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ON STATION EVALUATION OF THERMOSTABLE NEWCASTLE DISEASE VACCINE AND SURVEY ON COMMUNITY KNOWLEDGE ON NEWCASTLE DISEASE RISK FACTORS IN THREE DISTRICTS OF WEST GOJAM, ETHIOPIA

MSc THESIS

BY

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DEPARTMENT OF VETERINARY CLINICAL STUDY

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BISHOFTU, ETHIOPIA

ON STATION EVALUATION OF THERMOSTABLE NEWCASTLE DISEASE VACCINE AND SURVEY ON COMMUNITY KNOWLEDGE ON NEWCASTLE DISEASE RISK FACTORS IN THREE DISTRICTS OF WEST GOJAM, ETHIOPIA

A THESIS SUBMITTED TO COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF VETERINARY SCIENCE IN TROPICAL VETERINARY EPIDEMIOLOGY

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Tadiose Habte** titled **On Station Evaluation of Thermostable Newcastle Disease Vaccine and Survey on Community Knowledge on Newcastle Disease Risk Factors in Three Districts of West Gojam, Ethiopia** and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Tropical Veterinary Epidemiology

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LIST OF ABBREVIATIONS

ACIAR = Australian Centre for International Agricultural Research

DOC = Day Old Chicks

DZARC = Debrezeit Agricultural Research Center

EID = Embryo Infectious Dose

GIT = Gastro Intestinal Tract

HI = Haemaglutination Inhibition

HR = Heat Resistance

IBD = Infectious Bursal Disease

IM = Intra Muscular

ND = Newcastle Disease

NDV = Newcastle Disease Virus

NVI = National Veterinary Institute

OEV = Oil Emulsion Vaccine

OIE = Office International des Epizooties

OTC = Oxy-Tetra Cyclin

PA = Peasant Association

USD = United State Dollar

VVND = Velogenic Viscerotropic Newcastle Disease

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ABSTRACT

Cross sectional survey was conducted on 96 randomly selected farmers in Mecha district from April to May 2015 in order assess the driving forces which contribute for the occurrence of ND. Ninety nine percent of farmers are practicing scavenging poultry production with no separate housing. Eighty nine percent of farmers reported ND as a major disease condition. Although ND is not age specific in the study area, based on the survival time baby chicks, growers and broody hens are more susceptible to the disease. ND is more common at the end of dry season. Even if the cause of ND is not well understood by farmers the loss is high that might be related with poor bio-security and vaccination. Any health technology was not received by 84.4% of farmers. The screening test shows 66.2% sero-conversion. An Experimental study was also conducted at DZARC with proper experimental set up. Two breeds, local and koekeok chickens were used in four treatment and a control groups with three replications each. The replications were 12 local and 19 koekoek chickens. The four treatments uses I2 vaccine through eye drop, water, parboiled barley and litter spray. Pre vaccination serum was collected at day 1, 14 and 20 while post vaccination was taken at day 36, 46 and at pre-challenge. Sample was also taken 8 days after the challenged with wild ND strain. Pathogenic Index HI and survival rate were used to detect the protection level of the treatments. The result shows, the antibody response of chicken and the pathogenic index was not significantly different between breeds but protection was higher in all treatments than the control. The pathogenic index was lower in ocular and spray vaccinated chicken. Survival rate of the chicken is significantly lower in control groups but higher chicken vaccinated with ocular and spray route. Therefore spray vaccination is the better choice for route of

thermostable (I2) vaccines in village production system. This route of vaccination is preferred over the others because it easy to administer, effective and can also be performed by trained farmers.

Key words: I2, ND, vaccination, and chicken

1. INTRODUCTION

Ethiopia, in general, is endowed with a huge livestock population in Sub-Saharan Africa. It has been estimated that livestock supports the livelihoods of about 80% of the 60 million rural population (FAO 2004). In terms of poultry, 94% of the country's poultry population comprises indigenous birds (CSA. 2013), which are favored by household producers due to their perceived traits, such as their adaptability to local agro-ecological conditions, taste, low price and input requirements as compared to exotic or improved breeds (Alemu *et al*. 2008). The fact that almost all of the poultry in Ethiopia comprises indigenous (i.e., local) birds reveals that the poultry subsector is strongly dominated by small-scale, household-level poultry. According to Tadelle *et al*. (2003), small-scale, village poultry production in Ethiopia contributes almost 99% of the national egg and poultry meat consumption, although this figure recently might have been changed slightly due to the emerging commercialization in peri-urban agriculture.

Poultry is an interesting tool to respond rapidly to poverty gaps if included in rural development strategies. It has fast generation interval and high reproductive rate. It is prolific, easy to rear and their output can be generally expanded more rapidly and easily than that of other livestock. Different scales of poultry productions are available in Ethiopia: scavenging, large, small-scale and commercial. The 3 production systems have their own specific chicken breeds, inputs and production properties. Each can sustainably co-exist and contribute to solve the socio-economic problems of different target societies (Duguma, 2009). The indigenous flocks are said to be disease resistant and adapted to their environment. However, the survival rates of the Ethiopian indigenous chicks kept under natural brooding conditions considered low. Disease and predators are known to be the major causes of mortality in the country (Holye, 1992; Negussie, 1999). According to Negussie (1999), Newcastle disease accounted for the largest proportion of overall flock mortality to be 57.3% followed by fowl pox 31.6%, coccidiosis 9.4% and predator loss 1.7%.

In Ethiopia Newcastle disease (ND) appear to be the most challenging avian disease. The circulating strains of NDV are capable of causing 90-100% mortality in unprotected flocks (Serkalem *et. al.,* 2005), (OIE, 2013) . How the virus was introduced into the country is still unknown. The disease is transmitted from bird to bird and from farm to farm mainly via aerosol but contaminated feed and water, feces from sick bird and egg and carcass from infected birds are also means of transmission (OIE, 2012).

The common control strategies of the ND around the globe are vaccination, strict quarantine, slaughter and disposal of all infected and exposed birds and disinfection of the premises. Vaccination is generally very cost effective intervention and given a high priority by farmers in most developed nations where infrastructure and veterinary service are well known/available (Alexander *et al*., 2004).

Vaccination has a cost include price of the vaccine, time spent designing the vaccination schedule and paying for the crew that administers the vaccines. Another major cost for vaccination, which is rarely considered, is due to the losses from vaccine reactions from the live type vaccines and local tissue reactions associated with the inactivated vaccine injections (Dias *et al*., 2001). Many trials have been conducted to develop village vaccination program and reduce cost of Newcastle disease vaccination for scavenging poultry production system (Nasser *et.al.* 2010).

Having thermostable vaccine is enable farmers to worry less on the logistics related with the cold chain. The immunogenicity of the thermostable I2 vaccine was mentioned by works of Nasser *et.al.*, (2010) in ethiopia and other work by Tu *et.al.* (1998) in Vietnam. Both report that chickens vaccinated with I2 vaccine with different route of vaccination and feed grains as a channel has a good protection effect.

Many routes of vaccination were tested and their effectiveness is evaluated but the practicality of those vaccines in the country is not well disseminated in the farming community. This might be related with poor veterinary service and less practicality of the vaccination routes by rural community of the country. Therefore, the following trial on

routes of vaccination was carried out in two breeds of chickens with the following objectives

General objective

To designing ND control and prevention strategies for the development of the poultry sector in village system.

Objective

- Determine the protection level of the litter based I2 thermo-stable Newcastle disease vaccine as compared to I2 in parboiled barley and water
- Compare between the litter based I2 thermo-stable Newcastle disease vaccine systems with intra ocular.
- Identify the deriving factors of ND infection in West Gojam village poultry production system.

2. LITERATURE REVIEW

2.1 Relevance of Village Poultry

Village poultry production keeps chicken using family labour. Village poultry plays a crucial role in most developing countries like Ethiopia because it makes up the largest percentage of the national poultry population. The sector is a major source of income and contributes to 20% of the protein consumed in these countries. Poultry also play a significant role in human society through their contribution to the cultural and social life of rural people (www.fao.org). The gift of a chicken is often in many parts of the country is a way of welcoming high status visitors or honoring affine and kin. There is a growing demand for animal products due to raising income of the population and urbanization in the country.

Village poultry keeping is a means in alleviating poverty and act as a vehicle for reach target of the county rural development goal. To reach this goal different stakeholders like development agencies, international agencies, governments and non-government organizations were involved. The pace and scope of such support have expanded over the last 20 years and some major initiatives have been undertaken. These development-oriented interventions are mainly focused on replicating commercial poultry innovations at a small scale household level through to development of innovation and support networks at national level (NPRCT, 2013 unpublished document).

Village chicken production also plays a significant role in the economy of the country. It improves the nutrition, income, food security and livelihood of smallholder poultry producers. In small holder farms poultry increase protein source in the community and improve income status of producers; because they have short generation interval and high rate of productivity. Further, 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 with religious taboos and its complementary role played

in relation to other crop–livestock activities. Chickens can be raised by poor smallholder-farmers which can include farmers affected by landlessness (Tadelle, *et al*., 2003).

2.2. Free Range Flock Management

Housing and Hygiene in Free Range

Poultry housing in free range system is of different standards based on the socioeconomic situations and living standards. Poultry hoses in this production system will help farmers to alleviate risks related with predator, bad weather and theft. The type of house might range from standard poultry houses built purposely for the chicken or can be simple night-basket. A perch can be prepared inside the house to let chicken to slip on it. Egg laying nests are set inside the house in order to minimize egg loss, to minimize the disturbance of hens while they lay eggs and to make egg collection easy (NPRCT, 2013 unpublished document).

Poultry house management is very important to prevent disease of chicken including endoparasites and ectoparasites of chicken. Proper management mainly focuses on taking hygienic measures. Hygienic measures include proper cleaning of drinker and drinkers, keeping nests clean by refreshing it with clean hay or straw and cleaning of the house. The house, the perches, and the nests should be thoroughly cleaned and must be treated with lime specially when there is disease outbreak. If the house is made of locally available materials and cheaper in its cost it is advisable to burn it down and construct a new one, this will prevent hiding of ectoparasites and other contaminants specially microorganisms in small cracks.

Feed and Water

Clean water and feed is a key factor for improved disease resistance in birds and on proper development of the body. The availability of scavenging feed resource is depending on the time of the year therefore supplementation is based on the time of the year in which feed materials are available or not. Supplementation is given in order to

improve body weight and egg laying ability of chicken. In supplementation of feed and water to chicken it should be clean and with good quality, the supplementation programs are early in the morning and in late evening when chickens are returned home from scavenging. The material used in supplementing chicken must be clean, so that infections do not spread through dirty feed and water.

In early age chicks need protein rich feed and supplementation feed should include protein rich feed ingredients. When it is necessary to supplement chicks with protein richer feed they should be fed separately from adult ones. It is also important to supplement clean water to chicks to prevent them die of dehydration.

Health

Contagious diseases are major diseases that can be easily introduced in poultry flock through contaminated materials and sick birds. This is the main reason why experts suggested that poultry producers should not buy chicken from live bird market or from uncertified or unknown sources, especially during outbreaks of diseases. When it is important to purchase chicks from outside source it is important to quarantine the chicks for two weeks in a shed or cage. The same structure can be used for the isolation of sick birds if quarantined chicks are not available there.

In serious disease conditions poultry farmer should isolate or kill sick and must ask veterinarian for help. After serious of investigation the farmer should give drugs or/and implement management measures recommended by the vet. Dead birds (or parts from dead birds) should be burned or buried deep enough (about 1 m) to prevent dogs and other animals from digging them up and spread the disease. If there are many sick animals, the cause of the disease must be established before introducing new birds or vaccinating chicken prior to disease outbreak.

When it is important to purchase chicks from outside source it is important to quarantine the chicks for two weeks in a shed or cage. The same structure can be used for the isolation of sick birds if quarantined chicks are not available there.

