracked sorghum. As indicated in figure (1,2,3,and 4) and Table 3 at fifteen weeks (105 days) of age a further drop in the antibody titer was also noticed. Only chickens in conventional treatment group were able to maintain GM titer above protection level ($\geq \log_2 2^3$). The least antibody titer was observed in chickens vaccinated with NDV-I2 mixed with cracked sorghum as carriers ($\log_2 2^{0.4}$).

After the third (2nd booster) vaccination (at 17 weeks of age or at 119 days) the antibody titer of vaccinated chickens increased again (Fig 1, 2, 3 and 4). Out of fifteen treatment group in eight of them the GM titer was $> \log_2 2^3$. The highest GMT of $\log_2 2^{7.1}$ was observed in chickens vaccinated by conventional method followed by NDV-I2 coated cracked maize and parboiled barely with GMT of $\log_2 2^{6.8}$ and $\log_2 2^{5.1}$, respectively (Fig 1, 2, 3 and 4). The difference between the latter two was not statistically significant ($p > 0.05$) while the immune response induced by conventional method of vaccination was statistically different from the other. NDV-I2 coated parboiled wheat and parboiled sorghum were also shown to induce nearly equal antibody titer of $\log_2 2^{4.5}$ and $\log_2 2^{4.1}$, respectively. NDV-I2 mixed with cracked sorghum was found to be poor in inducing antibody response in vaccinated chickens (Table 2).

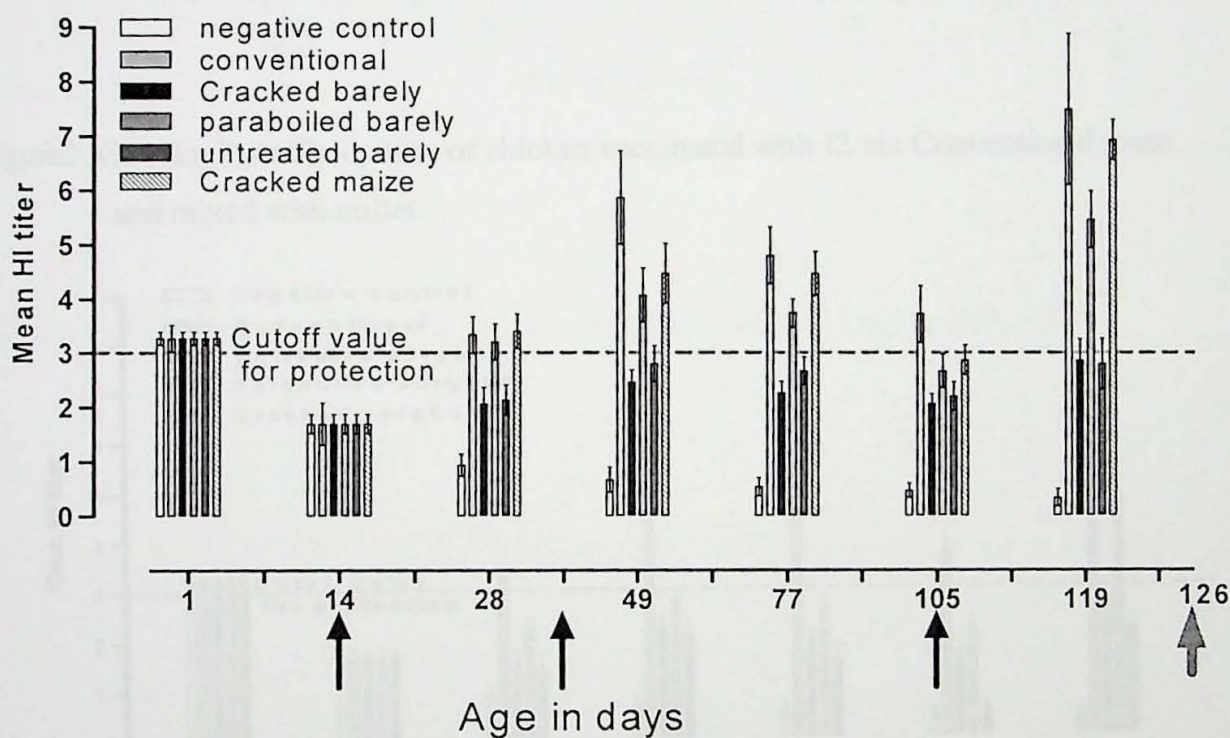


Figure 1. GM (log₂) antibody titre of chicken vaccinated with I2 via Conventional route and mixed with barely and cracked maize

* Black and red arrow indicate the day of vaccination and challenge respectively.

