

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

**A LONGITUDINAL STUDY OF EPIDEMIOLOGICAL OCCURRENCE OF
ANTIBODIES AGAINST NEWCASTLE DISEASE AFFECTING VILLAGE
INDIGENOUS CHICKENS IN HORO AND JARSO OF ETHIOPIA**

BY
BERHANU ABERA

JUNE, 2012
DEBRE ZEIT, ETHIOPIA

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**A Thesis submitted to the School of Graduate Studies of Addis Ababa University in
partial fulfilment of the requirements for the Degree of Masters of Veterinary Science
in Tropical Veterinary Epidemiology**

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LIST OF ABBREVIATIONS

APCs	Antigen presenting cells
APMV	Avian paramyxo virus
CMI	Cell mediated immune response
CPE	Cytopathic effects
DEAE	Diethylaminoethyl
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
HA	Haemagglutination
HH	Household
HI	Haemagglutination Inhibition
HN	Hemagglutinin/neuraminidase
ICPI	Intracerebral pathogenicity index
MHC II	Class II major histo- compatibility complex
ND	Newcastle Disease
NDV	Newcastle disease virus
NVI	National Veterinary Institute
OB	Outbreak
OIE	Office International Des Epizooties
SAS	Statistical Analysis System
UK	United Kingdom
vND	virulent Newcastle Disease

ABSTRACT

A longitudinal study to monitor epidemiological occurrence of antibodies against Newcastle disease (ND) virus, potential risk factors for these occurrence and outbreaks in scavenging village chickens was conducted in eight villages within two districts of Oromia region. A total of 985 blood samples were collected from 800 individual birds in 400 household chicken flocks during two sampling periods. None of the birds enrolled in the study was vaccinated against ND. A haemagglutination inhibition (HI) test was used to determine antibody titres against ND virus. The bird-level ND seroprevalence pooled across all samplings was 11.1 %, while accounting for each season 10.1% and 13.8% of wet season and dry season, respectively. The bird-level incidence rate for the period between the first and the second sampling was 4.3%. The flock-level ND seroprevalence (at least one bird tested had antibodies against ND virus) pooled across all samplings was 19.4%. The result indicated that village chickens in Horo and Jarso were exposed to ND virus; there was a higher seroprevalence of infection in Jarso than Horo despite insignificant association either in flock-level or bird-level. Univariate risk factor analyses indicated that flock-level seroprevalence of ND was significantly associated with different market-sheds, villages, offering of rain feed, frequency of contact during wet season, mode of waste disposal, and housing ($p < 0.05$). None of the factors investigated was significantly associated with bird level incidence. Management had significant association with disease outbreak. Thus, despite occurring year round; in current study outbreaks during rainy season were mainly due to management factors and severe at times of climatic stress and had been known to worsen the outcome of the outbreak. The experimental study investigated the effect of concurrent parasitic and viral diseases on the immune response; as well as differences in protection between indigenous ecotype and improved breeds of chickens. This was done using the antibody response towards ND vaccine virus in both chickens ecotypes/breed with and without parasites. Multivariate analysis showed that there was a significant reduction in NDV specific antibody production post vaccination in individuals with parasites compared to antihelmintic treated chicken in both breeds. However, this finding confirmed that there was a significant dramatic reduction in NDV specific antibody production post vaccination in indigenous ecotype compared to improved breeds ($p < 0.05$).

Though breeds differ genetically in their immune responsiveness to the antigen, and being protected following challenge; difference in their susceptibility to the virulent challenge virus needs to be ruled out.

Key words: Incidence, Indigenous, ND, Outbreak, risk factor, seroprevalence

1. INTRODUCTION

The impact of village chicken in the national economy of developing countries is significant owing to its low cost of production (FAO, 1997; Gondwe, 2004; Abdelqader *et al.*, 2007; Abubakar *et al.*, 2007). Chickens are among the first 10 agricultural commodities with the highest contributions to total value of production. A 20 percent increase in chicken production could result in a four percent reduction in the severity of poverty which indicates that Village chicken production is a reliable and viable tool for eradication of rural poverty and food insecurity (Branckaert and Guèye, 1999).

In overall; animal production in general and chicken production in particular play important socioeconomic roles in developing countries (Alders, 2004; Kondombo, 2005). Rather than caged and large-scale poultry production, 85% of all households keep chicken under free range/ extensive system that are generally owned and managed by rural poor people, providing a reliable source of cash income (Guèye, 1998; Bangnol, 2001; Sonaiya, *et al.*, 2004; Abubakar *et al.*, 2007).

Recent estimates in 2007 put the poultry population in Ethiopia at around 38 million with native chicken representing 95.44%, hybrid chicken 3.92% and exotic breeds 0.64% (CSA, 2008). Despite the fact that traditionally reared poultry account for approximately 99% of the chicken population in Ethiopia, little research has been carried out on traditionally reared poultry. According to Tadelle (2003), in Ethiopia, village chicken production systems are characterized by low input-low output levels. A range of factors such as suboptimal management, lack of supplementary feed, low genetic potential and high mortality rate are the major causes for the apparent low output level. High mortality rate is usually caused by factors such as disease, predators, and poor management and nutrition (Ashenafi, 2000).

In Ethiopia, poultry diseases are considered to be the most important factor responsible for reducing both the number and productivity of chickens (Tadesse, 2000). Poultry disease though a veterinary problem, its transmission, control and impacts are contingent

upon several socio-economic, cultural and institutional factors. Poultry diseases such as Newcastle disease (ND), Fowl cholera, Coccidiosis, Salmonellosis, and nutritional deficiency are considered to be the most endemic and the ones to incur huge economic losses (Sonaiya, 1990; Alemu, 1995; Ashenafi, 2000; Tadesse, 2000).

By common agreement of all but a very few of those who have studied village poultry, Newcastle disease is the single greatest constraint on the production of village poultry (Alders and Spradbrow, 2001; Alexander, 2001). ND is contagious and destructive illness of chickens that occur as a fulminating disease, inflicting heavy losses. They are economically important, since they cause high morbidity and mortality, reduce egg production, deteriorate egg quality, and impair live performance (Tadesse *et al.*, 2005).

Serological surveys in conjunction with isolation studies have shown that velogenic NDV strains are endemic everywhere among free-range village flocks, even in isolated villages and possibly in isolated flocks because they are comprised of different species and are in continuous contact with wild birds (Spradbrow, 1993/94).

The spread of NDV within and between village poultry populations is relatively slow due to a low contact rate. The major mode of transmission of ND appears to be by the faecal-oral route. There is also report that some of the ecto-parasites, especially tick and mites, are vectors of other poultry diseases such as Newcastle disease, avian pastuerellosis, Fowl pox, and possibly chlamydia (Ruff, 1999).

Furthermore; ND epidemic in rural poultry depends upon a number of factors. These include NDV pathotype, age, structure and immunity of the host population, breed susceptibility, concurrent disease and seasonal influences (Awan *et al.*, 1994). Moreover, it has been reported that parasitic infection or their concurrent infections result in immunosuppression, especially in response to vaccines against some poultry diseases (Permin *et al.*, 1997).

According to Glukhov's (1984) study on the effect of *A.galli* infection on immunity to ND, the antibody level declines more rapidly in infected chickens. Newcastle disease is caused by viruses in the serotype avian *paramyxovirus type 1 (APMV-1)* belonging to the

members of the genus *Avulavirus*. The importance and impact of NDV is directly related to its virulence (Orsi *et al.*, 2009). Newcastle disease is particularly complicated in that different isolates and strains of the virus may induce enormous variation in the severity of disease, even in a given host such as the chicken. The virus occurs in three pathotypes: *lentogenic*, *mesogenic*, and *velogenic*, reflecting increasing levels of virulence (OIE, 2000; Alexander, 1998). The highly virulent form of Newcastle disease (vND) is one of the most important poultry diseases worldwide. Outbreaks of vND have a tremendous impact on backyard chickens in developing countries (Dennis and Alexander, 2003).

Appearance of vND viruses in different parts of the world (OIE, 2008) requires repeated and expensive use of live and/or inactivated vaccines. In spite of this, there are many recent reports of considerable economic loss due to mortality and cost of control of the diseases (Leslie, 2000; Capua *et al.*, 2002). Village chicken farmers are disheartened by the loss of large numbers of their chickens to these disease outbreaks that often occur on an annual basis. The occurrence of the diseases can be serologically detected since sero-conversion occurs after infection but the ease of obtaining a definitive diagnosis by isolation and identification of the causative organism precludes the need for sero-diagnosis (OIE, 2008).