Vaccination is one of the means to prevent disease in the flock. Vaccination of chicken using available vaccine (like Newcastle disease vaccine, fowl typhoid vaccine and Pox vaccine) in the area is necessary to prevent disease and mortality in scavenging system. All birds must be vaccinated against those vaccines. But it is not a common practice in most developing countries like Ethiopia. In Ethiopia there are some irregular vaccination programs against Newcastle disease in village system but they were not successful. Because due to so many factors such as lack of awareness, poor veterinary service and farmer's perception towards vaccination are the major causes problems related with vaccination.

2.3. Newcastle Disease

New castle disease is a disease caused by a virus in; Order Mononegaviraie, Family Paramyxoviridae, Genus Avulavirus ,Species New Castle Disease virus. The virus has four strains; mesogenic, velogenic, apathogenic enteric and lentogenic. The severity of the disease varies depending on the strain of the virus. The Velogenic strain is highly virulent and produces severe nervous and respiratory signs, spreads rapidly and causes up to 90% mortality. The monogenic strain has intermediate virulence and is associated with coughing, affects eggs quality production and results in up to 10% mortality (Victor *et al*., 2013).

NDV infections have been established in at least 241 species of birds representing 27 of the 50 orders of the class. All birds are probably susceptible to infection, but, the disease observed with any given virus may vary enormously from one species to another. It also be reported that Newcastle disease infect other animals other than birds, ranging from reptiles to humans (Alexander, 2000).

Controlling of Newcastle disease requires immediate response. The major response during ND outbreak is tightening the bio-security measures in order to contain the outbreak as much as possible and to prevent disease from spreading to other sites (Field Manual of Wildlife Diseases). Major bio-security measures are strict quarantine (delay

introduction of new birds to the flock for 30 days), slaughter and disposal of all infected and exposed birds, and disinfection of the premises. It Pests such as insects and mice should be controlled, human traffic should be limited, and the introduction of new animals with unknown health status should be avoided (Adwar & Lukešová, 2008). Therefore management techniques and hygienic practices at the farm level will minimize the risk of disease (Marangon & Busani, 2006). Otherwise large amount of the virus are shed from infected birds and contaminate the surrounding environment. Carcass contamination can last long time due to its heat- stability characteristics of the virus in the carcass. But, control of the disease can be challenged if; wild life is involved and highly virulent strain is involved (OIE, 2013).

Even if vaccination is effective means of controlling Newcastle disease it is advisable to understand the type of strain used according to the epidemiology of the disease and according to the strategies that we are going to perform in the given epidemiological area (Indi *et al.,* 2014). There are four major strains of Newcastle disease virus. But one of the four is not used in vaccine production. To mention them:

Lentogenic strains: This will cause low virulence, low mortality and loss of egg production and some respiratory signs (FAO, 2005 and Adwar & Lukešová 2008). Vaccines produced from these strain of the virus include; Hitchner B1, F, VG/GA, Clone LaSota and LaSota vaccines. The B1 vaccine was first developed by Hitchner and licensed to use in 1950 (Marcelo, 2012).

Mesogenic Strains: These have Moderate virulence; the mortality might reach up to 50 percent and also cause loss of egg production (FAO, 2005 and Adwar & Lukešová 2008). This strain comprises Mukteswar, Komarov and Roakin vaccines. The main drawback of this strain is that the vaccine from this strain can cause severe illness and mortality in vaccinated chicken (Marcelo, 2012).

Velogenic strain: It is known with its high virulence and causing disease and high mortality. It is the only strain that is not subjected to vaccine production (FAO, 2005 and Adwar & Lukešová 2008).

Apathogenic enteric strain: A form that usually consists of a sub-clinical enteric infection (Adwar & Lukešová 2008). Vaccines in this group are V4, PHY.LMV, Ulster 2C, and VH**.** They are the most common commercially available vaccine strains which can be used in many production systems (FAO, 2005 and Marcelo, 2012).

2.4. Newcastle disease Vaccine selection and vaccination strategy

A vaccination program for a given area is tailored for local conditions such as: the type of poultry production, the densities of different bird species, the prevailing disease situation, the availability of the vaccine itself, the presence of exercising other vaccines, the prevalence of other diseases, the resources available and the costs (Marangon & Busani, 2006).

In breeder flock prevention of Newcastle disease can be achieved by vaccination of the flock using mesogenic strains of Vaccine at day 10 by the aerosol or eye drop route. This expedient is only justified if birds have previously received one or more live attenuated lentogenic vaccines (Simon & Emeritus, 2005). Studies in 1970 shows higher HI titers (better protection) can be achieved when the combination of live and inactivated vaccines are used than live or inactivated alone (Marcelo, 2012). But in areas with a defective cold-chain the V-strain live thermostable mutant ND can be distributed to subsistence and backyard flocks (Simon & Emeritus, 2005).

During vaccination of birds dose of the vaccine play a great role in the immune status of chicken. In the birds vaccinated for ND the higher antibody titer was observed in chickens vaccinated in both eyes. Chicken vaccinated with cloned 30 strain is not getting the protective immunity but other vaccines show protective antibody level. Lassota is more immunogenic than hitchnable B1. Recombinant vaccine of ND and bronchitis with

boaster fort dodge ND and vaccinating with Lasota two times give significant protection level than other vaccinations (mean titer is > 840 after the second vaccination in both cases). Vaccinating M-strain IM at day 60 also brings a significant increase in the immune response of chicken (Banu *et al.*, 2009).

There are three types of vaccination strategies (Marangon & Busani, 2006), these are:

a) Routine vaccination program which held in areas where the disease is endemic. It is effective to reduce mortality and also it is very helpful in eradication campaigns.
b) Emergency vaccination is an option during introduction of new infection in unaffected area and also the disease has the potential to spread easily
c) Preventive vaccination is a measure that may be applied wherever a high risk of introduction and further spread of a contagious poultry disease has been identified. Prophylactic vaccination should be applied as long as the risk of infection exists.

Different strains of Newcastle disease vaccines can be selected to be used in different production system and different epidemiological areas. To select an appropriate vaccine strain for a given production system or epidemiological area it is advisable to know the characteristic futures different vaccines produced in many part of the world.

All the vaccines seen above are known of their moderate immunogenicity and their easiness on administration. Therefore on selection process of vaccines especially for village system is mainly based on the transportability and cost. The I2 and HVT vaccines are known of their transportability and lower cost.

Live vaccines are the most commonly preferred vaccines used in poultry production. These vaccines are selected to be used due to their broad advantages. The most common advantages of live Newcastle disease vaccines are; it is easy to apply, relatively inexpensive, and give moderately good immunity to individual bird and to the flock in general (FAO, 2005). Vaccinal reactions to them vary according to the vaccine strain used in the process of production. Among the live vaccines, the heat resistant vaccines

require less stringent transport requirements in the field, and they have also been widely used in villages (Adwar & Lukešová 2008). Lasota and Hb1 are the two most common live Newcastle disease vaccines used in many parts of the world. While NDV4-HR and I2 are the two most common live thermostble vaccines used in many production system (Nega *et al.*, 2012 and Zelalem *et al*., 2014).

Live vaccines can replicate in the host. The advantage of this vaccine is that it is not necessary to vaccinate every bird individually because the vaccinal virus can spread on its own from one to another and also it easy to apply with drinking water. But the disadvantage is that, the birds can react to the vaccination in manifesting some of the signs of the disease. The severity of this reaction depends on strain of the vaccine or presence of concurrent infection with other pathogens (Bell J.G., 2000). The other main advantage of live vaccines is their ability to immunize birds that are not vaccinated for the disease directly but has a chance to contact with vaccinated birds. The contact immunization potential is high in thermostable vaccines than vaccines need cold chain and high care in dilution and application. Vaccination of birds is not means that birds are getting the desired 100% protection or in other word it will not prevent mortality of chicken 100%; therefore after vaccination if there is an outbreak Newcastle disease there might death on be some chicken in immunized flock..

Vaccination decreases the incidence of velogenic viscerotropic Newcastle disease (VVND) in commercial poultry worldwide. But these vaccination regimes are not feasible in free-range and backyard systems of poultry production practiced in many developing countries (Folitse *et al.*, 1998). The reason behind is ND vaccines were produced for commercial flocks, the cost of production is very high due to use of costly technologies in the process of production. Costs related with production include; cost of specific pathogen free egg, cost of adjuvants and costly control program for every batch of vaccine. Therefore in order to minimize the COST of the vaccine most commercial vaccines are produced in large doses (Spradbrow, 2005).

Vaccination under village conditions could not yield 100% protection (Spradaw., 2005). But there is a significant increase in the survivality of chicken in village in which vaccination program was implemented (Tran, 2000). Therefore it is advisable to set criteria for use of vaccine in a rural setup. The selection of a ND vaccine for use in rural chicken will depend on the local conditions in each country. Selection criteria will include – ease of use, cost, thermostability, immunogenicity, availability and transportability. In circumstances where the cold chain is weak or absent, the only reliable option will be the use of thermostable ND vaccines; i.e. the live vaccines NDV4-HR (Ideris *et al.*, 1987) and I-2. In most cases where farmers are to contribute wholly or partially to the cost of the vaccine, the price 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 (Adwar & Lukešová, 2008).

Inactivated vaccines give very good immunity without vaccinal reactions and have been widely used, but are relatively expensive and require considerable attention to training when used by non-veterinary personnel. Live vaccines are easy to apply and relatively inexpensive, and give moderately good immunity. Vaccinal reactions to them vary according to the vaccine strain. Among the live vaccines, the heat resistant vaccines require less stringent transport requirements in the field, and they have also been widely used in villages (ACIAR,1998 and Alders, 2002). The selection of a ND vaccine for use in family poultry will depend on the local conditions in each country. Selection criteria of vaccine for village use include (Copland and Alders, 2005) it should be easy to use, it should be thermostable, its COST should be lower; it should have high immunogenicity capacity, good transportability and it should be available easily. Due to their characteristics future thermostable vaccines are the most suitable vaccine strains to be used in village production system.

2.5. Thermostable Live ND Vaccine

Thermostable vaccines are vaccines which are resistant to environmental heat (FAO, 2005). The presence of this vaccine enables distributors and users to reduce the problems associated with inadequate cold chains in the field (Adwar & Lukešová 2008). Even if this vaccines are resistance to heat it has to be handled as a biological product, that is, you cannot expose the vaccine to sunlight and frequent shifts in temperature and still expect it to remain active (ACIAR,1998).

Thermostable vaccines are prepared from strains that are infective after stored outside cold chain for short time. Thermostable vaccines are prepared in two forms; from naturally thermostable variant or by increasing thermostability artificially in the laboratory (FAO, 2005). Among thermostable vaccines the Australian NDV4-HR is the first thermostable vaccine produced for small scale producers (ACIAR, 1998). To use the NDV4-HR in village system is difficult due to the cost of production of the vaccine and legal issue related with giving seed vaccine strain for developing countries.

According to the study performed in Mozambique; the Australian NDV4-HR and I-2 provide protection to all vaccinated birds and other birds in contact with vaccinated ones against a local virulent strain. The same study also reviled that individual vaccination in eye drop has a better protection level than administering in the drinking water or by oral drench. This shows that NDV4-HR and I-2 are efficacious, immunogenic and suitable in the control of ND (Dias, *et al.,* 2001). In other study conducted in Pakistan the researcher compared five lasota strain brands. Thermostability of the vaccine was also measure by incubate it at 4, 25, 40 degree centigrade for 24 hours but no change in HI results in vaccines stored in different temperature (Abbas *et al.*, 2006).

The ACIAR project funded by Australian government also produces a seed virus similar to NDV4-HR that could be made available without cost to laboratories in developing countries (ACIAR, 1998 and Bensink And Spradbrow, 1999).

Before producing this vaccine the project tries to test 45 isolates of a virulent ND were examined for antigenicity, safety and ability to spread. The most promising of these isolates were checked for their thermostability and the more resistant isolates selected for enhanced heat resistance. The result of this study was strain I-2, which was amplified in eggs from a disease-free flock to form a master seed. The seed was tested for safety and for freedom from bacterial contamination. Strain I-2 has undergone laboratory tests in several countries and has proved to be protective against local virulent strains of the ND virus (Alders and Spradbrow, 2001). In Vietnam, after extensive laboratory and village trials, it has been officially recognized as the ND vaccine for village chickens (TU *et al.,* 1998). In Tanzania, it has given protection for at least two months after vaccination (Wambura *et al.,* 2000). Field records in Mozambique indicate that I-2 ND vaccine provides approximately 80 percent protections in the field in the face of an outbreak, when given every four months via eye-drop (Alders and Spradbrow, 2001). In the study by Nega *et al.,* (2012) mortality of chickens was reduced by 82% after vaccination of chicken for ND.