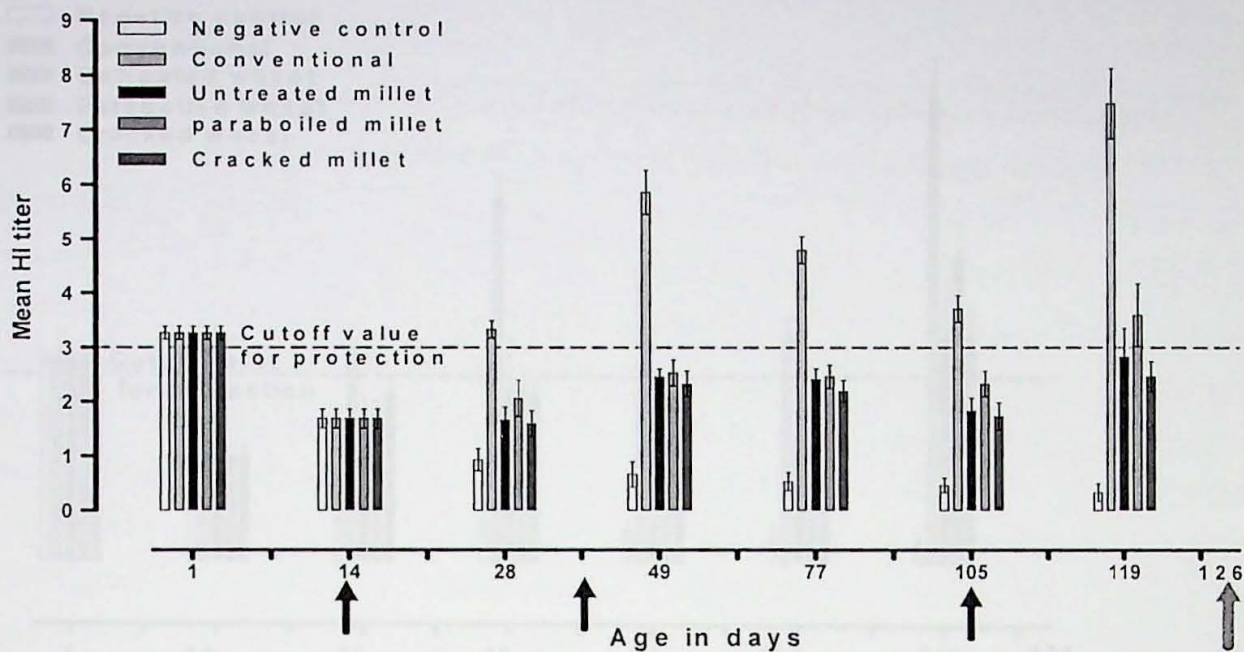


Figure2. GM (log2) antibody titre of chicken vaccinated with I2 via Conventional route and mixed with millet.

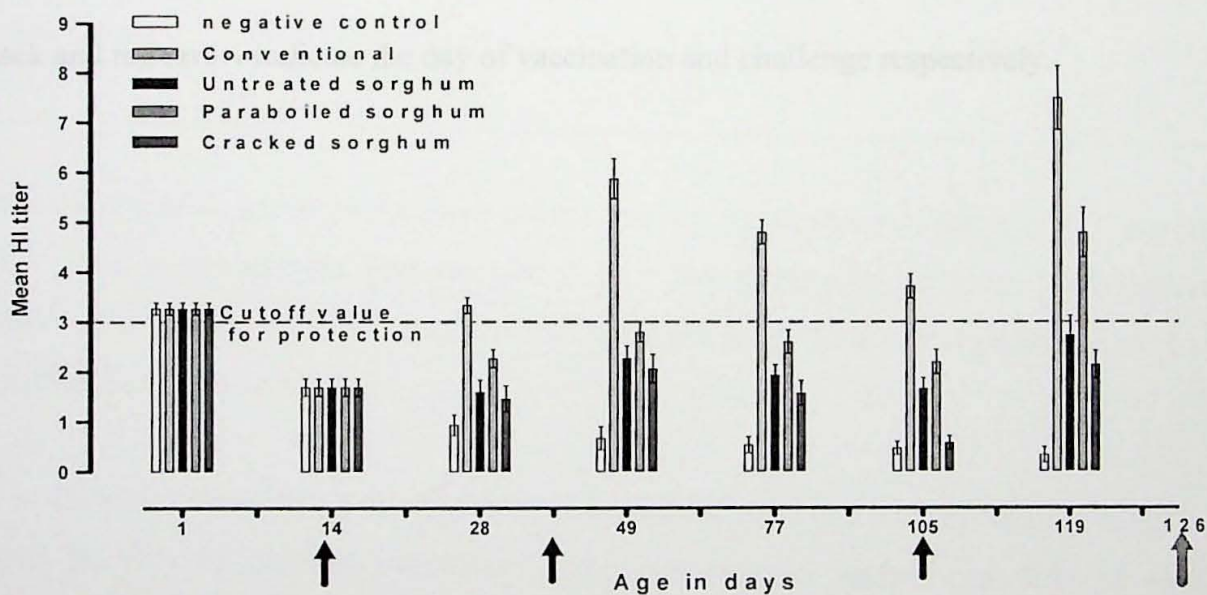


Figure 3. GM (log2) antibody titer of control and vaccinated chickens with I2 via conventional route and mixed with sorghum.