The aim of the current study was therefore to determine the seroprevalence and incidence of ND, identify potential risk factors that might be associated with ND seroprevalence and incidence, characterize chicken disease outbreaks in naturally exposed non-vaccinated village indigenous chickens between seasons in Ethiopia, and investigate avian immune response towards ND vaccine (Lasota vaccine) between non-treated and de-wormed chickens.

2. LITRATURE REVIEW

2.1. Indigenous chicken production in Ethiopia

Indigenous chicken is of interest as poverty reduction tools because the small, scavenging poultry production system is the most widespread animal production system and a good way to uplift rural communities, as chickens are not as expensive to maintain as other livestock such as cattle, sheep and pigs (Samuel & Spradbrow, 1991; Frands Dolberg, 2003). The impact of village chicken in the national economy of developing countries and its role in improving the nutritional status, income, food security and livelihood of many smallholders is significant owing to its low cost of production (FAO, 1997; Gondwe, 2004; Abdelqader, 2007; Abubakar *et al.*, 2007).

There is no generally accepted definition of rural poultry production, (Tadesse *et al.*, 2005) but it is characterized by minimal human involvement, with birds scavenging in the backyard for food, and no investments beyond the cost of the foundation stock, a few handfuls of local grain, and possibly simple night shades, but no veterinary medical attention (Dessie and Ogle, 1996). Alemu and Tadelles (1997) also reported that the local chicken in Ethiopia vary widely in body size, conformation, plumage color, comb type and feather cover.

Table 1: Population of chicken in different regions of Ethiopia

Region	Native	Hybrid	Exotic	Total
Tigray	302,9519	366,727	78,149	3,474,34
Amhara	9,983,180	339,046	46,049	10,368,4
Oromia	11,983,42	545,062	75,165	12,603,6
Somale	106,414	0	0	106,534
Benishangul-Gum	735,343	7,260	0	742,858
SNNPR	6,437,286	65,978	13,862	6,517,16

Source: Dawit, A. *et al.* (2009). Each breed include cocks, cockerels, pullets, laying hens, non-laying hens and chicks

The low productivity of indigenous chicken is characterized due to low egg production performance, production of small sized eggs, slow growth rate, late maturity, small clutch size, an instinctive inclination to broodiness and high mortality of chicks (Teketel, 1986; Abera, 2000). Despite their low productivity, indigenous chicken are known to possess desirable characteristics such as thermo-tolerance, resistance to some diseases, good egg and meat flavor, presence of hard egg shells, high fertility and hatchability as well as high dressing percentage (Aberra, 2000).

2.2. Definition of Newcastle disease

The current OIE definition (OIE, 2000) is: Newcastle disease is defined as an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence:

a) The virus has an Intra Cerebral Pathogenicity Index (ICPI) in day-old chicks (*Gallus gallus*) of 0.7 or greater.

Or

b) 'Multiple basic amino acids' have been demonstrated in the virus (either directly or by deduction) at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term 'multiple basic amino acids' refers to at least three arginine or lysine residues between residues 113 to 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characterization of the isolated virus by an ICPI test. In this definition, amino acid residues are numbered from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene, 113–116 corresponds to residues –4 to –1 from the cleavage site" (OIE, 2008)

2.2.1. The importance of Newcastle disease in village chickens

The major striking problem of village chicken production systems in developing countries is the high mortality rate which could reach as high as 80-90% within the first weeks after hatching, due to diseases and predation in general and Newcastle disease in

particular (Wilson *et al.*, 1987). Newcastle disease is an important avian disease that had an economic impact on the poultry industry. This disease affects the international trading of poultry and poultry products around the world (Wanasawaeng *et al.*, 2009). In some countries, this disease can be controlled; nevertheless, it can cause economic loss due to the expense of vaccination and disease surveillance (Alexander, 1997).

Table 2: Relative importance of family poultry diseases

	Ethiopia	Burkina Faso	Indonesia	Bangladesh
1	ND	ND	ND	ND
2	Parasite	Trichmonas	CRD	Fowl Cholera
3	Coccidiosis	Fowl Pox	Fowl Pox	Coccidiosis
4	salmonellosis	salmonellosis	Coccidiosis	Fowl Pox
5	Fowl Pox	Fowl cholera	Fowl Cholera	Pullorum
6	Fowl cholera	Parasites	Pullorum	Parasites

Source: *Drawn based on available data*

The importance of Newcastle disease in village chicken is indicated by the fact that Newcastle disease has a local name in many countries, for example, NCD is locally known as “*Fengel*” in Ethiopia, “*Konoku*” in the Western Region of Ghana, “*Twase Obgo*” in the Greater Accra Region. In Mozambique, it is known as “*Muzungo*” in the Shangaan language, in much of Asia, ND is known as “*Ranikhet*” disease (Alders & Spradbrow, 2001; Tadesse *et al.*, 2005).

Furthermore; the importance and impact of a NCD virus isolate is directly related to its virulence (Orsi *et al.*, 2009). The occurrence of highly virulent NDV (vNDV) having an intercelebral pathgenisity index of 0.7 in day-old chicks (*Gallus gallus domesticus*) is currently defined as a notifiable disease (OIE, 2000). The first documented evidence of NCD in Ethiopia dates back to 1978, when an outbreak occurred in then-Eritrea in the

northern part of the country (Tadesse *et al.*, 2005). Outbreaks of ND are unpredictable and discourage villagers from paying proper attention to the husbandry and welfare of their chickens (Alders & Spradbrow, 2001).

So many isolation studies, the longitudinal study (Thitisak *et al.*, 1989), and the reported regularity of recurrent outbreaks of ND provide strong evidence for the continuous presence of NDV in village poultry populations.

Maintenance of the virus in the population requires some form of reservoir(s). The nature and relative importance of these reservoir(s) of NDV in a typical village environment is poorly understood (Martin, 1992); however, the factors probably involved in the maintenance of infection are: presence of carrier chickens, constant introduction of susceptible birds, other poultry species, wild birds, favourable physical environment and heterogeneity of NDV (Awan *et al.*, 2007).

ND has been presented as the major constraint to rural poultry production by many authors throughout the world. This experience demonstrates the importance of ND as the main pathological factor responsible for high rural poultry mortality (Awan *et al.*, 2007).

2.3. Newcastle Disease Virus

2.3.1. Physical structure of paramyxovirus

Virions contain a lipid bilayer envelope that is derived from the plasma membrane of the host cell in which the virus is grown and possess two surface proteins which mediate the entry and exit of the virus from its host cell. In general they are important to the identification and behavior of the virus (Alexander, 1998).

Hemagglutinin/neuraminidase (HN) is important in the attachment and release of the virus from the host cells, in addition to its serologic identification. The other very important surface protein is the fusion (F) protein, which has a critical role in the pathogenesis of the disease. Fusion proteins and attachment proteins appear as spikes on the virion surface. Matrix proteins inside the envelope stabilize virus structure (OIE, 2000). The nucleocapsid core is composed of the genomic RNA, nucleocapsid proteins,

phosphoproteins and polymerase proteins. It can be spherical, filamentous or pleomorphic (OIE, 2000).

2.3.2. Newcastle disease virus classification

The three virus families *Rhabdoviridae*, *Filoviridae* and *Paramyxoviridae* form the order Mononegavirales. Newcastle disease is caused by APMV-1 viruses, which, with viruses of the other eight APMV serotypes [APMV-2 to APMV-9], have been placed in the genus *Avulavirus*, sub-family *Paramyxovirinae*, family *Paramyxoviridae*, in the current taxonomy (Mayo, 2002). According to Alexander (2003), the virus can be classified into five pathotypes based on the clinical signs induced in infected chickens:

Viscerotropic velogenic (VV): high mortality with intestinal lesions,

Neurotropic velogenic (NV): high mortality following nervous signs,

Mesogenic: low mortality, respiratory and nervous signs, reduced egg production,

Lentogenic: Mild or inapparent respiratory infections, deaths confined to young chickens,

Asymptomatic enteric (apathogenic): inapparent intestinal infection (Alexander, 2003).

2.4. Epidemiology of Newcastle disease

2.4.1. Host range

From the available literature, Kaleta and Baldauf (1988) concluded that in addition to the domestic avian species, natural or experimental infection with NDV has been demonstrated in at least 241 species of free-living and caged birds from 27 of the 50 orders of birds. However, this disease also affects other types of poultry, game birds raised in captivity, companion birds, birds in zoos, and wild birds. It seems reasonable to conclude that the vast majority of, if not all, birds are susceptible to ND infection and the disease seen with any specified strain of virus may vary considerably with host. Chickens are the most important host for Newcastle disease virus. However, village flocks are not limited to domestic chickens. We must be aware of the possible role of other avian species (John *et al.*, 2003).