The I2 vaccine 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. I-2 vaccine produced in Mozambique will retain its activity for eight weeks at 28°C when freeze-dried and stored in the dark (Bensink And Spradbrow, 1999 and ACIAR, 1998).

A study results indicated that ND can be controlled in rural chickens effectively with the use of I-2 ND vaccine administered via eye drop. Since new chicks hatch almost every two to three months, revaccination of chickens at three-monthly intervals will ensure that these newly hatched chicks are protected against sporadic outbreaks of ND throughout the year (Awuni, *et al.,* 2004).

It is advisable to conduct a risk analysis of the options available as the basis for the selection process. The risk analysis will also form part of the vaccine registration process. This analysis should be done in sufficient detail for all stakeholders to understand the

risks and benefits associated with each option. The analysis will require more time and investigation in countries that opt to produce the ND vaccine locally. In countries where ND is endemic, the high mortalities associated with ND outbreaks will most likely indicate that the risks of not controlling the disease are far greater than the possible risks associated with a ND vaccine that is locally produced (Alders, 2002).

Proper cold chain should be used if available even if thermo-stable vaccines are transported and stored in the area. Freeze-dried vaccine stored at 4–8°C will retain high titer for a longer period than that stored at ambient temperature. At 4–8°C, the vaccine should maintain an adequate titer for at least one year. When taking the vaccine to the field, it should be placed in a cool box with ice or an ice pack. The vaccine should not be frozen (unless the instructions specifically indicate that it may be frozen). Freeze-dried vaccine packaged under vacuum rather than with nitrogen will lose the vacuum and gain moisture if the vial is frozen. The rubber cap on the vial contracts when frozen enabling moist air to enter the vial. When this occurs, the shelf life of the vaccine is reduced. These vaccines are thermostable, but attention to the conservation of the vaccine once removed from refrigeration will ensure optimal results. The vaccine should always be kept away from sunlight. When transporting the vaccine in the field, it should be wrapped in a damp cloth and carried in a covered open-weave basket, this allows evaporative cooling which helps to keep the vaccine cool and the cover prevents contact with sunlight, the date the vaccine leaves the cold chain should be recorded as it will remain effective for 2–3 months only, the vaccine should be stored in a cool, dark location (Adwar & Lukešová 2008, Alders *et al.*,1994).

2.6. Administration of Live Thermostable ND Vaccines

An adequate level of protection should be reached when administering thermostable Newcastle disease vaccine to chicken. To reach this protection level a standard dose must be applied to chicken (106 EID50/bird). EID50 (50 percent embryo infectious dose) is a laboratory measure of the content of living infectious virus in a vaccine. It has been demonstrated that birds that received a higher oral dose of thermo-stable vaccine

generated a higher immune response when confined in cages with wire floors (Alders *et al., 1994*). It has been demonstrated that birds that received a higher oral dose of the NDV4-HR vaccine generated a higher immune response when confined in cages with wire floors. The same report indicated that the dose responsiveness to oral vaccination was no longer apparent when groups of vaccinated chickens were housed together on litter. The explanation for this is that the vaccine virus replicated and excreted in the faeces and the birds then re-infected by the virus from the environment (Adwar & Lukešová 2008, Alders *et al.*, 1994)

But even though the thermostable vaccine can survive at ambient temperatures, attempts to improve its conservation will result in a slightly higher vaccine titer at the time of vaccination and consequently a higher and longer-lasting immunity. This is particularly important when birds are not housed together at night (Adwar & Lukešová 2008).

We have to use the right route, method and frequency of administration. An improper vaccine application is considered one of the most common reasons for vaccination programme failure. The choice of method will also depend upon other factors such as the type of production, bird species, and size of the flock, length of the production cycle, general health status, maternal immunity, vaccines to be applied, and costs (Marangon & Busani, 2006). But there is no difference in the administration of a vaccine from day-old chicks to adults.

The immune response induced by live ND vaccines increases as their pathogenicity increases. According to Alexander, 2000 in order to achieve an optimal level of protection without severe adverse reactions, vaccination programmes should include the sequential use of progressively more virulent live vaccine strains or live vaccines followed by inactivated vaccines (Marangon & Busani, 2006).

2.7. Improving the Potency of the Vaccine

120 white leghorn chickens primed with a lentogenic Newcastle disease (ND) live vaccine at 7 days of age were divided into three equal groups of 8 weeks of age and vaccinated with a live mesogenic ND vaccine (NDV). One group received only Newcastle disease mesogenic vaccine in normal saline, the second group received with groundnut oil as adjuvant and the third group received RDVK with liquid paraffin as adjuvant. Sera were collected at different time points for the assessment of antibody level against ND virus (NDV) by the haemagglutination inhibition (HI) test. The commonly used non-adjuvanted could not evince 100% protective HI titre beyond 11 weeks of age but in both the adjuvanted groups 100% protective HI titre was evident upto 20 weeks of age. On challenge at 20 weeks of age both the adjuvanted groups withstood challenge but in the non-adjuvanted group 80% of chickens withstood the challenge. A significant difference in immune response between the adjuvanted and non-adjuvanted groups was seen but not between both the adjuvanted groups. The advantage of vegetable oil (groundnut oil) as an adjuvant for live mesogenic ND vaccine has been discussed (Parimal *et al*, 1999).

In the study which tries to develop a single vaccination regime for long lasting protection of chicken from Newcastle disease. The result revealed that there is no significant difference among birds vaccinated with; killed-in-oil emulsion plus live virus, experimental vaccine plus live virus; killed-in-oil, live vaccine and oil emulsion and live virus. (George A. 2007 & Folitse *et al.*, 1998).

Thermo-stable vaccines may be diluted using locally available potable water. It is recommended that the water is boiled and left to cool overnight in a non-metallic container before use. Chlorinated tap water is unsuitable. If, however, this is the only water available, let the treated tap water stand overnight to allow the chlorine to dissipate or add one teaspoon of powdered milk per 10 litter of water to neutralise the effects of the chlorine. Once the freeze-dried vaccine has been diluted, it is advisable to follow a simple schedule for eye drop administration. If the diluted vaccine is used at the day of dilution

one drop is enough for a single bird but if it has to be used in the next day after dilution two drops must be used but the diluted vaccine is not effective after this; therefore storing it is not worth nothing (Dias *et al*., 2001).

The thermostable live ND vaccines spread from vaccinated to unvaccinated birds when housed together The degree of spread under field conditions is less when birds roost in trees and horizontal transmission should not be seen as a reliable substitute for vaccinating village birds (Alders and Spradbrow 2001, Catley *et al*, 2004, Bell., 2004, Awuni., *et al*, 2004).

Thermostable vaccines can be administered via eye-drop, drinking water, certain feeds and injection. The same dose is given to birds of all ages, from day-old chicks to adults. Different studies shows different vaccination level in different vaccination routes (Dias *et al., 2001*, Nasser *et al.,* 2000, Eidson and Kleven, 1976)

2.8. Comparison between Different Routes of Administration

Eye Drop Administration of Vaccine: In a research performed in Mozambique indicate that most farmers preferred to adminster thermostable vaccines intraocular even though it entails the capture of birds. In their opinion, eye-drop administration produces a greater survival rate, has a lower frequency of administration and is easy. It is effective because the vaccine passes through harderian gland which is very important in the immune response of chicken and also the vaccine guarantee administration to each individual bird (ACIAR, 1998). It is important that the eye-dropper used be made of virus-friendly plastic and that it is calibrated to ensure that one drop contains one dose. Calibration of the eye-dropper and administration of the eye-drop to the bird is done with the dropper in a vertical position to make sure that drops of a uniform size are produced. The vaccine should be administered once, with revaccination every 3–4 months (Adwar & Lukešová 2008).

Administration via Water: It is the easiest means of vaccine administration but it should be given twice in two weeks interval and booster vaccination at least every three months (Adwar & Lukešová 2008, ACIAR, 1998) because, it provokes lower immunity. If it is in a rural setup it is advisable to give the vaccine early in the morning when birds released from their home (night shelter) (ACIAR, 1998).

Administration via Feed: This route of administration is mainly performed at remote areas with poor veterinary service using thermostable vaccines. The procedure needs selection of suitable grain that channel the vaccine fine. The calculated dose is 7-8 gram of selected grain will moist with 1 ml of the vaccine for a single bird. This kind of vaccination should be given twice (ACIAR, 1998).

2.9. Advantage of Live Thermostable Vaccines

There are some characterstics which make live thermostable vaccines to have advantage over inactivated vaccines. The common characterstics are (Awuni *et al.* J.A., 2004)

- it is cheap and affordable
- It need no cold chain for transportation, therefore it is vaccine of choice for rural community,
- It is being produced locally and can thus be made readily available to farmers at their convenience
- Since it need no skill it can be given by the farmers and this will increase the total return

2.10. Safety of Thermostable Vaccine

The avirulent live ND vaccines (I-2 and NDV4-HR) are not administered an overdose. They are harmless to both bird and handler. Field records indicate that the I-2 ND vaccine provides approximately 80% protection in the field in the face of an outbreak, when given every 4 months via eye drop (Marangon & Busani, 2006). Both the I-2 and NDV4-HR vaccines produce no evidence of clinical respiratory signs, weight loss, and mortality

in young chickens or egg production drop after vaccination. The safety performance of the original V4 (avirulent) vaccine is superior to both the HB1 (lentogenic) and La Sota (mesogenic) vaccine strains (Adwar & Lukešová 2008 and http://www-naweb.iaea.org/nafa/aph/public/16-strategies-a.pdf).

2.11. Cost/Benefit Analysis

In most cases where farmers are to contribute wholly or partially to the cost of the vaccine, the price of the vaccine will be a major factor. The lower the 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. Locally produced freeze-dried I-2 ND vaccine is usually cheaper than imported freeze-dried live and inactivated thermostable vaccines, but it is more expensive than the "wet" vaccine. The freeze-drying process, the special vials, caps and labels all increase the price of the vaccine. However, freeze-dried vaccine does have a longer shelf life than "wet" vaccine (Marangon & Busani, 2006 and Alders *et al*, 1994).

Cost/benefit analysis is the most important component of vaccination program which is performed before implementing it to the given locality. Vaccination program costs include the costs of vaccines, vaccine delivery, monitoring, laboratory testing, and all other related activities. The lower price of the locally produced vaccine (particularly the "wet" I-2 vaccine) will increase the number of birds that can be vaccinated with the funds available. In addition, locally produced vaccine requires much less foreign exchange (Marangon & Busani, 2006 and Alders *et al.,* 1994).

In a study conducted in eleven countries which consider ND vaccination as intervention. The intervention brought high return on investment. The total profit gained in the study areas (in USD) was 0.18-10 in Cameron, 33.46 in Ivory COST, 67 in Ghana, 2326 in Tanzania, 11093-1534 in Uganda, 642- 2190 in Sudan, 369 in Kenya, 10.1 in Madagascar, 140 in Morocco, 51-70 in Mauritius. The net return is high in some countries in which farmers are trained to administer the vaccine by themselves (Klos R.

et al., 2004). This shows that the cost related with vaccination can be compensated with the higher investement return rate.

2.12. Vaccination Failure

Vaccination failure is suspected when clinical disease occurred or altered productivity or unusual vaccine reactions. Vaccinated birds are also getting diseased when conataminated vaccine is used to immunize birds. Therefore, good manufacturing practices should ensure that vaccines are highly unlikely to be carriers of virulent ND virus (FAO, 2004). There are some factors related with vaccination failure. The type of production system commercial poultry sector has a significant effect on disease prevention and control (Marangon & Busani, 2006). To mention some reasons which cause vaccination failure:

The Vaccine Itself: These are factors related with the vaccine and the other inter-related components of the vaccine. A vaccine of moderate-to-poor titer may give satisfactory results if very carefully applied, while it may be a disaster if poorly applied (Nasser *et al.,* 2000, Paul, 1985).