* Black and red arrow indicate the day of vaccination and challenge respectively

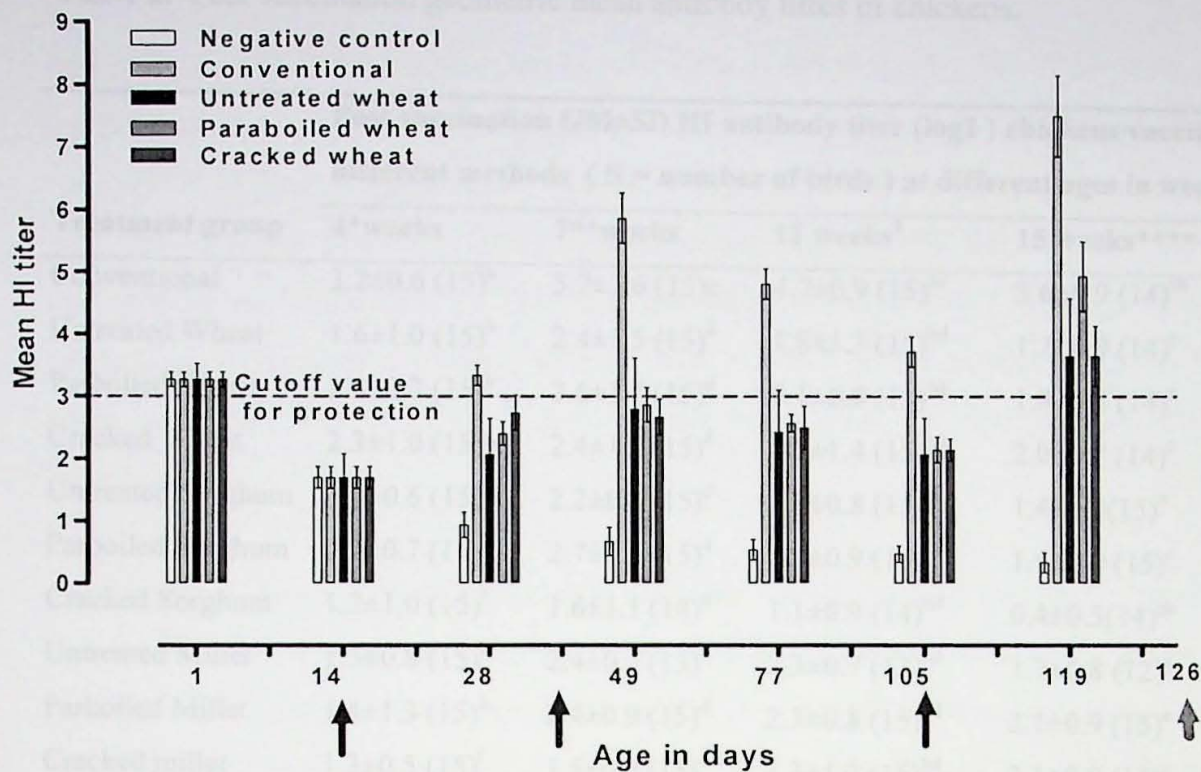


Figure 4. GMT (log2) antibody titer of chicken vaccinated with I2 via conventional route and mixed with wheat.

* Black and red arrow indicate the day of vaccination and challenge respectively.

Table 2. Post vaccination geometric mean antibody titres of chickens.