Ducks deserve special consideration. Ducks are reported to be readily infected with Newcastle disease virus and to be capable of spreading the virus. At FAO (1997) meeting in Morocco, it was observed that in Tanzania Newcastle disease is a greater problem in chickens in villages where ducks are also kept. Turkeys can also be infected with Newcastle disease virus, but are apparently not very susceptible to disease (John *et al.*, 2003). There may be more unusual hosts for Newcastle disease virus. Again from Indonesia there has been an account of the apparent replication of Newcastle disease virus in rice field crabs (Kingston and Dharsana, 1977).

2.4.2. The virus host interaction

Newcastle disease virus reacts with avian hosts in various ways. When non-immune domestic chickens and highly pathogenic strains of Newcastle disease virus meet, the common sequel is an acute disease with mortality close to 100%. Surviving birds will often have defects that are attributed to neural lesions. The birds will often be paralyzed or have twisted necks (Spradbrow, 2000). This severe disease, attributed to strains of Newcastle disease virus that we call *velogenic*, occurs in both village and commercial poultry. It is the only form of the disease that is of consequence in village chickens. The syndromes attributable to viral strains of lesser virulence would not be noticed in village flocks (Kaleta and Baldauf, 1988).

In commercial chickens, strains of Newcastle disease virus of moderate virulence (*mesogenic* strains) cause lower rates of mortality in mature chickens, but severely deplete egg production. Strains of low virulence (*lentogenic* strains) cause little mortality except in young birds, but do reduce egg production (Spradbrow, 2000). These strains also interact with other pathogens, especially respiratory pathogens, to produce severe clinical disease. Most Newcastle disease vaccines are living vaccines, drawn from the ranks of lentogenic or even mesogenic viruses. Their use may alter the epidemiology of Newcastle disease. It is also recognized avirulent strains of Newcastle disease virus that cause no clinical signs when they spread between chickens of any age by natural routes. Lentogenic, mesogenic and avirulent strains also circulate naturally in some countries, modifying the epidemiology of Newcastle disease (Spradbrow, 2000).

2.4.3. Avian Immune response against Newcastle disease

The immune mechanisms can be divided into the innate and the adaptive responses. The innate response mainly consists of Natural Killer cells and phagocytizing cells such as macrophages and heterophil granulocytes (Skamene and Gros, 1983).

In birds macrophages play an important role being the first line of cellular defence towards invading pathogens (Skamene and Gros, 1983). In the lungs and air sacs macrophages do not exist and here the first line of cellular defence consists of heterophils (Latimer *et al.*, 1988; Toth and Siegel, 1986). Natural Killer cells are a population of non-adhering and non-phagocytizing lymphocytes capable of inducing lysis of target cells (Trinchieri, 1989; Sharma and Schat, 1990; Davison, 1996).

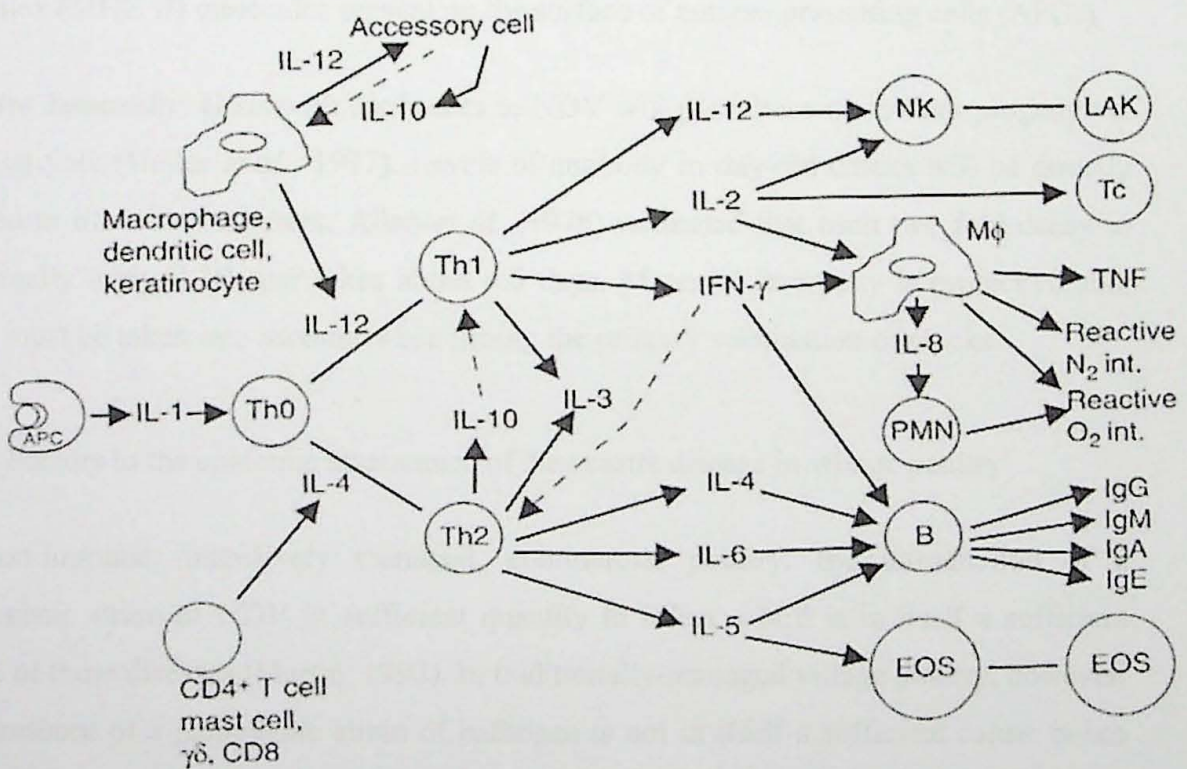


Figure 1 T cell subsets and cytokine interactions in response to infection

Source: Kaiser (1996).

In contrast to the innate response an immediate adaptive immune response is dependent upon a previous infection with the pathogen. The defence mechanism can be divided into active immunity (*Cell-Mediated Immunity and Humoral Immunity*) and passive immunity (Trinchieri, 1989; Sharma and Schat, 1990; Davison, 1996).

Active immunity: Several experiments have been made in the attempt to determine the immunological mechanisms that become activated when birds are infected with Newcastle disease virus. Many have tried to clarify the role of the humoral immune response and the cell mediated immune response (CMI) in protection of the bird (Timms and Alexander, 2003).

Both responses are activated by activated T helper (Th) cells. The activation is brought about by the attachment of peptide fragments to class II major histocompatibility complex (MHC II) molecules present on the surface of antigen presenting cells (APCs).

Passive Immunity: Hens with antibodies to NDV will pass these on to their progeny via the egg yolk (Heller *et al.*, 1977). Levels of antibody in day-old chicks will be directly related to titers in the parent. Allan *et al.* (1978) estimated that each two fold decay in maternally derived HI titer takes about 4.5 days. Maternal immunity is protective and, thus, must be taken into account when timing the primary vaccination of chicks.

2.4.4 Factors to the epidemic appearance of Newcastle disease in village poultry

In non-immune, intensively managed, commercial poultry, the introduction of a pathogenic strain of NDV in sufficient quantity to infect a bird is in itself a sufficient cause of these diseases (Martin, 1992). In traditionally-managed village poultry, however, the presence of a pathogenic strain of pathogen is not in itself a sufficient cause; hence the occurrence of epidemic disease depends on a combination of factors (Martin, 1992).

2.5. Diagnostic techniques of Newcastle disease

Detection and pathotyping of avian isolates are extremely important because the appearance of virulent pathogen has significant economic consequences related to vaccination and eradication, impairing the ability of a given geographic region to export poultry products and to obtain evidence to support epidemiological investigations (Stram *et al.*, 1998). None of the clinical signs or lesions may be regarded as pathognomonic, and the wide variation in disease with strain, host species, and other factors means that at best, these can serve as only a suggestion of infection with NDV.

2.5.1. Isolation and Identification of the virus

The ethiopathogenic diagnosis should be conventionally based on isolation and biological characterization of field samples (Stram *et al.*, 1998). At present, the only unequivocal method of ND diagnosis, which also allows characterization of the infecting strain, is virus isolation. It is possible to use primary cell cultures or even cell lines for routine isolation of NDV (Dennis and Alexander, 2003).