Vaccination is said to be good if the titter produced in response to the vaccine is protective and stable for the targeted period of time. Live virus vaccines are known of producing adequate titter but the stability is affected by the success of lyophilization and the temperature under which it is stored. Periods of validity must me strictly followed, or the vaccine re-titrated (Paul, 1985).

The other factor related with the vaccine is the serotype and the biotype of the virus used in the production of the vaccine (Naqi S.A., *et al*., 1980). The biological characteristics or strains used in live-virus vaccines have a great influence on the process of immunization (Paul, 1985). As general rule, the greater the invasiveness of an organism the greater will be the immunity produced.

Inactivation of the virus and the adjuvant used in the vaccine production process is very important. These factors have similar importance for inactivated vaccines as do liophilization and titre for live vaccine. Type and quality of emulsion can influence the serological response to oil-adjuvant vaccines.

Administration of the vaccine The Vaccine Itself: Unvaccination is the major cause of vaccination failure (Paul, 1985). Also the routes of vaccination affect the outcome of the vaccine. When intra-muscular and aerosol vaccination are compared for Newcastle disease vaccination, live aerosol vaccination is very effective than inactivated intra-muscular routes (Beard & Easterday, 1967, Winterfield *et al*., 1980). But in case of day old vaccination using mild strain from non immune mild vaccine strain it can simply kill the chicks.

The other factor to consider in mass administration of vaccine to a flock is its uniformity of the flock. Even with individual application, problems of uniformity of application can occur due to poorly adjusted vaccinating materials. Live vaccines which allow some lateral spread of the immunizing virus among birds reduce the necessity for uniformity at time of application.

Administration of recombinant vaccine may affect the response of the ND virus vaccine, especially when they contain viruses which have the same target tissues as ND (Paul, 1985).

Immunization against infectious disease is rarely dependent on a single inoculation; but most vaccination needs multiple administration of the vaccine. The importance of the vaccination program lies in the immunological phenomenun called the "anamnestic response". This refers to the ability of the lymphoid tissues to recognize and respond to antigens to which they have already been exposed. This response is usually more prompt and greater than the response which occurred when the bird was first exposed. The diluent used for live virus vaccines is very important to ensure that an adequate titre of virus actually reaches the birds.

Factors Related with the Bird: Previous exposure status of the bird to the virus and passive protection may affect the response to vaccination. Passive immunity comes about in two ways: the first is through hyper immune sera and the second one is transmitted from the breeder bird to her chick via the yolk and protects the chicks until the age between 14 - 30 days. The passive immunity passes from maternal immunity to baby chicks can influence the response to vaccination (Nasser *et al.*, 2000, Paul, 1985 and ACIAR, 1998).

If a vaccine fails to fully protect against a disease because the birds were infected prior to or soon after vaccination this is only an apparent vaccine failure (Paul, 1985). Stress of any sort is well known to reduce disease resistance and can also be expected to affect response to vaccination. Disease conditions such as infectious bursal (Gumboro) disease virus, Chick Anaemia Virus and Marek's disease virus are known of their immune-compromization (Muskett *et al*., 1979).

In general vaccination failure is the major problem in many part of the world. The measures used in the measurement of the efficacy of the vaccine affect an apparent variation in response to vaccination is simply due to variation in the method should be kept in mind (Paul, 1985). Also vaccines cannot reasonably be expected to project 100% of the flock under commercial poultry conditions. The actual protection obtained will be determined by the sum of all the factors which can affect vaccine efficacy (Paul, 1985).

3. MATERIALS AND METHODS

3.1 Experimental Trial

3.1.1 Experimental Study Area

This experiment was conducted at Debre Ziet Agricultural Research Center (DZARC) which is located 1850 meters above sea level with an annual average rain fall of 800mm and annual average temperature ranges from 12.3^{o}C to 27.7^{o}C (CSA, 2008).

3.1.2 Setting and Hatching Chicken

The experiment used 190 local (Horo ecotype) and 295 KoeKoeK (South African breed) one day old chicks. The day old chicks were hatched at DZARC from fertile eggs collected for seven days from both breeds. The eggs collected each day were stored in a cool egg storage room until setting. Proper disinfection procedure was carried out prior to setting. The eggs are transferred to hatchery unit at day 18 and DOC was collected at day 22.

3.1.3 Management of Experimental House

Separate pens were used for all treatment groups and control. The experimental house and pens were thoroughly washed with water and sprayed with 10% of formalin. After drying, clean new litter was spread over the floor. Equipments including waterier, feeders was cleaned, disinfected and introduced to the house.

3.1.4 Management of Chicken

During brooding the room and brooder temperature was maintained with a source of 250 watt infrared bulb per treatment group. Clean water and formulated feed was provided

according to their requirement at their stage of development. The feed was formulated and prepared at the research center. The chickens were visited regularly.

3.1.5 Experimental Design

Ten sampled chicks were sacrificed and serum sample was collected at day one from both breeds. Blood sample was also collected again from 10 chicks at day 14 and 20 from both breed in order to get information on the level of maternal antibody transferred to the baby chicks. The remaining 180 chicks from local (indigenous) chicken and 285 chicks from koekoek breed were divided in to 15 equal groups. A single treatment has three replications. In this setup 4 treatments and 1 control were used. The parboiled barley treated with thermo-stable I2 vaccine (Nasser. *et al.,* 2010) was used as a reference. The sample size per treatment was calculated based on RCT sample size calculation (Chan 2003). Management of chicken was similar in all treatments.

Table1. Experimental setup for I2 ND vaccination trial and viral challenge.

Treatment	Breed	No. chickens vaccinated	No. of the vaccinal viral dose	Interval of vaccination*	No. chickens challenged	No. of the challenge viral dose, IM route
Eye-drop	Horro	36	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
	Koekeok	57	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
Water	Horro	36	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
	Koekeok	57	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
Feed	Horro	36	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
	Koekeok	57	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
Spray	Horro	36	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
	Koekeok	57	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
Naive	Horro	36	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)
	Koekeok	57	10^6	Day 21, 36	10	0.4ml of (10^9HA unit)

* two weeks interval

3.1.6 Vaccination

The experiment uses different route of vaccination on the four treatment groups. The treatments mentioned below were given twice in 15 days interval at day 21 and 36. The vaccine was purchased from NVI. The vaccination was carried out after 3 weeks of age to override effect of maternal immunity (Nasser. *et al.,* 2010).

Eye vaccination: Eye vaccination of chicken is set using a standard dose calculation for a one eye one drop vaccination dose calculation (NVI manual). The vaccine was then administered to the chicks in one eye using a sterile pastor pipette by catching individual chicks.

Water vaccination: A water vaccination is given to chicken using distilled water. The chicks were kept without water for 2.5 hours prior to vaccine administration. A dose calculation is used for the vaccine administration is set by calculating 10 ml per bird in the first vaccination and 20 ml per bird in the second vaccination. The dose calculation is based on the manual by NVI.

Feed vaccination: Parboiled barley preparation was adopted from Nasser *et al.* (2010). Parboiled barley preparation was adopted from Nasser *et. al.,* (2000). One kg of grain is added to 1.75 litres of boiling water and left for 5 minutes. It was cooled using water. The grain was sun dried. It was cracked it manually. Then 1 kg with 4 liters of water twice in a day and leave it soaked overnight. Then dried it using sunlight and use it for the treatment. The prepared barley was then sprayed using a fine sprayer in the ratio of 1 ml per 10 gram of grain. The feed was given to the birds by calculating 10 gram of feed per bird. This shows that the dose of the virus required for a single bird was calculated per 1 ml of the reconstituted vaccine.

Litter spray vaccination: I2 vaccine was used to spray the litter where experimental chicken were kept. A 1 ml per bird ratio was used in each breed. The vaccine was bought from national veterinary institute (NVI).

3.1.7 Serum Collection

1-2 ml of blood was collected from experimental chicken at each bleeding days. Blood sample was collected by sacrificing in DOC but jugular vein and brachial vein was used to take blood using 23-gauge needle after disinfecting the site with cotton soaked in 70% ethanol. The whole blood collected from chickens was labeled and allowed to clot under normal atmospheric condition in the syringe. Then, the clear serum was harvested into labeled cryovials and stored at -20°C until HI test carried out. Blood sample was collected at day 1, 14, 20, 36, 44, 51, 58, 65 and 82. The bleeding at day 1, 14 and 20 were before the first vaccination as the 1^{st} vaccination was given at day 21. The bleeding at day 36 was immediately before 2^{nd} vaccination on the same day. The bleding on day 44, 51, 58 and 65 was after 2^{nd} vaccination. The challenge virus was administered at day 65. Post challenge bleeding was done on survivors of the deadly velogenic viral challenge.

3.1.8 Haemagglutination Inhibition Test

The collected sera at pre and post vaccination and post challenge were tested to monitor the level of antibody in the body using heamagglutination inhibition test. The test was performed following the method described in OIE (2009) manual for hemaglutination and inhibition test and the protocol of national veterinary institute (NVI). The antibody level for each serum sample will be recorded using well designed recording sheet.

3.1.9 Source of Virus

Wild virus was collected from chicken embryo at NVI vaccine quality laboratory. The wild virus which collected from Haromaya by NVI was tested for haem-agglutination before administration to the birds in order to check the level of its potency.

3.1.10 Challenge with Virulent Field Virus

Ten chickens from each treatment was isolated and challenged three weeks (at day 65) after the second vaccination with wild strain of ND virus. The challenge viral dose was in accordance with the work of Darminto and Daniels (1992) and Khalafall *et. al.,* (2004). The virus was given via Intra Muscular route in the breast muscle (Khalafall *et. al.,* 2004) and (Nasser *et al.,* 2010). The birds were kept under close observation for 15 days. Numbers of dead and live birds was recorded.

Standard bio-security measures like restriction of movement, proper disinfection and disposal of dead chicken were implemented in-order to prevent the spread of disease to other flock in the research center.

3.1.11 Pathogenicity Index Measurement

The pathogenicity index for the challenge virus was measured using tools adopted from Tizard, 2004. Pathogenic index is a tool to evaluate the protection period of the vaccine from the disease outcome. The pathogenic index of the vaccine in the five treatments was measured. The pathogenic index was set based on the time taken until an event is occurred in individual animal. To follow individual chicken each chicken were wing tagged. Then the chickens were followed for 15 days and occurrence of an event is recorded. For pathogenic index measurement for categories were used according to Tizard (2004) and Mishra *et al.,* (2001). Category 0was given to the chicken whent there was no any clinical signs; 1 for inappetence and depression, 2 for discharges and nervous signs, and 3 for dead chickens.

3.2 Survey

3.2.1 Study Area

The survey was conducted in Mecha Woreda; west Gojam Zone. The area is located 1900 meter above sea level. According to CSA 2013 the area is characterized by population of 287,459. The zonal poultry population is around 2, 285, 5 4 2 chicken. The survey mainly focuses on the production system, disease status and risk factors which predispose chicken for ND. The area purposely selected for its potential in poultry production and also characterization of chicken is well done. It also reported that chicken in the study area are known for their growth potential and better weight gain (Halima *et al.*, 2006). The area is also known of its untouchable population genetic potential with more than 93% genetic heterozygosity (Halima *et al.*, 2009). It is also reported that ND as potential trait for poultry production. Therefore this paper will fill the gaps of those outputs in the area of chicken health and risk factors which predispose chicken to disease.

3.2.2 Household selection

A total of three representative peasant associations (PA) were selected randomly from seven markets shed PA's. The sampling technique for the seven PA put in consideration chicken production potential and road accessibility. Farmers list was taken from the three PA's and households are selected randomly from the list without prior information about the household. Simple random sampling technique was applied to draw 32 households from each PA. A total of 96 village chicken owner households were interviewed using a pre-tested structured questionnaire.