Treatment group	Post vaccination GM $\pm$ SD HI antibody titer (log ₂) chickens vaccinated by different methods (N = number of birds) at different ages in weeks				
	4*weeks	7**weeks	11 weeks [¥]	15 weeks****	17*** weeks
Conventional	3.2 $\pm$ 0.6 (15) ^a	5.7 $\pm$ 1.6 (15) ^c	4.7 $\pm$ 0.9 (15) ^{ac}	3.6 $\pm$ 0.9 (14) ^{ab}	7.1 $\pm$ 2.4 (14) ^a
Untreated Wheat	1.6 $\pm$ 1.0 (15) ^b	2.4 $\pm$ 1.5 (15) ^d	1.8 $\pm$ 1.3 (15) ^{bd}	1.7 $\pm$ 0.9 (14) ^c	3.3 $\pm$ 1.2 (14) ^f
Parboiled Wheat	2.3 $\pm$ 0.7 (15) ^b	2.6 $\pm$ 1.1 (15) ^d	2.1 $\pm$ 0.8 (15) ^{bd}	1.9 $\pm$ 0.8 (14) ^c	4.5 $\pm$ 2.1 (14) ^f
Cracked Wheat	2.3 $\pm$ 1.0 (15) ^b	2.4 $\pm$ 1.1 (15) ^d	2.1 $\pm$ 1.4 (15) ^{bd}	2.0 $\pm$ 0.7 (14) ^c	3.3 $\pm$ 1.8 (14) ^f
Untreated Sorghum	1.4 $\pm$ 0.6 (15) ^f	2.2 $\pm$ 0.8 (15) ^d	1.8 $\pm$ 0.8 (15) ^{bd}	1.4 $\pm$ 0.8 (15) ^c	2.4 $\pm$ 1.5 (15) ^{ed}
Parboiled Sorghum	2.1 $\pm$ 0.7 (15) ^b	2.7 $\pm$ 0.8 (15) ^d	2.4 $\pm$ 0.9 (15) ^{bd}	1.5 $\pm$ 0.9 (15) ^c	4.4 $\pm$ 1.9 (15) ^f
Cracked Sorghum	1.2 $\pm$ 1.0 (15) ^f	1.6 $\pm$ 1.1 (14) ^d	1.1 $\pm$ 0.9 (14) ^{bd}	0.4 $\pm$ 0.5 (14) ^{eb}	1.9 $\pm$ 1.0 (14) ^{ed}
Untreated Millet	1.3 $\pm$ 0.8 (15) ^b	2.4 $\pm$ 0.5 (13) ^d	2.3 $\pm$ 0.7 (13) ^{bd}	1.7 $\pm$ 0.8 (12) ^c	2.4 $\pm$ 1.8 (12) ^{ed}
Parboiled Millet	1.4 $\pm$ 1.3 (15) ^b	2.4 $\pm$ 0.9 (15) ^d	2.3 $\pm$ 0.8 (15) ^{bd}	2.1 $\pm$ 0.9 (15) ^c	3.2 $\pm$ 2.0 (15) ^f
Cracked millet	1.3 $\pm$ 0.5 (15) ^f	1.5 $\pm$ 0.6 (15) ^d	1.3 $\pm$ 1.0 (15) ^{bd}	2.1 $\pm$ 0.9 (15) ^c	2.5 $\pm$ 0.8 (14) ^{ed}
Cracked Barely	1.6 $\pm$ 1.2 (15) ^b	2.4 $\pm$ 0.6 (15) ^d	1.7 $\pm$ 0.9 (15) ^{bd}	1.5 $\pm$ 0.9 (15) ^c	2.5 $\pm$ 1.4 (15) ^{ed}
Cracked Maize	3.2 $\pm$ 1.2 (15) ^a	4.2 $\pm$ 2.1 (15) ^{ab}	3.9 $\pm$ 1.6 (15) ^{ac}	2.7 $\pm$ 1.1 (15) ^{fc}	6.8 $\pm$ 1.4 (15) ^g
Parboiled barely	3.0 $\pm$ 1.3 (15) ^a	3.7 $\pm$ 1.9 (15) ^{ab}	3.6 $\pm$ 1.0 (15) ^c	2.4 $\pm$ 1.2 (15) ^{fc}	5.1 $\pm$ 2.0 (15) ^g
Untreated Barely	1.7 $\pm$ 1.0 (15) ^b	2.5 $\pm$ 1.3 (15) ^d	2.4 $\pm$ 1.0 (15) ^{bd}	2.0 $\pm$ 1.0 (15) ^d	2.6 $\pm$ 1.8 (15) ^{ed}
Control	<1 ^f	<1 ^{ab}	<1 ^g	<1 ^d	<1 ^c

Means with the same letters in the same column are not significantly different at 0.05 confidence level. * = two weeks after the first vaccination, ** = two weeks after the second vaccination, ¥ = six weeks after the second vaccination, **** = ten week from the second vaccination, *** = two weeks after the third vaccination.

As it is shown in the Table 3 the highest percentage of chickens with HI titers $\geq \log_2^3$ were recorded in 80% of chicken vaccinated using conventional method, in 80% of chickens vaccinated with cracked maize and in 66.7% of chickens vaccinated with parboiled barely after the first vaccination. Nearly 50% the chicken vaccinated using NDV-I2 mixed with parboiled wheat, cracked wheat, untreated wheat and parboiled millet were able to produce HI titer of $\geq$ (at this time. The least percentage of HI titer $\geq \log_2^3$ was recorded in chicken vaccinated with vaccine coated cracked sorghum and untreated sorghum (Table 3).

Following administration of the first booster dose at seven week of age there was an increase in the proportion of chicken with HI titer greater than or equal to the protection level ($\log_2 2^3$) in comparison to the first vaccination. The highest percentage was observed in chickens immunized with conventional method (100%), cracked maize mixed with the NDV-I2 vaccine (86.7%) followed by parboiled barely (75%) and parboiled wheat (73%) (Table 3). Even though the percentage of vaccinated chicken with HI titer $\geq \log_2 2^3$ using cracked sorghum and untreated sorghum mixed with NDV-I2 is low, it was better than the titer induced during the first vaccination.