Cytopathic effects (CPE) are usually the formation of syncytia with subsequent cell death, with the CPE having some relationship to the strain's virulence for chickens (Reeve and Poste, 1971). Plaque formation in chick embryo cells is restricted to velogenic and mesogenic viruses unless Mg^{2+} ions and DEAE (Barahona and Hanson, 1968) or trypsin are added to the overlay. Because of relatively poor growth of NDV in most cell culture systems, they are impracticable for virus propagation for most purposes (Rott, 1985). The specific-pathogen-free embryonated chicken egg, however, represents an extremely sensitive and convenient vehicle for the propagation of NDV and is used almost universally in diagnosis (Dennis and Alexander, 2003). The extreme variation in virulence of different NDV isolates and the widespread use of live vaccines can be confirmed by international agreement, based on the intracerebral pathogenicity test (Banerjee, 1994).

2.5.2. Serology

In the absence of vaccination, the presence of specific antibodies against the ND virus indicates that the bird has been infected by the virus at some time, but not necessarily that it was suffering from the disease at the time of sampling (Alexander *et al.*, 2004; OIE, 2008). It is generally thought that following seroconversion antibody levels rise to a peak then decline to a lower but stable level. In practice, a high antibody titre is indicative of a recent infection. At present, two methods are used to measure antibody titres: the HI test is most widely used for detecting antibodies to NDV in birds, although many poultry producers are using Commercial ELISA kits to assess post-vaccination antibody levels (Alexander *et al.*, 2004). Good correlation has been reported between ELISA and HI tests (Dennis and Alexander, 2003).

Haemagglutination inhibition tests: In spite of detecting by a variety of tests including single radial immunodiffusion single radial haemolysis, agar gel precipitin, VN in chick embryos, and plaque neutralization; conventionally, antibodies to NDV and the other avian paramyxoviruses have been detected and quantitated by the HI test (Dennis and Alexander, 2003).

The Haemagglutination inhibition test is based on the principle that the haemagglutinin protein on the NDV envelope can bring about the agglutination of chicken red blood cells and that this can be inhibited by specific antibodies. When serum containing these antibodies is mixed with Newcastle disease virus, the antibodies bind to the haemagglutinin protein in the envelope of the virus (Alexander *et al.*, 2004). This blocks the haemagglutinin protein from binding with the receptor site on chicken red blood cells. Thus the haemagglutination reaction between the virus and the red blood cells is inhibited. By performing two-fold serial dilutions on the serum prior to testing, the concentration of the serum antibodies can be expressed as an HI titre to the log base 2 (Dennis and Alexander, 2003).

2.6. Control and Prevention of Newcastle disease

2.6.1. Vaccination

Vaccination is the only effective way of controlling (Alders and Spradbrow, 2001). Field work by Spradbrow (1993/94) showed dramatic reductions in poultry mortality after ND vaccination. However, vaccines currently in use are mainly of benefit to commercial poultry producers whose chickens are kept in large, single-age, confined flocks. When working with village chickens, it is essential that benefit:cost analyses of all interventions be done so that any ND control strategies are cost-effective. The main costs associated with the control of the disease are the purchase of the vaccine, transport and handling costs (Alders and Spradbrow, 2001). Ideally, vaccination against vND would result in immunity against infection and replication of the virus. Realistically, ND vaccination usually protects the bird from the more serious consequences of disease, but virus replication and shedding may still occur, albeit at a reduced level (Alders and Spradbrow, 2001).

2.6.2. Management procedures

Possibly the most important factors in preventing the introduction of NDV and its spread during outbreaks are the conditions under which the birds are reared and the degree of biosecurity practiced at the farm (Alders and Spradbrow, 2001). Although many biosecurity measures may often be regarded as costly, onerous, and time-consuming by those involved, if such measures are implemented there is no doubt that the introduction of ND viruses to poultry flocks and the spread to the rest of the poultry industry will be dramatically reduced. Such measures are also likely to reduce the spread of other endemic diseases that may affect the birds and reduce their yield and should be seen as an important investment in the profitability of poultry production (Dennis and Alexander, 2003).

3. MATERIALS AND METHODS

3.1. Study areas

The study was conducted in the two districts of Oromia regional State of Ethiopia representing different local chicken ecotypes of Ethiopia (PRA report, 2011).

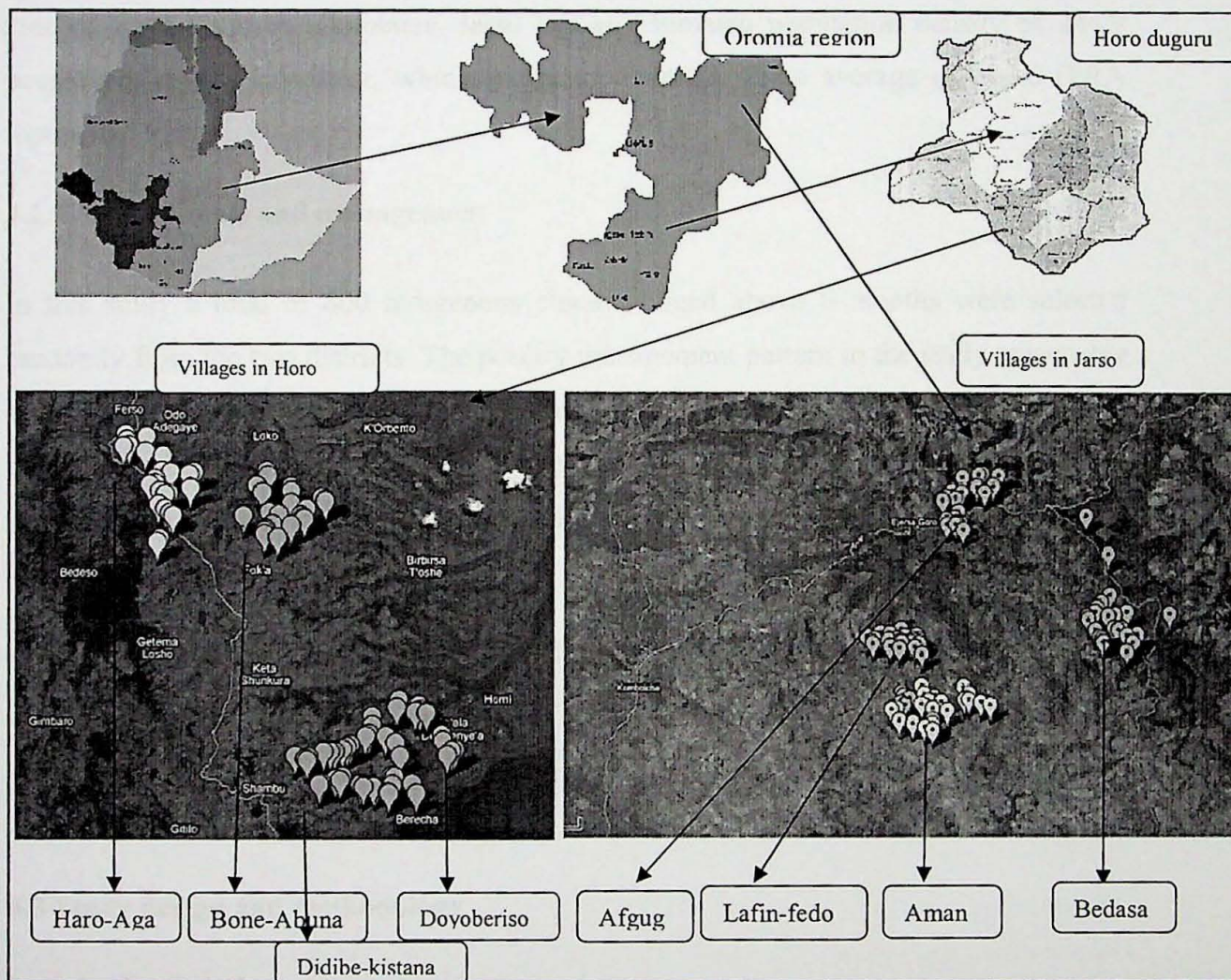


Figure 2: Households and villages in Horo and Jarso, Oromia region, Ethiopia

Source: Drawn with available and modified data

Horo: Having an area of 998.7 km² located in East Wellega zone. Shambu town is the administrative center of the district. The district experiences a temperate (Dega) type of

climate. It is second populous district in East Wellega zone. The crude population density was ~109 persons/km². Cultivable and grazing land covered 61.6% and 8.0% of the district respectively.