3.2.3 Field survey and serology

Structured questioner was developed (annex. 1) to gather information on current status of poultry production system and ND situation in the area. It also tries to cover vaccination and bio-security status of village (traditional) chicken and the effect of ND in the

production system. Serum samples of 65 were also collected from village chicken in the households and markets. The collected serum samples were screened for HI to determine status of Newcastle disease in the area.

3.3 Data analysis

The data collected from different sources (experiment and questioner survey) was summarized using Microsoft Excel and analyzed using Microsoft Excel and SPSS version 20. Descriptive statistics was used for computing all the parameters for the survey data. Cox regression survival analysis was used to analyze the differences in the survival rate between treatment groups and analysis of variance was used to compete between breeds and treatment variation in HI titter and protection.

4. RESULTS

4.1 Survey result

4.1.1 Household demography

The average family size of participant farmers was 5.2 of which 52.5% were male and 47.5% were females. Forty percent of the household members were children under the age of 15.

Table. 2. Respondent household characteristics

	Frequency	Percent
Age group participated		
20-40	38	39.6
41-60	54	56.3
above 60	4	4.2
Sex of respondent		
Male	58	59.4
Female	38	39.6
Educational status		
Illiterate	48	50
Read and write	18	18.8
Primary education	26	27.1
Secondary education	4	4.2
Main Occupation		
Farming	86	89.6
Other	10	10.4
Land Size Owned		
No land	8	8.3
Less than one hectare	68	70.9
More than one hectare	20	20.8

4.1.2 Importance of poultry production in the study area

The family use incomes from live chicken and egg sell for children school needs, cloth and other house hold inputs such as buying salt, coffee and other small household inputs. The income from selling of chicken and egg is also used for social obligations; like "Ikub" and "Edir".

Table 3. Ranking of purpose of poultry production

Purpose of poultry production	Primary	Secondary	Tertiary
Consumption purpose	42 (43.8%)	49 (51%)	5 (5.2%)
Household supplementation	45 (46.9%)	38 (39.6%)	0
Income purpose	9 (9.4%)	1 (1%)	1(1%)

4.1.3 Production system

The production system is of three types (Table 4.). The type of supplement is mainly of a single grain base (maize or finger millet or barley). Sometimes the chicken might provide with house leftovers and other grains when available.

Table. 4. Types of production systems in the study area

Production Sytem	Frequency	Percent
Scavenging	3	3.1
Conditional Supplemenation	40	41.6
Regular Supplementation	53	55.2

4.1.4 Chicken Housing

The problem of predators, fear of theft and lack of experience were the main reasons for not constructing separate poultry houses.

Table 5. Chicken housing and house cleaning practice

Poultry housing	**Frequency**	**Percent**
Separate	12	12.5
With human	74	77.1
With other animal	8	8.3
Other	2	2.1
house cleaning		
Daily	63	65.6
In 2 or 3 days interval	15	15.7
Weekly	6	6.2
Not at all	12	12.5

4.1.5 Flock Structure and Dynamics

The production system in the study area is mainly composed of local chicken. Local chicken were comprise 99% of the total poultry population in the households. The exotic and cross breed chicken are only one percent of the flock.

Table. 6. Flock characteristics

	Frequency	Percent
Base Stock		
Purchased	88	91.6
Other source	8	8.4
Source of Current Stock		
Own	79	82.3
Purchase	11	11.5
Other	6	6.2
Maximum flock size		
10 to 20	57	59.4
21 to 30	24	25
above 30	17	17.3

As it comprises 99% of the total chicken population the average age groups of local chicken is displayed in the table below.

Table. 7. Flock structure in the households.

	N	Mean	Std. Deviation
Local chicks	96	6.26	7.095
Local grower	96	2.05	4.368
Local pullet	96	1.10	2.034
Local layer	95	2.31	1.407
Local cock	96	1.06	1.304

Farmers report that the flock structure dynamicity as decreasing, increasing and remain unchanged. They also set some reasons for the decreasing trend on chicken population (Table 8)

Table 8. Cause of chicken loss in the study area during two years.

Flock dynamic	N	Frequency	Percent
Increasing in population	96	35	34.5
Remain unchanged in number	96	2	
Decreasing in population	96	61	63.5
Reason for decreasing in chickens			
Disease related loss	61	32	52.5
Disease and predator combined	61	19	31.1
Selling	61	6	9.8
Others	61	4	6.6
Total		61	100.0

4.1.6 Loss and seasonality of ND

Newcastle Disease outbreak is varying with season in all three Peasant associations (Table.9).

Table 9. Number of chicken lost in the households and ND seasonality

	Frequency	**Percent**
Loss Due To Fengil		
nothing	11	11.5
1 to 10 chickens	53	55.2
11 to 20 chickens	28	29.1
above 20 chickens	4	4.2

(Continued)		
ND seasonality		
End of rainy season	15	15.6
Beginning of rainy season	78	81.3
Any time of the year	3	3.1

19.8% of the respondents do not have an idea to which the disease outbreak related with ND occur in their chicken while 38.8 percent of the respondents think the disease is mainly transmitted from other chicken by contact. 11.5% of the farmers respond that the disease is transmitted by dogs that eat dead birds in the neighborhood during the time of an outbreak. Among the respondents 11.5% of them considered cleaning as a major cause of disease. Newly introduced chicken, market and human activities are also mentioned by the farmers as a potential means of disease transmit ion.

Table 10. Comparison of loss related with fengil in the three PA

(I) PA	(J) PA	Mean	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Ambomesk	Lehulu selam	6.75	-2.156	1.844	.736	-6.65	2.34
Rim	Ambomesk	8.91	3.656	1.844	.151	-.84	8.15
	Lehuluselam	10.41	1.500	1.844	1.000	-3.00	6.00

The use of traditional medicine and traditional practices to treat ND (Fengil) is less than 35% in the survey. The most widely used traditional medicines were (Semiza), Feto, Holly water and kerosene. From the response of 73.6% of the respondent farmers practicing traditional treatment the result was not satisfactory. Oozing a blood from brachial vein of the wing is the other most practice traditional practice. The effectiveness of this practice is claimed effective, but it need experienced person to perform it.

Table 11. Outbreak of ND and age group in risk

	Yes (%)	No (%)
Fengil Outbreak In The Last Two Years	83 (86.5)	13 (13.5)
Risk Of Age For ND		
Risk Pullet	16 (16.7)	80 (83.3)
Risk Baby Chicks	65 (67.7)	31 (32.3)
Risk Grower	17 (17.7)	79 (82.3)
Risk In Broody Hens	69 (71.9)	27 (28.1)

4.1.7 Bio-security and vaccination

The production system is characterized by mixed type of production. The response of farmers with relation to measures taken by them is shown in table (12)

Table 12. technology use and bio-security measures in the study area

Factors	**Yes (%)**	**No (%)**
Use any Technology	15(15.6)	81 (84.4)
Seasonality Of ND	93 (96.9)	3 (3.1)
Having Own Cock	35 (36.5)	61 (63.5)
Vaccinating Chicken	1 (1.0)	95 (99.0)
Taking Any Training	9 (9.4)	87 (90.6)
Contact With Chicken In The Neighbourhood	89 (92.7)	7 (7.3)
Contact With Other Animals	85 (88.5)	11 (11.5)

Table. 13. Village biosecurity measures status

	Frequency	Percent (%)
Measures Taken To Prevent Contact With Other Chickens		
Fencing	1	14.3
Housing	4	57.1
Other	2	28.6
Watering Material Cleaning		
Daily	42	43.8
Weekly	9	9.3
Not At All	45	46.9
Materials Used To Clean		
Nothing	45	46.9
Soap	3	3.1
Water	47	49.0
Disposal Of Dead Chicken		
In The Farm	1	1.0
Outside The Farm	31	32.3
Road Side	12	12.5
Abysm	48	50
Toliet	3	3.1

4.1.8 Characteristics of Newcastle disease

Farmers characterize Newcastle disease in the three villages with similar features but with different local names for different age groups. The call it “Engulch” in case of baby chicks but the name is changed to “Wetete” in adults. Chicks with “Engulch” can only survive for shorter time while “Wetete” gives some time for adult birds. Depression, dropping of wing, yellowish to green diarrhea and death are the common features

observed by most farmers while swelling around the eye, poor shell quality of the egg and nervous signs were observed only by few.

4.1.9 Screening Test

ND screening testing used in this study is based on haem-aglutination inhibition test. The study performed the screening test targeting the outbreak season. The test is set only to test an antibody for the disease. Screening test was performed in 65 apparently healthy chickens which are set to market in the area where an outbreak of the disease was seen.

The screening test show that 33.8 percent of the birds screened for Newcastle disease are showing no protective antibody in the body while the remaining 66.2% of the chicken have protective antibody titter. The chicken that are not taking any vaccination for ND in the past six month that are showing protective antibody for ND is considered as actively infected for the disease in the past few weeks.

Table 14. Screening test result of chickens (n = 65) from the three PA

Antibody titter	Frequency	Percent	Cum Percent	
0	19	29.2%	29.2%	
1:4	2	3.1%	32.3%	
1:8	1	1.5%	33.8%	
1:32	15	23.1%	56.9%	
1:64	11	16.9%	73.8%	
1:128	13	20.0%	93.8%	
1:256	4	6.2%	100.0%	

4.2. Experimental result

4.2.1 Hi titter in experimental animals

The result shows that there is no a significant difference in the antibody response between breeds. Local chicken (Horo ecotype) and Koekoek chicken has no a significant difference in protection (Table 15).

Table 15. Protective HI titter between two breeds

	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	7.202	1	7.202	2.257	.134
Within Groups	1333.795	418	3.191		
Total	1340.998	419			

Significantly higher HI titter of chicken in ocular and spray than in feed and water and the latter, in turn, differ significantly than the control group (Table 16).

Table 16. HI titters between treatments after vaccination

Treatment (1)	Treatment (2)	Mean Difference (1-2)	Std. Error	Sig.
Ocular	Water	18.889*	5.157	.003
	Feed	18.444*	5.157	.004
Water	Feed	-.444	5.157	1.000
Spray	Ocular	-3.056	5.157	1.000
	Water	15.389*	5.157	.030
	Feed	15.833*	5.157	.023
	Naïve	43.341*	4.969	.000
Naïve	Ocular	-46.397*	4.969	.000
	Water	-27.508*	4.969	.000
	Feed	-27.952*	4.969	.000

4.2.2. Pathogenic index

There is no significant difference (P= 0.82) in the pathogenic index between breeds but there is significant difference between treatments. The result of the experiment shows that control groups were the first in exhibiting the disease outcome in shorter time than the other four treatments.

Table 17. Mean Pathogenic Index of chickens under each treatment

Treatment	Mean	N	Std. Deviation
Ocular	0.18	20	.554
Water	0.74	20	1.122
Feed	1.35	20	1.105
Spray	0.42	20	.860
Naïve	2.43	20	.233
Total	1.02	100	1.159

Chickens in all treatments have significantly lower pathogenic index than that of chicken in control group. The pathogenic index is not significantly different between birds' vaccinated using spray, water and ocular route of vaccination but it is significantly lower in chicken vaccinated with barley (Table. 18)

The pathogenic index of the challenge virus in the four treatment groups is not significantly different in the two breeds of chicken. This shows that the breed effect on the pathogencity of the disease is not significant.

Table 18. Pathogenic index difference of different treatments

(I) Rx	(J) Rx	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
water	Eye	.559	.268	.234	-.19	1.30
Feed	Eye	1.167*	.268	.000	.42	1.91
	Water	.609	.268	.163	-.14	1.35
Spray	Eye	.236	.268	.903	-.51	.98
	Water	-.322	.268	.748	-1.07	.42
	Feed	-.931*	.268	.007	-1.68	-.19
Control	Eye	2.250*	.268	.000	1.51	2.99
	Water	1.691*	.268	.000	.95	2.44
	Feed	1.083*	.268	.001	.34	1.83
	Spray	2.014*	.268	.000	1.27	2.76

4.2.3 Survival rate of chicken after challenge

The experiment uses a wild strain of Newcastle disease virus to test the difference between the HI protection titter with relation to the simulated wild outbreak condition. The result from this study revealed that the survival rate of the chicken after viral challenge with wild ND virus higher in all treatments than the control group.