The result in Table 3 also shows that after the third (second booster) vaccination at the seventeen weeks of age all (100%) of the chicken vaccinated with NDV-I2 by conventional methods and using cracked maize induced HI titer $\geq \log_2 2^3$. Among vaccinated chickens using NDV-I2 mixed with parboiled barely 86.7% of them had HI titer of $\geq \log_2 2^3$. The percentage HI titer $\geq \log_2 2^3$ among chickens immunized by parboiled wheat and parboiled sorghum was 78.5% (Table 3). The least percentage was observed in chickens vaccinated with NDV-I2 mixed with cracked sorghum. Throughout the study period the GMT antibody in control groups is low and none of them develop titer $\geq \log_2 2^3$.

In the non-vaccinated negative control chicken 0% of the chickens had HI titer $\geq \log_2 2^3$ at all sampling stage (4, 7, 11, 15 and 17 weeks) during the six months of age. It indicated vaccinating chicks with different cereal grain coated with I₂ ND virus induce different degree of HI antibody titer as indicated above.

Table 3. Percentage of chickens with HI titers greater than or equal to $\log_2 2^3$

Treatment group	Number of chickens (%) with HI $\log_2 \geq 3.0$ of all chicks (N= 15)				
	4 weeks	7 weeks	11 weeks	15weeks	17 weeks
Conventional	12(80 %)	15(100%)	15(100%)	10(66.7%)	15(100%)
Untreated Wheat	7(46.7%)	8(53.3)	6 (40%)	5(33.3%)*	10 (71.4%)
Paraboiled Wheat	8(53.3%)	11(73%)	7(46.7%)	5(35.5)*	11(78.5%)*
Cracked Wheat	7(46.7%)	9(60%)	5(33.3)	4(28.5%)*	9 (60%)*
Untreated Sorghum	1(6.6%)	6(40%)	4(26.7%)	2(13.3)	7(46.7%)
Paraboiled Sorghum	6(40%)	8(53.3%)	6(40%)	5(33.5%)	9(60 %)
Cracked Sorghum	2(13.3%)	5(33.3%)	2(13.3%)	0(0.0%)	3(20%)*
Untreated Millet	4(26.7%)	6(50%)**	5(41.7%)**	3(25%)**	7(58.3%)**
Parboiled Millet	8(53.3%)	10(66.7%)	7(46.7%)	5(30%)	11(73.3%)
Cracked millet	4(26.7%)	7(46.7%)	5(33.3%)	3(30%)	8(53.3%)
Cracked Barely	7(46.7%)	9(60%)	6(40%)	4(26.7%)	8(53.3%)
Cracked Maize	11(73%)	13(86.7%)	10(66.7%)	9(60%)	15(100%)
Parboiled barely	10(66.7%)	12 (75 %)	8(53.3%)	9(60%)	13(86.7%)
Untreated Barely	6(40%)	9(60%)	5(33.3%)	6 (40%)	8 (53.3%)
Control	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

Where, * represents N= 14 ; ** represents N= 12

4.2. Monitoring vaccinated chicken survival rate following artificial challenge

In the current experimental study all the control and vaccinated chicken were challenged two week after the third vaccination at age of 18 weeks (126 day). Table 4 depicted the mean HI titer before the challenge, the morbidity and mortality of challenged chicken and percentage of survival after the challenge. Only 3(20%) out of fifteen unvaccinated negative control chickens survived the challenge, the rest (80%) of them were died. Chickens vaccinated with NDV I2 by

conventional method, cracked maize and parboiled barely showed 100% survival after challenge without any morbidity (Table 4 and Fig. 5). High protection rate was also observed in chickens vaccinated with NDVI2 by parboiled wheat (85%). Among chickens vaccinated with vaccine coated untreated wheat and parboiled sorghum 80% survived the challenge while 73.3% of chickens vaccinated using parboiled millet and resisted the challenge. The lowest survival rate and highest mortality rate next to negative control group was observed among chickens treated with vaccine coated cracked sorghum (Table 4). The overall differences in mortality rate between treatment groups were statistically significant ($p < 0.001$).