Jarso: located in East Hararghe Zone of Oromia regional state. Altitude ranges from 1050 to 3030 m above sea level; 9°25'N42°10' . Chat, fruits and vegetables are important cash crops. The woreda has an estimated total population of 123,556 with an estimated area of 504.54 square kilometers, Jarso has an estimated population density of 244.9 people per square kilometer, which is greater than the Zone average of 102.6 (PRA report, 2011).

3.2. Study animals and management

In this study a total of 800 indigenous chickens aged above 6 months were selected randomly from the two districts. The poultry management pattern in the study areas were entirely free-ranging traditional system where birds scavenge in the backyard, and no investments beyond the cost of the foundation stock, a few handfuls of local grain and possibly simple night shades were provided and with no veterinary medical attention at all, no vaccination and drugs use .

For each chicken samples sex, age, treatment history, and health status during sampling were recorded. Moreover; a numbers of socioeconomics, management, health and genetics questionnaires have been asked for each bird and household owners as it is prepared on the annex (Appendix-3).

3.3 Study design and methodology

Longitudinal study

A 9-month (from May 2011 to February 2012) longitudinal study was undertaken to elucidate the incidence rate and epidemiology of ND on indigenous chicken reared in rural poultry production within two distinct of different geographical and climate areas on the bases of detailed serological examination and structured questionnaires. During the study period 800 birds, which belonged to different age and sex categories, were sampled

, labelled them with leg rings and taken some photographs of birds in the first visit (May 2011) then they had been re-sampled in the re-visited (October, 2011) accompanied by modified revisit questionnaires. The sera were collected from unvaccinated chickens.

Questionnaire survey: Questionnaire survey had been conducted to have a birds-eye-view of poultry diseases in the afore-mentioned sites. In all study areas veterinary personnel and poultry owners were interviewed with a structured questionnaire. Emphasis was given on the frequent clinical symptoms manifested whenever outbreaks of poultry diseases occur in the respective study sites. Tentative diagnosis was made based on the classical disease manifestation and the epidemiological information whenever available. The farmers will be interviewed in their homes for their convenience with the assistance of field guides. Information on socio-economics of household, source of new birds, contact with other birds, loss of bird to disease, Housing, feeding and watering, treatment of sick birds, source of prescription, type of remedies used and method of preparation were asked on management and health questionnaire (annex 3).

Clinical examination of chickens (breathing, demeanor, neurological, discharge, ectoparasite burden), details of previous diseases, bird details (Age, Sex, Weight), origin, body condition score, recent treatment, owner's comments on productivity and other questions as described in annex -6 were collected at the time of sampling.

Sera collection and testing: Totally 800 chicken sera were collected from local chickens raised by 400 randomly selected households. About 1-2 ml of blood was collected from the humeral region of the wing vein with a syringe and needle of 3ml size. The syringe with blood would be then kept horizontally until the blood clotted. After clotting, the syringe was returned to a vertical but inverted position and left on a bench overnight to permit the serum to ooze out. The separated serum was transferred into eppendorf tubes, labeled, inactivated at 56°C for 30 min and stored at -20oC until use. The risk of misdiagnosing can be eliminated using control sera and a panel of reference antigens. The positive standard reference serum was obtained from the UK, NVI as well as from EARO.

Haemagglutination inhibition test: Presence of antibodies against NDV was detected by the HI test conducted in micro-titration plates as described by Allan and Gough (1974). The serum samples were diluted in serial twofold dilutions in PBS and then a fixed quantity of viral antigen is added to each well. Usually 4 HI units are used, according to the method of Allan and Gough (1974). The HI titre is the reciprocal of the highest dilution of serum which completely inhibits haemagglutination and is usually and most conveniently expressed as the logarithm to the base 2. Serum samples showing an antibody titre of less than 3 were considered negative. Since no vaccination has taken place, diagnosis of the infection can be made on this basis, although it cannot be determined exactly when it took place. Sequential samples taken at different times can indicate whether the titre is rising – indicative of a recent infection – or declining (Alexander *et al.*, 2004).

Experimental study

The design was conducted as a factorial longitudinal study to investigate the effect of concurrent parasitic burden and breed/ecotype difference on humoral immune response in chickens. This was done using the antibody response towards NDV in chickens with and without parasites as an indicator for changes in the humoral response.

70 indigenous chickens were selected in two rural villages. The chickens had been neither vaccinated against ND nor treated with antiparasitic drugs prior to the experiment. These chickens were divided randomly into four groups (Table 3).

Table 3 Grouping of chickens

	Improved breed	Indigenous	Total
Anthelmintic treatment +	26 Group 1	11 Group 2	37
anthelmintic treatment -	17 Group 3	16 Group 4	33
Total	43	27	70

Treatments and vaccination: Chickens in groups 1 and 2 were treated with anthelmintic in form of Levamisol (35 mg/kg) for two days, Piperazine (15 mg/ kg) and Amprolium 20% (0.3 g/l water) for fifteen days. All drugs were given p.o. to each bird individually using a syringe. These groups were treated with multi-vitamin and Oxytetracycline 20% (0.5 g/l water) for fourteen days. This was done to avoid other infections and new littermates potentially carrying latent infections of various kinds. Two weeks after the anthelmintic treatment was terminated, all groups were vaccinated against ND using the La Sota vaccine. Each bird was vaccinated receiving three drops of the vaccine intraocularly. The vaccinations were performed using an Eye-dropper calibrated according to the calibration methods described by Alders and Spradbrow (2001).

3.4 Sampling techniques and sample size determination

3.4.1. Sampling techniques

A multi-stage sampling procedure (purposive and random) were applied for the study; hence the district were divided into two based on their geographical difference then four villages from each were selected purposively. Therefore, a total of eight representative farmer villages had been selected based on sharing of the market shed, chicken production potential and accessibility.

A simple random sampling technique was applied to choose 50 households possessing 2 or more chicken from each village by giving equal chance for those farmers with different flock size, chicken husbandry systems and other related practices. Hence, samplings were done from these households and a total of 800 village chicken will be sampled.

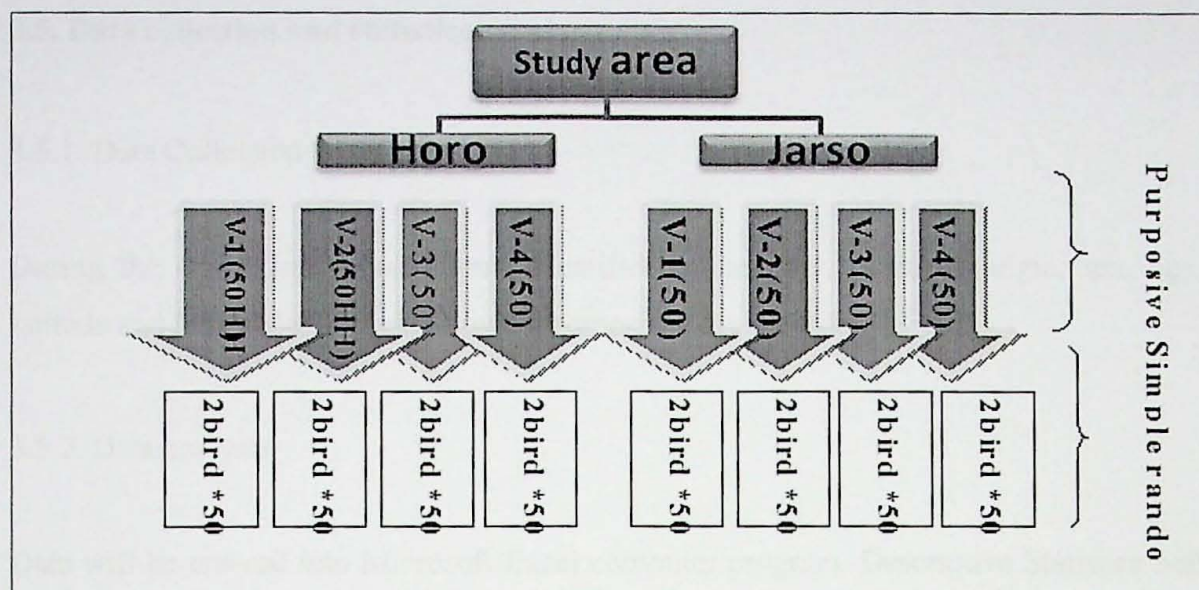


Figure3. Multi-stage sampling procedure in Horo and Jarso woredas, Ethiopia

3.4.2. Sample size determination

The number of study animals was determined based on the expected prevalence of Newcastle disease and the desired absolute precision following the formula published in Thrus-field (2005).

$$n = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2}$$

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Z (commonly 1.96^2 at 95% CI) constant from normal distribution at a given CI

Therefore; the total number of samples to be collected with 50% prevalence rate, absolute precision value of 5% and 95% confidence interval were 384. But in order to increase the accuracy of the study the sample size had been increased to 800 individual chickens.