The result from this experiment shows that chicken under the four treatments have better survival time than that of chickens under control group in each breeds, but between breed difference in the survival of the field challenge is not significantly different (P=0.6).

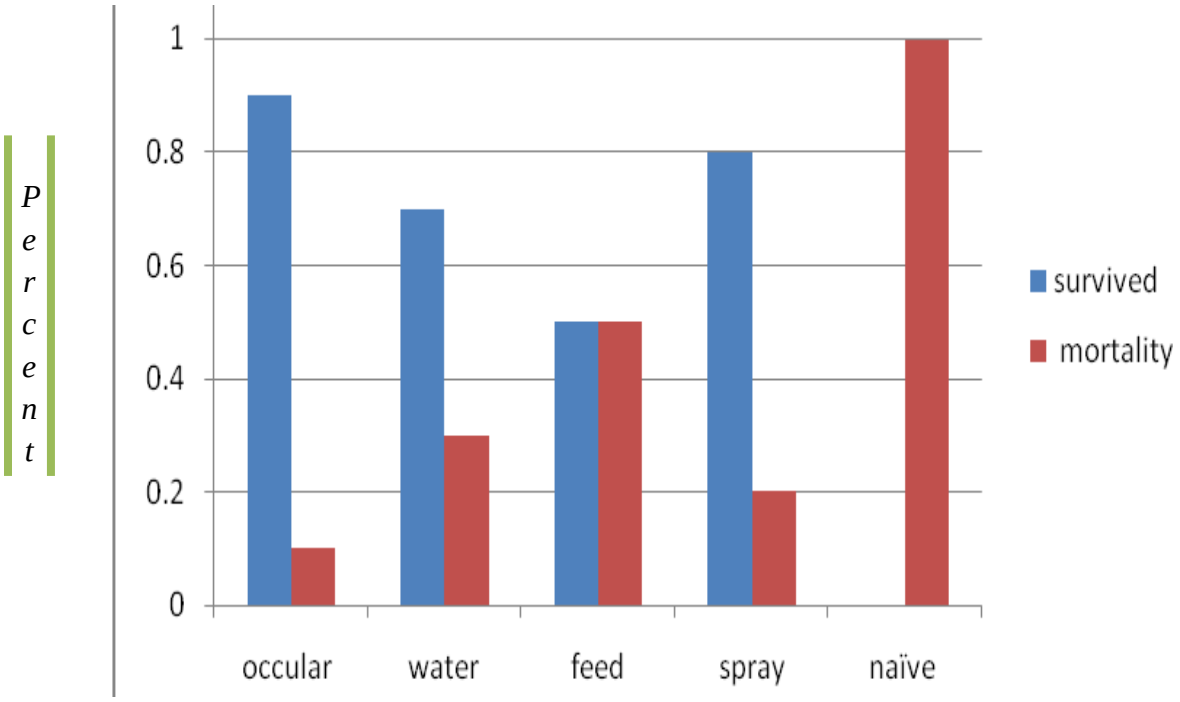

Fig. 1. Post challenge survival (percent) of chicken in the five treatment groups

Survival probability of chicken in different treatments groups is measured using mortality probability of chicken with relation to their HI titter.

4.2.4. Relationship between mean HI titer and mortality of experimental chickens

The picture below shows the mean titter of HI for the four treatments with relation to the control group is high. The result shows that the loss related with Newcastle disease in unprotected flock was up to 100%.

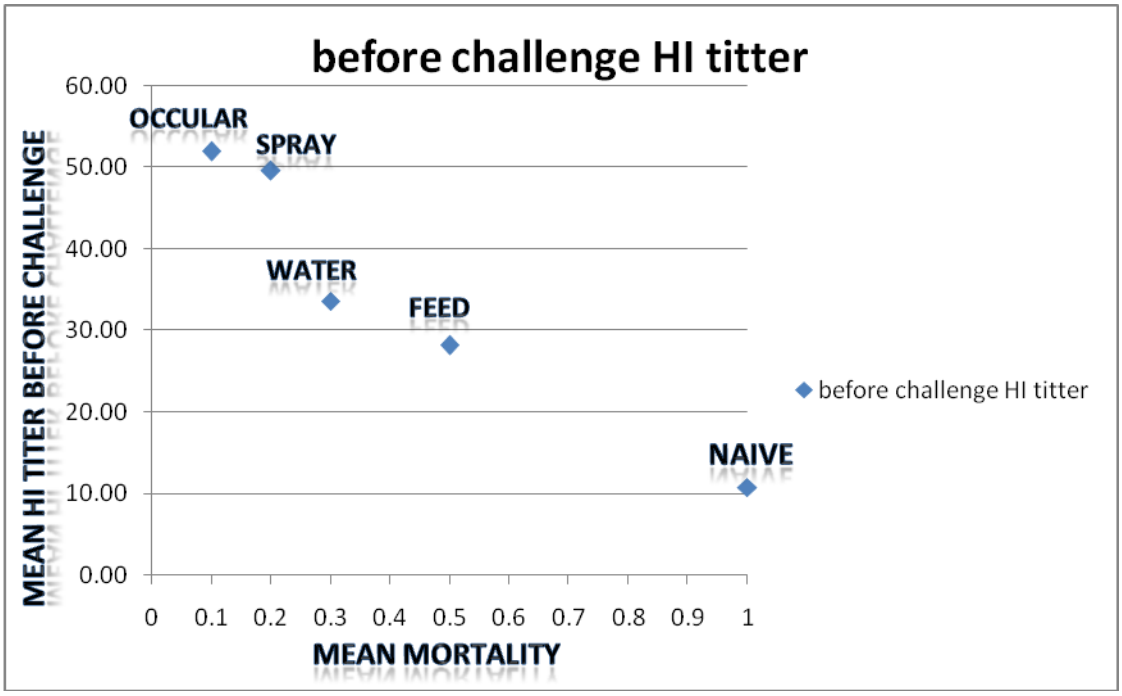

Fig. 2. Mean HI titter before the challenge

The survival time of chicken under the experiment challenged with wild strain of ND is lower in chicken under control group while it is longer in chicken in chickens under ocular and spray route of vaccination. Survival rate of chickens which takes vaccine through vaccine treated feed is relatively lower than other treatment groups.

The survival curve showed that more than 75% of the chicken in the ocular, spray and water treatment groups survived the mortality. The survival rate of chicken in the control treatment was zero percent after 8 days post challenge (Figure 3).

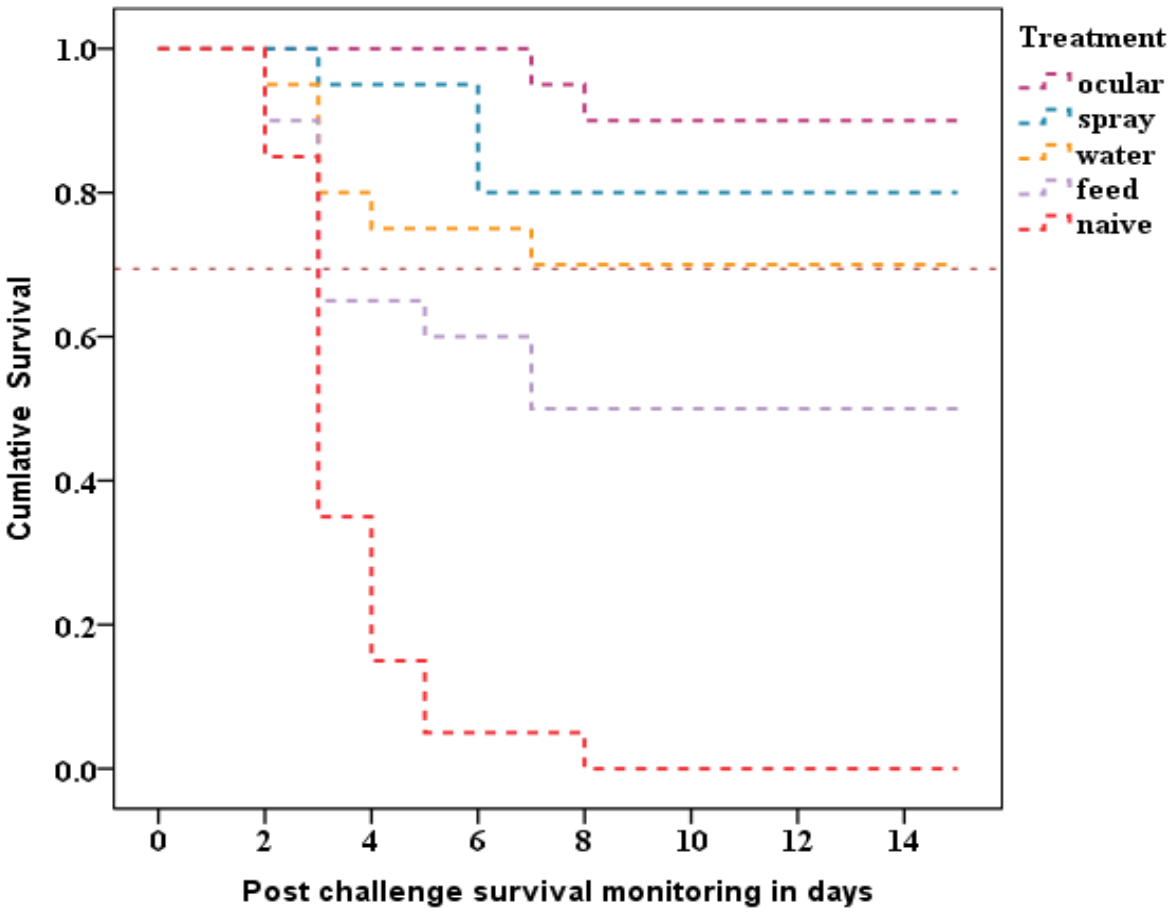

Fig.3. Post challenge survivality of chicken

5. DISCUSSION

5.2. Survey

Farmers participated in the survey were well experienced in poultry production since it is the initial investment applied by many households. The number of chicken owned by farmers was varying between male and female headed households. This might be related with distribution of labor. Single mom's having huge responsibility in both in farm and out farm activities therefore the number of chicken that they keep might be limited. The other attributer is economic position of female-headed households which force them to limit their flock size. This is in agreement with project of (FAO) in Afghanistan.

The number of younger generation is higher than that of other age groups in the community, but the cultivated land that is owned by farmers is very small and it is getting decreased from time to time (Derek *et. al.,* 2013). Therefore leaning livelihood on crop production is not sustainable for the newly emerging generations. Improved family production is a means that can be performed in small land and generate income. Therefore improved poultry farming is a means to improvement of the livelihood of farmers by alleviating poverty and increasing income resource for resource poor farmers (FAO, 1998) (Livestock master plan, 2015. Unpublished).

Pure scavenging, scavenging with conditional (seasonal) supplementation and scavenging with regular supplementation production system are the three most common poultry production systems in the area. This production system are known for their poor productivity and used only for home consumption and contribute few for house hold expenditures. As it is reported by Samson and Endalew, (2010) in mid rift valley of Oromia, Ethiopia the poorer intention of farmers towards poultry production is the main reason for the lower productivity of the chicken. Meager extension system and training services which are provided by experts to improve the productivity and management system is the other main factors which hinder poultry production in scavenging production system which is similar to (Dana *et al.,* 2006).

Raising improved chicken in the area is not a basic requirement by participant farmers. This is mainly due to the susceptibility of the chicken to the disease and predator. This disease is mainly related poor disease prevention, control and poor nutrition. In both conditional and regular feed supplementation of feed the type of supplementary feed is not based on the requirement of the chicken rather it base only on the type of available grain in the house. Supplementing chicken with per their requirement is important to improve production performance and improve disease resistance of chicken. A study by Kondombo, (2005) in Burkina Faso shows that the productivity and survival rate of local chicken is improve significantly when supplementation of concentrated feed was in practice. Therefore to improve production and survival of chickens under village system towards improved family poultry the technology should be go with improved management and disease prevention and control technologies.