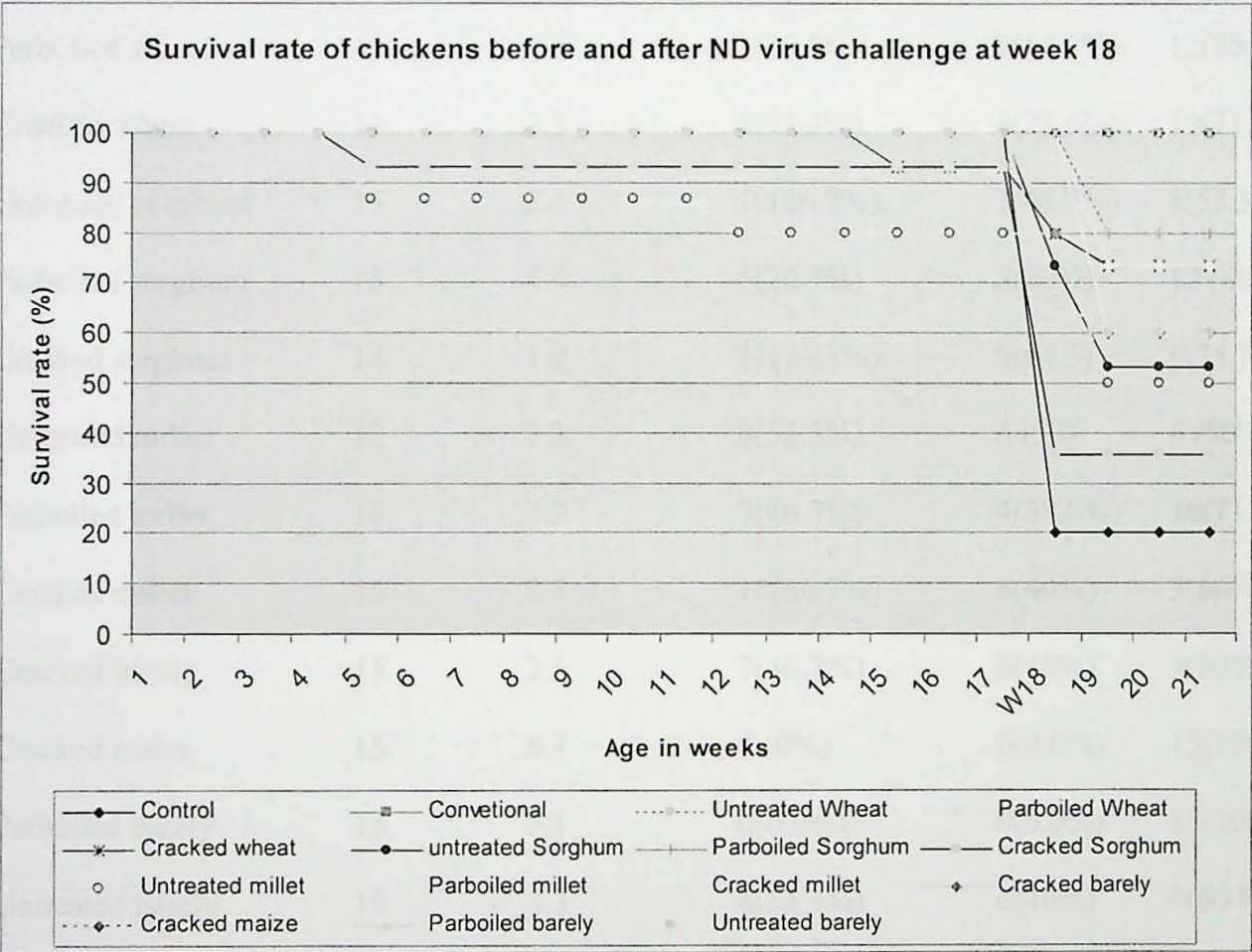


Figure 5: Survival rate of chicken before and after ND virus challenge at week 18

Table 4. Morbidity, mortality and survival rate of ND virus challenged chickens after three times vaccination.

Method of vaccination	No. Chickens	Mean HI titer	Morbidity rate	Mortality*	Survival
Control	15	0.0	14 (93.3%)	12(80%)	3 (20%)
Conventional	15	7.1	0(0.0%)	0(0.0%)	15(100)
Untreated wheat	15	2.6	6 (40.0%)	3(20%)	12(80%)
Parboiled wheat	14	4.5	5(30.0%)	2(14.3%)	12(85.7%)
Cracked wheat	14	3.3	8(53.3%)	4(28.6%)	10(71.4%)
Untreated sorghum	15	2.4	10(66.7%)	7(46.7%)	8(53.3 %)
Parboiled sorghum	15	4.4	6(26.7%)	3(20%)	12 (80%)
Cracked sorghum	14	1.9	11(73.3%)	9(64.3)	5(35.7%)
Untreated millet	12	2.3	8(53.3%)	6 (50)	6 (50%)
Parboiled millet	14	3.2	7(46.7%)	4(28.6%)	10(71.4%)
Cracked millet	15	2.5	10(66.7%)	6(40%)	9(60%)
Cracked barely	15	2.5	7(46.7%)	6(40%)	9(60%)
Cracked maize	15	6.8	0 (0%)	0(0.0%)	15(100%)
Parboiled barely	15	5.1	0(0.0%)	0(0.0%)	15(100%)
Untreated barely	15	3.3	8(53.3%)	6(40%)	9(60 %)

* $p < 0.0001$

4.3. 2. Clinical signs and postmortem lesion in dead chickens after challenge.

The ND challenged chickens were followed for the appearance of clinical signs for 1 month. In the majority of chickens except for those under treatment group of conventional, cracked maize and parboiled barely different clinical sign were observed. The most frequent clinical signs were gasping, paralysis of the wings, depression, tremor, abnormal colour and rough surface of the egg and inappitient. Moreover, post-mortem examination was also conducted in dead chickens. The findings from all examined dead chickens were hepatomegaly, cecal heamorrhage, proventricular haemorrhage, splenomegaly and tracheitis.