3.5. Data collection and statistical analysis

3.5.1. Data Collection

During the study, individual animal identification number, place of origin, sex, age, latitude and longitude, household owner response were recorded

3.5.2. Data analysis

Data will be entered into Microsoft Excel computer program. Descriptive Statistics will be used to summarize data and analytical methods like Chi-square and multivariant and univariant regression analysis were used to test the effects and determine associations. 5% level of significance ($p < 0.05$) was used to determine significant differences. The SPSS Statistical software was used to do the statistical procedure. For all the analysis performed confidence interval of 95% and $p \leq 0.05$ were set for significance.

4. RESULTS

4.1 Household Seroprevalence and incidence of Newcastle disease

Data on flock/household level Newcastle disease seroprevalence (at least one bird tested had antibodies against ND virus) were presented in Table 3. The overall flock-level ND seroprevalence revealed in this study was 19.4%. seroprevalence of 17.1% and 21.9% was recorded during wet and dry seasons, respectively.

During the wet season, among the total seroprevalence, 47.1% and 52.9% were found in Horo and Jarso, respectively. Similarly, in dry season Horo accounted 32% of the total seroprevalence where as Jarso do 68%. There was no significant association between season, district and seropositivity ($p>0.05$). Similarly, the current study showed that Shambu market-shed had significantly higher seroprevalence (24.0%) than Sekela market shed (8%) during wet season ($p<0.05$).

Table 4. Household-level seroprevalence in different market shed and seasons in Horo and Jarso, Ethiopia

parameters	Wet season					Dry season				
	N	%+ve	p	OR	CI	N	%+ve	p	OR	C
Districts Horo	100	16.0		1		91	20.9		1	
Jarso	99	18.2	0.683	0.857	[0.409,1.795]	87	23.0	0.734	0.884	[0.434
Horo Shambu	50	24.0		1		47	19.1		1	
Sekela	50	8.0	0.037*	3.632	[1.082,12.183]	44	22.7	0.675	0.805	[0.293,
Jarso Ejersa	50	14.0		1		49	32.7		1	
Ahmed	49	22.4	0.28	0.562	[0.198,1.596]	38	10.5	0.020*	4.121	[1.247
Overall	199	17.1				178	21.9			

Despite none of households infected in Aman by NDV during dry season, the highest seroprevalence was recorded in Aman (41.7.0%) during wet season. Bedasa had the highest ND seroprevalence of 50% during dry season, while Lafin-Fedo had the least

seroprevalence of 4% during wet season. There was a significant difference between villages and market-sheds relating to seropositivity of Newcastle disease ($p < 0.05$).

Figure4. Strategic protocol to determine ND seroprevalence in different villages of Horo and Jarso

sampling area	woreda	marketshed	villages	Seasonal seroprevalence/%	
				Wet	Dry
study area	Horo	shambu	Didibekista	16	16
			Doyoberiso	32	22.7
		sekela	Haro-aga	4	25
			Boneabu	12	20.8
	Jarso	Ejersa	Afgug	4	16
			Bedasa	24	50
		Ahmed	lafinefedo	4	16.7
			Aman	41.7	0

4.1.2. Household incidence

The flock-level Newcastle disease incidence was percentage of household seronegative in the first survey and seropositive in the second survey within the same population. A total of 165 seronegative household from the last sampling period on April/ May 2011 was re-sampled on Sep/Oct 2011 for antibodies against ND virus. Only eight households were tested as ND seropositive, indicating a flock-level incidence level for ND of 4.85%. The incidence of ND was significantly higher in Jarso (8.64%) as compared to Horo which had an incidence of 1.2 % ($p < 0.05$).

Among the four Market-sheds, Ejersa-Goro market-shed had the highest flock-level incidence of 11.6%, while Ahmed-Imam and Shambu market-shed had incidence of 5.3% and 2.6%, respectively. There was no statistical significance between market shed and incidence of ND in this study (Table 4).

Table 5. Flock-level incidence of Newcastle antibodies in village indigenous chickens in Horo and Jarso, Ethiopia

Market-sheds	Household incidence						
	N	Incidence	SE	p	OR	LCI	UCI
Shambu	38	2.63		0.482	1	2.63	
Sekela	46	0	1.247	0.563	0.486	0.042	5.603
Ejersa-Goro	46	11.63	5.926	0.998	0.000	0.000	.
Ahmed-Imam	35	5.26	0.868	0.321	2.368	0.432	12.991
Woredas(Horo and Jarso)	165	4.85		0.026*	4.963	0.024	0.097

Mixing of chicken was among the risk factors significantly associated with ND incidence in free-ranging village chickens with overall p-value of 0.019. Chicken having contact with neighbour chicken were more at risk than chickens having no contact at all during wet season.

Table 6: Univariant analysis of frequency of contact with other chickens for ND incidence at flock level in Horo and Jarso, Ethiopia

Frequency of contact with other chickens	Household incidence					
	B	SE	p	OR	LCI	UCI
Never at all			0.517			
Rarely	2.246	1.183	0.058	9.45	0.930	96.007
Less than 1hr few day/week	-17.060	5.863	0.998	0.00	0.000	.
Greater than 1hr fewday/week	2.351	1.185	0.047	10.50	1.029	107.166
Less than 1hr everyweek	3.045	1.533	0.047	21.00	1.041	423.512
Greater than 1hr everyweek	-17.060	2.321	0.999	0.00	.000	.
Unknown	-17.060	2.321	0.999	0.00	.000	.

4.2. Birds-level seroprevalence of Newcastle disease

Serum sample from individual bird showing an antibody titre of $\geq \log_2^8$ in HI test was considered as seropositive.

Comparison was made on the seroprevalence of wet and dry season as well as between Horo and Jarso. The bird-level Newcastle disease seroprevalence pooled across all samplings in wet season, dry season and both season was 10.1%, 13.8% and 11.8 %, respectively.

In wet season the bird-level Newcastle disease seroprevalence in each of the two sampling areas (Horo and Jarso) was 9.5% and 10.7%, respectively where as in dry season it was 13.0% and 14.6% (Table 6).

Despite the absence of significant association between bird-level seroprevalence and the two districts during wet season, In Horo, seroprevalence of ND antibodies was significantly higher (15%) in Shambu market-shed compared to Sekela. Similarly; in Jarso, Ejersa-Goro had significantly higher seroprevalence than Ahmed-Imam market-sheds ($p<0.05$).

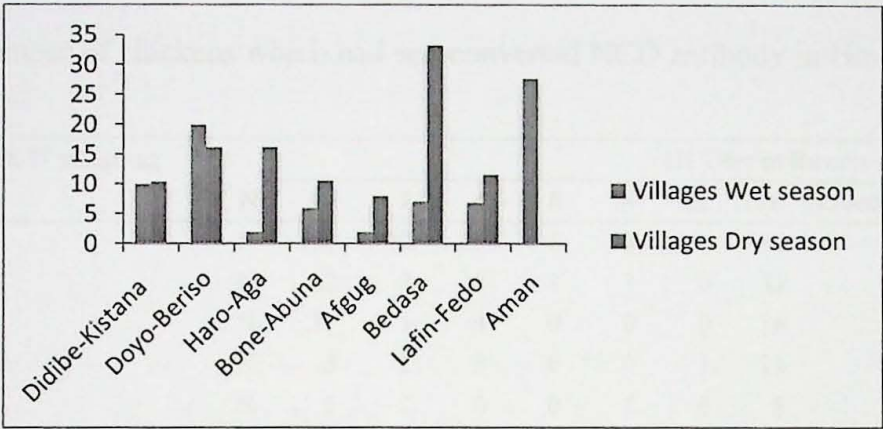
Table 7: Bird-level seroprevalence of Newcastle disease antibodies in indigenous chickens during the wet and dry seasons in Horo and Jarso, Ethiopia

Sampling areas	Wet season			Dry season			Total	
	N	%	p	N	%	p	N	%
woredas	398	10.1	0.713	356	13.8		754	11.8
Market-shed in Horo	200	9.5	0.009*	178	13.0	0.976	378	11.1
Market-shed in Jarso	198	10.7	0.097	178	14.6	0.023*		12.5
Villages in Horo	200	9.5	0.016	178	13.0	0.000*	21.9	11.1
Villages in Jarso	198	10.7	0.000*	178	14.6	0.000*		12.5

The highest seroprevalence in Horo was recorded at Doyo-Beriso (20%) in the wet season, but Haro-Aga (16.2%) in the dry season. However, the highest seroprevalence in Jarso was recorded from Aman (27.7%) and Bedasa (33.3%) during wet and dry season,

respectively. In the current study, the difference in seroprevalence between villages was statistically significant ($p<0.05$).