Housing facilities in the surveyed area were baskets for chicks and other locally available materials (mainly wood from eucalyptus tree) aiming at keeping the birds at night. These facilities were located on the floor or in the rafter space within the residence. Housing is essential to chickens as it protects them against predators, theft, rough weather (rain, sun, cold wind, dropping night temperatures) and to provide shelter for egg laying and broody hen. Confinement of chicken during disease outbreak is an important means of disease prevention and control. The confinement of scavenging chicken without providing an adequate amount and quality of water and feed will not guarantee disease prevention and control as well as productivity (Kondombo, 2005). In combating of Newcastle disease in village production system housing of chicken at the time of an outbreak and proper provision of feed might be considered as disease prevention methods. Farmers in the study area set some reasons not to construct separate housing for their chicken. This can be solved by proper training on the advantages of housing and in the methodology of constructing poultry houses easily from locally available material (DZARC training manual). Constructing house will help farmers to change the scenario in the village system.

There are different reasons for the decreased flock sizes in most households participate in the study. The Decreasing flock dynamics is mainly related with disease and predator. This is in agreement with (Tadelle, *et al*., 2003). But there are other reasons which contribute to the decreased flock dynamics in households participated in the study. Culling of chicken when they are becomes source of conflict between neighbors and cause damage to the vegetations in the backyard was also mentioned as a reason for unwanted sell. Giving more attention to other farm activities that are believed to fetch more cash might lead to less productivity. Although disease and predator are set as a threat to the sector in villages of the study area 69.8% of the farmers use their own flock as a base for the current flock in hand. The remaining chicken owners get the chicken from purchase, gift or markets. These were mentioned by some farmers as the main means of ND (Fengil) transmission.

The result also shows, the traditional family poultry system in the study area is mainly comprises of young chickens (baby chicks and growers). The number of pullets and cocks was very small. Newcastle disease is a disease of chicken of all age groups (OIE, 2013), but the susceptibility of the chicken for the disease outcome might vary between different age groups. In this study the susceptibility of baby chicks' growers and broody hens to ND is higher than that of other age groups. This might be related to the lower immune status of the chicken at this particular age. The lower immunity of these chickens might be related to the content of the food they consume is used for growth and development by their body. A relatively long survival time of other age groups give farmers' time to consume chicken with mild clinical signs and also to sell chicken with apparently healthy condition. These measures are not recommended as a control and prevention measure of ND, because the sold chicken might be source of ND in the area where are introduced.

The incidence of Newcastle disease is higher at the end of dry season and at the end of rainy season. The result of this study is in agreement with studies by (Komboi *et al.,* 2013) and (Njag *etal,* 2010) who report that the occurrence of ND (Fengil) is higher in dry season than that of wet season. Therefore seasonal prevention and control strategy is

needed for proper prevention and control of disease. Contact of chickens in the neighborhood is the main cause of disease transfer. Contagious disease can also be introduced to farm by contact with other animals (OIE, 2013). Even if farmers know the season and were experienced enough for long time in poultry production they are unable to control the ND in their farm. This shows that having an experience on village production system is not a guarantee to control or prevent ND in their flock.

Isolation of sick chicken when they manifest a sign of disease is the main measure recommended by most professionals to prevent a spread of disease in a given flock (OIE, 2012). The applicability of this control strategy in the study area is only on quarters of the households. The other disease prevention strategy which is less practical in the village system is chemo prophylactic. The efficiency of this measure is in question by most users. This might be related with the disease condition circulated in the area. In most viral diseases, although the chickens are taking the chemo-prophylaxis the probability of chicken to survive the outcome is very rare. In case of viral disease chemo prophylactic measures only can prevent bacterial complication.

The presence of cock in the farm has advantage and disadvantage with relation to disease transfer in the village system. Cocks are mainly giving service to fertilize the egg. In the village system the cock is not only give service to hen in the household it also mate hens from the neighborhood. The advantage that a farmer might have by having a cock in the in the house is the hens are not going anywhere in seek of cock. This will decrease the chance of contact of the flock to other chicken in the village and will have lower risk of disease at the time of outbreak. The disadvantage of having cock in the house is the cock might go out of the compound and get contact with other chicken specially hens and brought the disease to chicken at home.

Providing chicken feed and water using properly cleaned watering and feeding material is important in the control and prevention of disease in the flock (Alders *et al.,* 2010). Cleaning of materials must be performed using clean water and suitable detergent with

good disinfecting or cleaning ability, but cleaning measures is not being practiced in most households involved in the study.

The other means of controlling ND (Fengil) in chicken production is periodical and timely administration of a vaccine for the disease. The vaccine should be given to all breeds and age group in the village system according to vaccine producer manual. Poor diagnosing ability of the farmers and unavailability of vaccination program set for villages system are the main reason for high loss related with diseases such as ND and fowl typhoid (Solomon, 2008).

A higher antibodies titter to ND were identified in chickens from villages of the study area, which agrees with similar studies in Botswana, Mexico and Ethiopia (Zeleke *e. al.,* 2005, Mushi *e. al.,* 2001 and Gutierrez-Ruiz *et al.,* 2000) and it is higher than a research by Serkalem *et al*, (2005) which reported 28-32% sero-positive rate which is much lower than that of the current study. This might be related with the timing of this study is on time of outbreak.

The presence of Newcastle disease virus antibodies in the sera of the chicken in this study was an indication of previous exposure of the chickens to the virus (Duguma, 2009). Since all of the chickens sampled were over three months of age the presence of maternal antibodies can be ruled out for such antibodies are known to disappear after the age 3-4 weeks (Murphy *et. al.,* 1999). In the study performed by Bereket *et al*, (2014) vaccination of chicken is practiced only in less than 15% of households in Bahir Dar district where the veterinary service is much developed than our study district.

Village poultry production is not growing as it is expected due to several factors. Those factors, which are responsible to hamper growth of poultry sector, are described in detail (Duguma, 2009) that is ranging from improper management and poor health of the chicken to poor marketing system. This factors that hinder the development of poultry sector in the country can alleviated through formal training which is not practiced by farmers. It is believed that training of farmers with close supervision will increase

production and productivity as it is practiced in other African countries (Kyeema foundation, 2010).

Technology is the main input for the development of village poultry sector. Health, feed, and breed are the three most important technologies which improve productivity of chicken in the village system. Having improved breed with good productivity will not improve the productive system. Improved breed in the village system must be of good productivity and good disease resistance (Msoffe et. al., 2002). The chickens that were distributed in the study area were good in production but unable to survive the periodic outbreak of Newcastle disease (Fengil). The need of breed and health technologies by farmers in the study area is showing similarity with other studies performed in Benin (Epiphane et. al., 2012).

The growing literacy rate in the area and the emphasis given by the Woreda administration for poultry production as the result of the new livestock master plan are the two main powers that will disseminate intervention technologies that can improve the disease status of the area and the income from poultry sector in particular.

It is indicated that although vaccination generally provides good protection against disease and mortality, but it may not provide sufficient protection against virus transmission so as to be able to prevent or halt epidemics of Newcastle Disease. Their finding was of considerable interest as it brings into question the epidemiological effectiveness of current vaccination programs implemented throughout the country in un-organized manner.

5.1. Experimental vaccination trial

The protection level of the vaccine in the naive treatment groups in this study was in agreement with Musa *et al.* (2010) as the HI titter of chickens that were not taking no vaccination have unprotected antibody titter. The high mortality of chicken in naive group had similarity trend with control groups (naïves) of other works elsewhere (FAO,

2005), (OIE, 2013), (Hussain *et al.*, 1988) and (Nasser *et al.,* 2010). According to the FAO, 2005; NDV can cause 100% mortality in devastating outbreak condition which discourages people to engage in poultry farming and prevent them to spend their time and money. The manual prepared by OIE, 2013 reported that Velogenic strain of Newcastle disease can cause up to 100% mortality in un-protected flock.

In study which performed using bran, ground grain and water as a vehicle by (Abdu *et.al,* 2012); water vaccination was more protective than vaccination using feed as a channel. The difference in the immune response of chicken after getting vaccinated with water and feed is the time taken to take the formulated vaccine is taking longer in feed than that of water. This is mainly related with inadaptability of the chicken for the feed that the vaccine is constituted. Study by Musa *et al,* 2010; the mortality of chicken that were vaccinated with vaccine treated sorghum is devastating (up to 100% mortality), this is different from the result of the current study. The finding of this study on treated barley is different from the findings of Nasser *et al.,* (2010) which report more than 90% protection. This might be due to the number of animal under the challenge and the difference in the type of chicken used in the treatment. Broiler chicken was used by Nasser *et al.,* (2010) and according to Mozaffor *et al.,* (2010) broiler chicken have higher sero conversion for Newcastle disease than that of layer chickens.

In this investigation in on hand, better results were obtained when chicks were vaccinated via eye drop and spray route, resulting in high antibodies and good protection. This agreed with the findings of vaccination trials conducted in other African countries, using the same or other thermostable vaccines of ND (Musa *et al,* 2010; Hussain e*t al.,* 1988, Foster *et al,* 1997, Khalafall *et al.,* 2004). On the other hand, chicks vaccinated by water showed remarkably lower immune responses and protection rates as compared to ocular and spray vaccination but higher to vaccination of chicken using feed as a channel. The reduced response of the birds to vaccines that are given by oral routes is mainly due to virus viability be lost at the gastrointestinal tract (GIT), unless high amount of NDV is contained in the vaccine (Shuaib *et al.,* 1985). It is also reported on Spradbrow (1992) that the viral load excreted from orally vaccinated chicken was little or zero after the

second vaccination when faecal extracts possessed neutralizing activity, probably associated with IgA antibody. The same research paper also report that vaccination of chicken twice brings lower protection level while immunization of chicken trice and above was giving higher protection.

Based on these findings, the intra ocular rote administration of I2 vaccine is recommended for the vaccine application especially for village chickens where number of chickens in a flock is small. However, to implement conventional vaccination methods chickens are difficult to catch which is also reported by Latif *et.al.*, (1992). But spray vaccination which can be performed by middle level professional easily is a simple means of vaccinating chicken. Following the virus administered by spray it follows the natural route of infection, it reaches the upper respiratory tract through the naso-lacrymal duct where it multiplies to induce the required immune responses. This technique can also be practical on commercial production system that has large numbers of chickens to be immunized all at once.

6. Conclusions and Recommendation

6.1 conclusion

ND is responsible for massive rural chicken loss that makes farmers to loss their trust in poultry production as a means to alleviate poverty and improve family nutrition. In the study area, disease control and prevention especially ND is one of the required interventions by most farmers. The current experimental ND vaccination trial of this study provides an alternative vaccine administration routes towards prevention and control of ND disease with a potential for significant improvement in the livelihood of poor people in the study area. Accordingly, protection level of intraocular and spray vaccination is better than that of water and feed vaccination. However, the litter spray route is the easiest, affordable and highly effective means of vaccinating village chickens.

6.2. Recommendation

- Newcastle disease prevention and control with routine vaccination program should be of the first priority in village production system.
- The prevention and control of the disease should take in to consideration the seasonal characteristics of the disease and village poultry production system characteristics. Among the vaccination routes tested, litter spray vaccination of thermo-stable vaccine is the preferable one for scavenging small scale production system where ocular vaccination application is very difficult.
- In addition to proper vaccination program of chickens, training on management practices; village bio-security and nutrition must be implemented with.
- To complete the output of this result, on farm evaluation of ND vaccination and training of farmers in the study area should be implemented and the effect of the intervention should be quantified.

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8. ANNEX

Annex 1. Structured questioner

This questionnaire is intended to obtain primary data to assess effect of Newcastle disease in village production system. we thank you for your valuable time.