5. DISCUSSION

Velogenic strains of ND virus are widely distributed in African countries. As the result ND is the frontline constraint of village chickens in many developing countries (Iroegbu and Amadi, 2004). Control of the disease can only be achieved practically by vaccination. However, the conventional methods of vaccination which are so far successful in commercial poultry production system are not economically feasible and technically appropriate for village chicken flock in their feral nature (Echeonwu et al., 2007). In recent years the advent of thermostable vaccine such as I-2 resulted in the emergence of in feed oral ND vaccine delivery system. The role of the carrier feed would be simply to adsorb the virus from an aqueous suspension and to release it in a viable form in the digestive tract of chickens (Spradbrow, 1993/4). The efficacy of in feed vaccines can be tested by feeding chickens and thereafter by measuring both the antibody response and resistance to challenge (Oakeley, 1998).

According to Spradbrow (1993/4) the efficacy of any vaccine is determined mainly by assessment of the level of antibody produced in the target animals and ability of the vaccinated animal to resist exposure to the virulent agent when compared with unvaccinated control. These parameter were determined in the current experimental work. The suitability of different carrier grains feed to deliver viable vaccine virus in order to stimulate the production of protective antibody amongst chickens was the main focus of this work. The result in the current study have shown that chickens vaccinated with the thermostable NDV-I2 vaccine using conventional routes, with cracked maize and parboiled barley as vaccine carrier were completely (100%) protected against challenge with a virulent wild NDV strain. On contrary, 80% of unvaccinated (control) chickens were died following challenge with virulent strains. In this study it was not only mortality that was protected using these three methods of vaccine delivery but also the clinical morbidity was not noticed. It is evident from this experiment that it is possible to induce protective immunity in chickens using cracked maize and parboiled barley. This finding is consistent with Nasser *et al.*, (2000) who reported complete protection against challenge in chicken vaccinated with ND vaccine strain I2 via conventional route (drinking water) and parboiled barley.

Similarly previous work conducted in Nigeria also indicated that cracked maize was suitable vaccine carrier grains with substantial protection (Olabode *et al*, 2010). Moreover, the author also reported that the residue from the production of maize porridge has been used as a carrier for ND V4 strain vaccine which is similar to our NDV-I2 strain.

The reported protective titer for Newcastle disease vaccination is the HI titer of $\geq \log_2 2^3$. This was based on the level of HI antibody detected in the chicken serum (Alexnader *et al*, 2004; Spradbrow, 1993 / 4). By implication antibody titer less than $\log_2 2^3$ would not be protective. In this study about 20%, 46.7% and 60% of chickens vaccinated by using cracked, untreated and parboiled sorghum, respectively were able to attain the HI titer of $\geq \log_2 2^3$ following the third vaccination. This technically indicates only 20%, 46.7% and 60% of chicken vaccinated with above three preparation of sorghum, respectively, could be protected if applied under field condition. And this is well below the recommended protection level of 80% (Tizard, 2000). But when chickens were challenged by wild NDV strain only 35.5 , 53.3 and 80% of them survived (Table 3). Although the HI antibody titer induced was lower, the level of protection observed in chickens vaccinated with vaccine coated parboiled sorghum quite sufficient. This shows that very weak correlation exist between HI titer and level of protection. Alexander (1998) suggested that apart from serum antibody, secretory antibody (IgA) of mucosal surface and cell mediated immunity also play role in resistance to challenge. Ibrahim *et al.*, (1981) have also shown that no correlation existed between the HI titers due to ND vaccination and resistance to challenge. Holmes (1979) also suggested that protection of respiratory tract is independent of hummoral immunity. Thus the present study seems to indicate that a low NDV- HI antibody titer does not necessarily mean susceptibility to virulent NDV. High antibody titer, on the other hand, usually indicate protection (Alexander *et al.*, 2004).

Previously a similar trial conducted in Tanzania using I2 vaccine coated parboiled sorghum proved successful but other experiment conducted in same country Tanzania (Foster *et al.*,1999), in Nigeria (Echeonwu *et al.*,2008) and Ethiopia (Naser *et al.*, 2000) showed that I2 vaccine coated on parboiled sorghum gave low level of protection. This is not in agreement with 80% protection obtained in the current study (Table 3). This variation could be explained as suggested by (Oakeley 2000) that various grains that have grown in different agro- ecology and on

different soil characteristics could have difference in their vaccine virus carrying abilities. This in turn could be due to their physico- chemical variations especially due to their surface properties and chlorine content. This fact is further elaborated by variation regarding the success of using untreated barely as carrier for NDV-I2 between the current study and previous work in Ethiopia by Naser *et al.*, (2000) and Rushton (1995). In the current study among chicken vaccinated with NDV-I2 by untreated barely gave 60% protection following the challenge with a virulent Ethiopian NDV strain. On the other hand, Rushton (1995) reported complete (100%) protection using untreated barely.