Figure 5 Bird-level seroprevalence of NDV in different village of Horo and Jarso, Ethiopia



4.2.1. Chicken involved in the longitudinal study

Households from 400 chickens of the last sampling period on April/ May 2011 were re-visited on September/October 2011 for further data collection and antibodies against NDV. Among them, 206 chickens were survived, while 194 chickens had been lost from the flock following different reasons such as died due to disease, sold, predator, consumption, missed to follow up and accident (Figure 6).

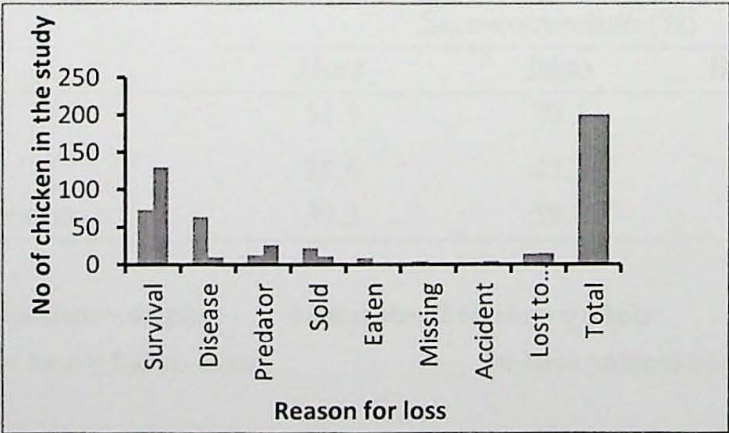


Figure 6 Chicken involved in the longitudinal study

Seroconversion of antibody titer:

A total of 206 chicken serums from those survived were sampled on October 2011 for further HI titer against ND antibodies. Of which, 106 chicken serums had been seroconverted, while 100 serum had no change in antibody titer (Table 7).

Table 8 Number of chickens which had seroconverted NCD antibody in Horo and Jarso, Ethiopia

HI titer in 1 st sampling	HI Titer in Revisit sampling								Seroconversion
	N	<2	2	4	8	16	32	Total	
<2	N	93	22	16	6	0	0	137	44
2	N	22	2	6	1	1	0	32	30
4	N	11	1	4	0	0	0	16	12
8	N	5	2	3	0	0	1	11	11
16	N	5	2	0	0	1	0	8	7
32	N	0	0	1	0	0	0	1	1
>64	N	0	0	0	1	0	0	1	1
Total	N	136	2	29	30	2	2	30	106

There was a significant difference in the percentage of seroconversion samples in different sampling areas accounting for 39.3% and 59.8% of Horo and Jarso, respectively ($p < 0.05$). Similarly, there was statistical difference between seroconversion and different market-sheds. Ejersa-Goro market-shed had higher seroconversion of 70.1%.

Table 9. Repeating birds which had sero-converted NCD antibody

Villages	Sero-conversion (%)		
	Horo	Jarso	Both region
Market- shed-1	51.1	70.1	
Market- shed-2	25.6	47.3	
Overall seroconversion	39.3	59.8	51.5

Note: Market-shed 1 for Horo = shambu

Market-shed 2 for Horo = sekela

For Jarso = Ejersa- Goro

for Jarso =Ahmed Imam

Bird level incidence

The bird-level ND incidence is percentage of chicken seronegative in the first survey and seropositive in the second survey within the same population.

185 seronegative chickens from the last sampling period (first survey) on May 2011 were re-sampled on October 2011 for ND antibody test. Hence, the overall bird-level incidence rate for the period between the first and the second sampling was 4.3%. The incidence of positive samples detected by HI was 1.3% and 6.4% in Horo and Jarso districts, respectively.

Among the four Market-shed groups, Ejersa-Goro market-shed had the highest bird-level incidence of 8.1%, while Ahmed-Imam and shambu had incidence of 4.3% and 2.5%, respectively. Similarly, Bedasa had the highest incidence of 8.8% followed by Afgug (7.1%) and Lafin-Fedo (6.7%).

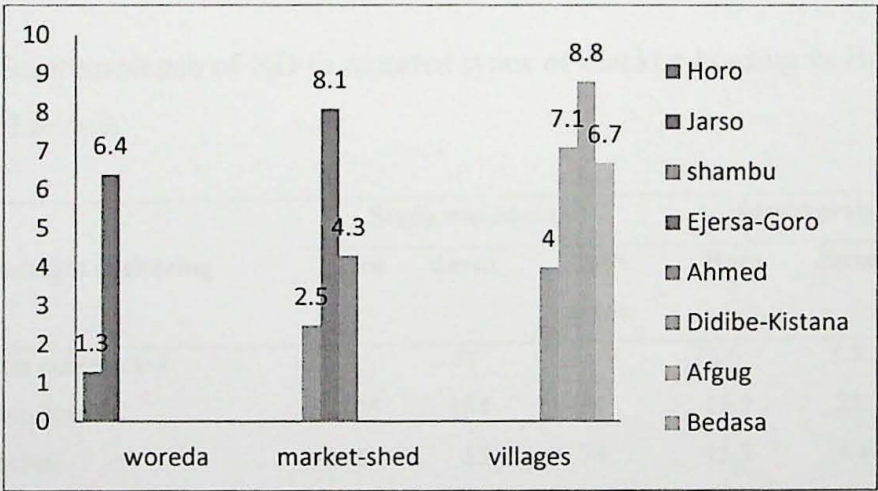


Figure 7 Bird-level incidence of ND antibody in Horo and Jarso Woredas, Ethiopia

The current study also tried to compare the risk factors that were associated with ND incidence during the study period. However, bird-level incidence of ND which was usually thought to be highly influenced by factors such as source of the chicken, physiological status of the chicken, sex, body weight and parasitic burden were not statistically significant(see Table 13).

4.3. Risk factors for seroprevalence of Newcastle disease

Household and management characteristics of interviewed village chicken owners were presented in accompanied with laboratory results (See Table 12).

4.3.1. Chicken housing as potential risk factor for Newcastle disease

The majority of the respondents indicated that they kept their chicken at various night sheltering places either the main house or kitchen including perches inside the house (259) and perches in kitchen (74). In Jarso, 9.14% of the respondents provided separate overnight houses for their chicken, while only 3.7% of the respondents in Horo did so.

Higher seroprevalence (31.2%) of ND antibody was found in households keeping their chicken at perches on veranda as compared to those keeping at various night sheltering places. The differences in the seroprevalence, however, were not statistically significant (see Table 12).

Table 10: Seroprevalence of ND in reported types of chicken housing in Horo and Jarso woredas of Ethiopia

Type of house/night sheltering	Study woredas (N)			Seroprevalence (%)		
	Horo	Jarso	Both areas	Horo	Jarso	Both areas
Separate shelter constructed	7	17	24	28.6	5.8	12.5
Perches in main house	105	154	259	15.2	23.3	20.1
Perches in kitchen	59	15	74	15.3	6.67	13.5
Perches on veranda	16	0	16	31.2	0	31.2

4.3.2. Flock structure

Average flock size of chickens per household in the study area was 7.82±6.75(mean±SD) chickens and varied between 2 and 44 chickens. Higher seroprevalence (21.0%) of ND was found in households having flock size of <7 compared to those having more

flock size (>12 flock size). However, the difference in the seroprevalence was not statistically significant (see Annex).