Questioner number _____ Date of interview----------

General Information

1. Study District _____ agro ecology _____ Town _____
2. Peasant Association (PA) _____
3. Respondent Name _____ sex ___ Age_____ Tel_____
4. Who is giving the interview? A. Husband B. wife C. child D. other (specify)

Household Demographic Characteristics

1. Marital status A= Married B= Single C=Widowed D=Divorced
2. Education level A= not able to read and write B= Read and write through informal education C=Elementary D= high school E= Higher education
3. What is your main source of livelihood? A. Farming (crop and livestock) B. Salaried employee C. Self employed (off-farm activities like working in other farm, producing charcoal etc.) D. Casual worker E. Others (specify) _____
4. Experience in poultry production (in years) _____
5. Family size (all age groups are in years)

Description	Total	<2	2-15	16-40	41-60	>60
Male						
Female						
Total						

6. Current livestock holding in the house hold

No	Livestock type	Amount (number)	Breed type		
			Local	Cross	Exotic
1	Cattle				
	• Cows				
	• Oxen				
	• Heifers				
	• Calves				
2	Sheep				
3	Goats				
4	Equines				
	• Horse				
	• donkey				
	• mule				

7. Land owner ship A. Owned ___ B. Rented _____ C. shared in _____
   D. shared out _ E. Other _____
8. Current total land size owned by you? _____
9. Type of cultivated crop in the last cropping season _____
10. Source of income (in the past one production season)

Source of income	Amount earn/ month or year(ETB)	Rank (A-H)
Poultry (*)**		
Dairying **		
Sheep and goat **		
Crop sale**		
Wage , nonfarm activities **		
Other livestock **		
Remittances		
Other (specify)		
Total		

11. What is the porpose of keeping poultry (Rank)?

A. For home consumption B. Cultural purposes C. For income generation D. to supplement household income E. recreational purpose F. Others (specify)_____

12. Any cultural or religious belief to rear a special type of chicken A. Yes B. No

13. If yes; specify the type of cultural/religious belief to rear a special type of chicken _____

14. If yes; specify the type of cultural/religious belief not to eat chicken meat and eggs _____

15. If yes; specify the type of cultural or religious belief not to sell chicken and eggs _____

16. What type of poultry production system do you practice?

A. Traditional (Scavenging only) B. Scavenging + Seasonal/conditional supplementation C. Semi scavenging (Scavenging + Regular supplementation)

D. Intensive system

17. Why you prefer this kind of production system? _____

Flock

1. Source of the base stock A. purchase from market B. gift C. family

D. purchase from neighborhood E. others _____

2. What is the current flock structure?

flock composition (age in week)	Blood level			Purpose
	Local	50% Cross breed	Exotic	
Chicks (< 8)				
Growers (8-18)				
Pullets (18-23)				
Layers				
Cock				

3. Source of current stock? A. own B. purchased (market) C. family D. neighborhood (purchased) E. other _____

4. Is the flock dynamics show increasing or decreasing trend? A. increasing B. decreasing

5. If decreasing why? A. disease related mortality B. predator C. shortage of scavenging feed D. Other; specify _____

6. If disease is responsible which disease/s is/are common (local name)?_____

7. If it is increasing; what do you think the main reason behind this? _____

8. What was the maximum flock size you kept at one time? _____

Feed and Feeding

1. Major feeding system A. scavenging B. housed feeding C. rationed D. scavenging with regular supplementation E. scavenging with conditional supplementation F. other

2. How does the availability of scavenging feed resource vary over an average year?

Jan	Feb	March	April	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

A=Excess available B=A lot of feed available C= Adequate feed available D= Shortage E= Extreme shortage

3. If you supplement your chicken, frequency of feeding _____ per day and watering _____per day

4. Who is responsible on feeding of chicken? 1= Men 2=Women 3= Boys 4= Girls

5. What are the common supplementary feeds?

Protein sources	Energy sources

6. Do you mix the above feeds before providing it to your chicken? A. Yes B. No

Reason for both _____.

7. Do you think supplementation will improve production? A. Yes B. No

Justify it _____

Technologies

1. Is there any poultry technology intervention in your area (breed, vaccine, medication etc.) introduced in the past? A. Yes B. No

2. If yes what are the most common technologies introduced for poultry production?

No	Technology	Rank	Remark/ introducers
1	Breed		
2	Feed related		
3	ND Vaccine related		
4	Management related		
5	Modern health service (Medication etc.)		
6	Others (specify) _____		

Remark= Effect of the technology on ND incidence A. directly related B. indirectly related

3. Which technology benefited you the most (why)? _____

3.1. If no, why? _____.

4. Source of technologies

Sources	Breed	Feed	Vaccine	Health	Training	Remark
Agricultural bureaus						
NGO						
Private farms						
Higher institutes						
Research institutes						
Other						

8. What kinds of technologies are required more? _____

9. Why? _____

10. What are the limitation factors which hinder you to access them? _____

Housing

1. What type of housing do you have for your chickens?

A. Built separate house for birds B. Share the same house with people

C. Share house with other animals D. Others (specify) _____

2. Does housing differ for different breeds of chickens? A. Yes B.No

3. Does housing differ for different age groups? A. Yes B. No

4. Farmer perseverance on housing has an impact on productivity and health of chicken? A= No B= Little C= Good D= Very good E= Other----------

5. Is your chicken get the chance to get contact with chickens in the neighbor?

A. Yes B. No

6. If No what are the measures taken to implement this? _____

Health

1. What are the common problems related with chicken production in your area? Rank them

1st _____ 2nd _____ 3rd _____

2. If disease is among the four; what are the common disease conditions? (Local names)

3. Please characterize each disease by symptoms

	disease Name (Local names)	Symptoms observed q=3	measures taken*	Occurrence (months)	Treatment
1					
2					
3					
4					

**A. taking to vet B. do nothing C. use traditional practices D. selling D. use it for home consumption E. others*

4. Is there disease outbreak in the past two years? A. Yes B. No

5. If yes, for which disease (number)? _____ and damage caused _____

6. If traditional practice is among the measures taken what are the common practices? _____

_____.

7. Which measure of intervention is working efficiently? _____

8. What are the measures of effectiveness of the intervention measure? _____

9. How many chicken you lost from your flock due to ND outbreak in the last two years?

10. Is there any risk difference between different age groups? A. Yes B. No

11. If yes; high prevalence age groups? A. Baby chicks B. growers C. adults

12. Is there any risk difference between sexes? A. Yes B. No

If yes, in which sex it is high A. Male B. Female

13. Have you observed any variation in disease resistance b/n chickens? A. Yes B. No

14. If yes what the unique characteristics of these birds? _____

15. Is there any traditional medicines used to treat ND? A. Yes B. No

16. If yes List them _____

17. Are those traditional medicines effective? A. Yes B. No C. I can't say any thing

18. Do you treat your chicken by yourself ? A. yes B. No

19. If no, where did take them? _____

20. If you treat by your own from where do you get the drug (commercial)? _____

21. Is there problem of cure after therapy? A. Yes B. No

22. Seasonality of ND

	Months											
	Jan	Feb	March	April	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
High Risk												
Low Risk												

23. What do you think the possible causes of disease transmition in your farm?

A. human activity B. rodents C. other animals D. insects E. wild birds

F. newly introduced chicken G. market H. Others specify _____

Selection criteria

1. Which characteristics of laying hens are most important to you? _____
2. Which characteristics of brooding hens are most important to you? _____
3. Do you have your own cock A. Yes B. No
4. Which characteristics of cocks are most important to you? _____
5. Plumage colour of your preference (rank, 1=most important)

5.1 Hen A. _____ B. _____ C. _____

5.2 Cock A. _____ B. _____ C. _____

6. Which plumage colours do you dislike? A. _____ B. _____ C. _____
7. Reason for plumage colour preference A Aesthetic value B. High market value C. Cultural and religious value. D. Other (Specify_____
8. Which comb type do you prefer? A. Single.... B. Double.....

Inputs/Services

1. Accessibility

Inputs	1= Yes	2= No	Source
Supplementary feed			
Veterinary services			
Extension services			
Vaccination services			
Market			
Training			
Others			

Training

1. Have you ever taken training on how to manage poultry production?

A. Yes B. No

2. If yes, who gave you the trainings? A. BOA experts B. Research centre C. Ngo's D. Private Farms E. Higher institutions F. Others (specify)_____

3. Who from the family members took training and how many times did you take in the last two years?_____

4. What were the contents of trainings (multiple response)? A. Improved feeding B. Improved health management C. Improved housing D. On improved breeds E. On marketing issues F. On general managemnt practices G. Others (Specify)_____

Marketing

A. Buying

1. Do farmers buy chickens? A. Yes B. No
2. What purpose? Which months?

Rearing/ breeding: ----------

Ceremonies/Rituals: ----------

Household consumption: ----------

Others: :----------

3. From whom do you buy these chickens?

A. Neighbors B. Village market C. Woreda market D. Traders E. others

4. What are the criteria set to buy chicken? _____
_____

5. Do you have any measure which should be taken for newly introduced chicken? _____.

B. Selling

1. Who is responsible in selling chicken and chicken products?
2. Live chicken _____
3. Egg _____
4. Is there a difference in price due to the place of sale? What is the price difference?

Type chicken	Price (range) of the animal when sold and buyers at the			
	Farm gate	Village/bush market	Woreda market	Remarks
Growers				
Pullets				
Layer				
Cockerels				
Egg				

5. What are the major reasons in which farmers sell their chickens? _____.
6. Egg production per year (calculate)_____
7. Does this vary with seasons? A. Yes B. No
8. If yes, indicate the changes_____
9. Type of birds (age/sex/color) most demanded by different buyers? _____
10. Once you have made the decision to sell, how long does it take you to find a buyer? ___
11. For what purposes is the revenue from egg/chicken sell used in the order of importance? _____.
12. What happen to unsold chicken? _____

13. In which months does the demand for chicken and chicken product increase/decrease?

	Months											
	Jan	Feb	March	April	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
High demand												
Low demand												

14. Factors affecting seasonality in chicken and chicken product demand in order of their importance?

1. _____

2. _____

3. _____

15. Average selling price of chicken and egg in different occasions

Type	Male birds			Female birds			Egg		Remarks
	small	Medium	Large	small	medium	Large	Local	exotic	
Christian festivals									
Muslim festivals									
Traditional festival									
Year round									
Scarification									
Wet season (kiremet)									
Dry season (bega)									

Vaccination practice

1. Is there poultry vaccination campaign held in your area in the past 12months? A. Yes B. No
2. Do you vaccinate your flock for ND ever? A. Yes B. No
3. Do you vaccinate laying hens? A. Yes B. No
4. Route of vaccination _____
5. Who is responsible in the administration of the vaccine? _____
6. Is the vaccination effective? A. Yes B. No
7. How do you express the effectiveness? A. excellent B. good C. fair
   D. poor E. very poor
8. What are the minimum and maximum age groups vaccinated? _____

Summery on Bio-security measures

1. Is there another village chickens in the neighborhood area? A. Yes B. No

   1.1. If yes; is there a chance of contact between these birds and your birds? A. Yes B.No

2. What are the measures taken to prevent contact between them? A. fencing of the compound B. housing C. other specify _____
3. Is there a chance of contact between production systems?
4. How often do farmers clean the house? A. Every day B. Every two days C. Weekly D. Every two weeks E. Monthly
5. How often you clean watering and feeding equipments? _____

_____

6. What do you use for cleaning materials in the farm? _____
7. Where do you dispose dead birds and chicken wastes? A. In the farm
   B. Outside the farm C. Communal disposing areas D. rivers F. Other specify _____
8. Did you keep all age groups together? A. Yes B. No

   8.1. If no; what is the reason for this _____

9. What solutions do you recommend to improve your poultry production system? _____

10. Thank you for cooperation. Finally if you have anything to say about chicken production in your area you are welcomed. _____ _____ _____.

Annex. 2. Pathogenic index

This chart is developed in order to record the pathogenic index of wild ND virus isolated from Harer on experimental units in DZARC poultry farm.

Breed ____ _____ Treatment ____ pen number ___

No	ID	25/02/15	26/02/15	27/02/15	28/02/15	01/03/15	02/03/15	03/03/15	04/03/15	05/03/15	06/03/15	07/05/15
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												