Cumming (1992) and Jackson (1992) suggested that heating, soaking, washing and cracking might be useful in developing a successful carrier feed. This is also proved in the current experimental work where parboiled grains induced higher immune response. Amongst chickens vaccinated with NDV-I2 vaccine delivered on untreated millet, cracked millet and parboiled millet 50%, 46.7% and 66.7% of them were able to produce HI titer of $\geq \log_2 2^3$, respectively, after the second vaccination. Up on administration of the third dose (2nd booster) of vaccine 58.3%, 53.3%, 73.3%, of chickens were able to produce HI titer of $\geq \log_2 2^3$. Intramuscularly injected challenge experiment results showed that the survival rate for the chickens vaccinated with untreated millet, cracked millet was 60 % and while it was 73.3 % for those vaccinated with parboiled millet. Previously 70% survival rate among chicken vaccinated with I2 using treated broken millet as the vaccine carrier was reported (Echeonwu *et al.*, 2007) which validate the result of the current study by parboiled millet. Generally the current study and work of Echeonwu *et al.*, (2007) indicates that up on proper treatment millet could be one of a good vaccine carrier.

The result obtained showed that around 50% of the chickens fed with vaccine coated untreated, parboiled and cracked wheat were produced HI titer of $\geq \log_2 2^3$. However, administration of second dose of vaccine induced HI titer of $\geq \log_2 2^3$ in 53.3%, 73% and 60% of chicken vaccinated with untreated wheat, parboiled wheat, cracked wheat (Table 2). Following delivery of the third dose of vaccine, untreated wheat, parboiled wheat, cracked wheat induced HI antibody of $\geq \log_2 2^3$ in 71.4%, 78.5% and 60% of vaccinated chicken, respectively. The challenge experiment using virulent ND virus showed that the minimum percentage (71.4%) of survival was recorded in chicken vaccinated using cracked wheat as a carrier for the vaccine

while it was 80% and 85.7% for those vaccinated with untreated and parboiled wheat. According to Spradbrow (1993/4) wheat was the base of large scale control program and now is used as the vehicle for oral vaccine that is being widely used throughout Malaysia. The result of the current study also confirms this even though cracked maize and parboiled barely are superior under Ethiopian context from current study.

A very important condition for successful development and use of any chosen food as vaccine carrier is the ability of such food to allow firm binding or adherence of the coated vaccine virus without interfering with the survival of the vaccine virus. In this regard different results have been reported from different country. Echeonwu *et al.*, (2008) reported that the virus coated onto the carrier foods offal without additive remained stable and immunogenic for 3 weeks (millet); 3.5 weeks (sorghum) and 5 weeks for maize at room temperature and Naser *et al* (2000) also able to recover viable vaccine virus after 14 hour at room temperature on parboiled barely. In the current study the vaccine virus coated on cracked maize, parboiled barley, parboiled sorghum and parboiled and untreated wheat was highly immunogenic after 6 hour exposure for room temperature. This concludes that grain could a potential vehicle of thermostable vaccine to vaccinate village chicken against ND.

Generally cracked maize, parboiled barley, untreated and parboiled wheat as well as parboiled sorghum could be good carrier for I2 vaccine virus to use at large scale under Ethiopian field (village) conditions. Study in Malaysia showed that chickens vaccinated under laboratory conditions with food-based vaccine showed better than 90% protection (Aini *et al.*, 1990a). Under simulated village conditions (Aini *et al.*, 1992) and under real village conditions with vaccine delivered by farmers (Aini *et al.*, 1990b), protection rates were about 60%. This report indicates that pilot field trial at village level is necessary to evaluate the result of the current study at real village conditions.

6. CONCLUSION AND RECOMMENDATIONS

Newcastle disease is the major constraint of village chickens production in Ethiopia. Thermostable ND vaccine (I2) where getting cold-chain is difficult and oral feed based method of vaccination where skilled vaccinators inadequately exist will play a crucial role to reduce ND related mortalities. Vaccination with such ideal vaccine for village chickens improves productivity of village chicken hence contributing to poverty alleviation in the rural areas. The findings from the present study showed the I-2 virus coated cracked maize and parboiled barely were immunogenic and gave complete protection of chickens against challenge after keeping the virus - grain mixture for 6 hours at room temperature before vaccination. Vaccine coated wheat, parboiled sorghum and parboiled millet were also gave sufficient protection and take the next rank following the above grains. Chicken vaccinated by vaccine coated untreated and cracked millet and barely had limited protection. On the other hand, cracked sorghum and untreated sorghum were found unsuitable as carrier for the vaccine virus. Generally, the finding of current experiment indicated that grains could be used as vehicle for the I-2 virus in Ethiopia and farmers can travel 6 hours with the virus before vaccination. But single trial could not be warranty in large country like Ethiopia where grain characteristics could vary from region to region and moreover the result could vary in the field experiment at real village conditions.

Therefore in light of the above conclusions the following points are forwarded

- ❖ The result of the current study should be evaluated at real village condition on the backyard chickens
- ❖ Further research should be conducted on different grains from different agro-ecology and soil characteristics.

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8. SIGNED DECLARATION SHEET

“I, the signed, declare that this thesis is 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”

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