Table 11: Chicken flock size per household by different age and sex groups in Horo and Jarso woredas, Ethiopia

Chicken flock size/hh by different age and sex groups	Study districts		
	Horo (Mean±SD)	Jarso (Mean±SD)	H+J (Mean±SD)
Hen	2.56±1.364	3.30±2.205	2.93±1.866
Cocks	1.12±1.038	1.02±1.130	1.07±1.085
Pullets	1.28±2.243	0.57±1.179	0.93±1.827
Cockerel	0.79±1.596	0.32±.899	0.56±1.316
Chicks	3.87±5.193	1.10±2.565	2.49±4.324
Total No. of chicken/household	9.48±7.803	6.16±5.011	7.82±6.75

Out of the total flocks, hens accounted for 25.2% and 47% for Horo and Jarso respectively. Similarly, out of the total flocks counted, young chicks accounted for 42% and 53% for Horo and Jarso respectively.

Table 12: Chicken flock size per household in Horo and Jarso woredas, Ethiopia

No of flock size	No Households			Seroprevalence		
	Horo	Jarso	Total	Horo	Jarso	Total
<7	86	128	214	23.3%	19.5%	21.0%
7-12	47	42	89	14.9%	21.4%	18.0%
>12	58	16	74	13.8%	25.0%	16.2%
Total	191	186	377	18.3%	20.4%	19.4%

4.3.3. Feeding

The nutritional management practiced in the study woredas was predominantly scavenging with some sort of supplementary feeding. About 98.9% of respondents offered supplementary feeds to their chicken, while only 1.1% did not. Out of the total households, seropositivity accounted for 19.1% and 25% for supplemented and non supplemented households, respectively. 84.2% respondents indicated that additional

feeds (in addition to supplemented feed) were required during the rainy/wet season (July to September) than the dry season, whereas 15.8% did not. Significantly higher seroprevalence of ND was found for households that did not offer rain feed (29.4%) than those offered rain feed ($p<0.05$) (see Table 13).

Table 13: Seroprevalence of ND in reported feeding of chicken in Horo and Jarso woredas, Ethiopia

Type of feeding	No Households	Seroprevalence (%)
Supplemented	372	19.1
Not supplemented	4	25
More Rain feed given	272	16.9
Reduced rain feed	51	29.4

4.3.4. Confinement

Despite variations in season and frequency of confinement, most of the respondents in the study areas confined their chicken. Higher seroprevalence (20.3%) of ND was found in households that confined their chickens compared to those did not (18.5%). However, the differences in the seroprevalence was not statistically significant ($p>0.05$).

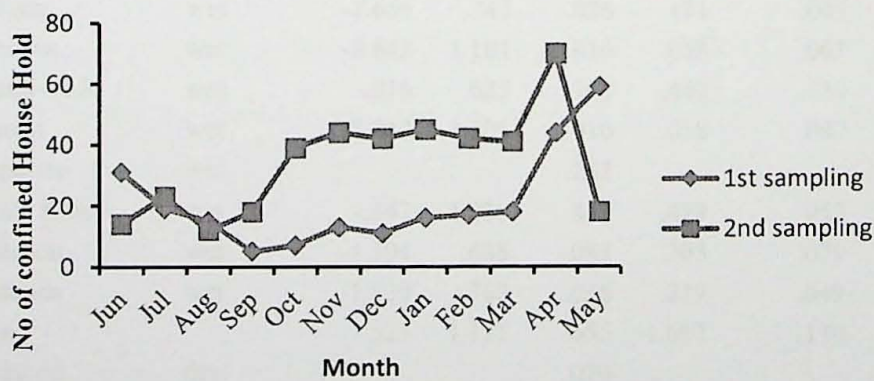


Figure 8. Variations in season and frequency of Chicken confinement in Horo and Jarso, Ethiopia

4.3.5. Contact with other birds

In Horo during rainy season, 46.9% of households had chicken mixing with neighbour chicken and the remaining 53.1% never mixed at all. In Jarso, 57.6% of the households had chicken regularly mixing with other chicken, and 42.4% do not mix at all.

In Horo, significantly ($p < 0.05$) lower seroprevalence of ND was found in households having chicken that never mix at all with others compare to those had contact (see Table 13). Considering contact during dry period, 46.9% and 53.1% of the respondents mix their chicken at least once and never contact at all with others, respectively.

Table 14: Univariable analysis of household seroprevalence in Horo and Jarso, Ethiopia

Parameter	level	Season	B	S.E.	p	OR	95.0%C.I.	
							Lower	Upper
woreda	Jarso		-.154	.377	.683	.857	.409	1.795
marketshed	Shambu	dry			.110			
	sekela	dry	.700	.646	.278	2.013	.568	7.136
	Ejersa-Go	dry	.916	.639	.152	2.500	.714	8.754
	Ahmed-Im	dry	1.416	.610	.020	4.121	1.247	13.625
Villages	Did-Kistana	wet			.008			
	Doyo-Beri	wet	-1.322	.685	.054	.267	.070	1.021
	Haro-Aga	wet	-.417	.596	.484	.659	.205	2.119
	Bone-Abuna	wet	-2.842	1.101	.010	.058	.007	.505
	Afgug	wet	-1.656	.742	.026	.191	.045	.817
	Bedasa	wet	-2.842	1.101	.010	.058	.007	.505
	Lafin-Fedo	wet	-.816	.625	.192	.442	.130	1.505
	Aman	wet	-2.842	1.101	.010	.058	.007	.505
Housing	separate	wet			.242			
	Main house	wet	-.847	1.029	.410	.429	.057	3.222
	Kitchen	wet	-1.194	.688	.083	.303	.079	1.167
	veranda	wet	-1.520	.762	.046	.219	.049	.974
supplement	yes		.523	1.171	.655	1.687	.170	16.743
RainFeed	reduced	dry			.070			
	same	dry	1.079	.489	.027	2.942	1.128	7.678
	more	dry	-.122	.541	.822	.885	.306	2.559
WetConta	yes		-1.561	.703	.026	.210	.053	.833
DeadDisp	Burn				1.000			
	Throw away	dry	-2.015	.983	.040	.133	.019	.916

4.4. Bird level risk factor

4.4.1. Sex

The present study indicated that out of the total chickens sampled, 64.2% and 35.8% were females and males, respectively. Moreover, 60.5% and 67.8% of the chickens were females in Horo and Jarso, respectively.

From the above analysis of bridging populations, it was evident that the risk of ND infection differed between households and was influenced by sex and age. Despite insignificant difference, the seroprevalence study showed that 13.2% of female and 9.4% of male chickens sampled had detectable antibodies to NDV showing evidence for sex as a risk factor in the occurrence of NDV in village chicken (Table 14).

4.4.2. Source of restocking to build the flock

The majority (81.1%) of the chickens were originated from own chicken/homebred and the rest were either purchased or gifted. Chickens were mostly purchased from the market in the same village (8.1%) and rarely from markets (7.6%) in neighboring villages (market shed) for flock rebuilding purpose.

Higher seroprevalence (14.8%) of Newcastle disease was found in chickens purchased from market compared to those restocking from homebred (12.1%) although the difference was not statistically significant (Table 14).

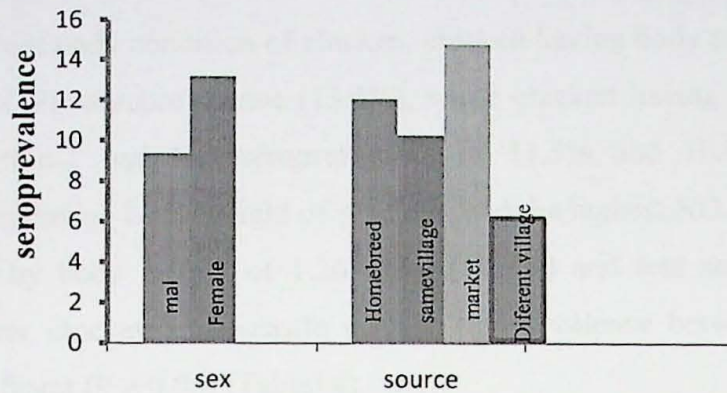


Figure 9: Seroprevalence of ND in chickens from different sources and sex

4.4.3. Physiological status of chicken

The physiological composition of the chicken in the current study resembled uneven distribution, with 62.4% of the composition of the chicken being chicken that were not in production at the period of sampling. Layer, brooding eggs and brooding chicks accounted for 23.5%, 3.8% and 10.3% of the total chicken population, respectively.

The current study showed that although the disease affects chicken of different age, sex and ecotypes indiscriminately, layers and brooding hens being the most vulnerable and affected groups, accounting for 14.9%, 3.7%, and 13.5% of layer, brooding egg and brooding chick, respectively. However; the seroprevalence of ND was not statistically significant (Table 14).

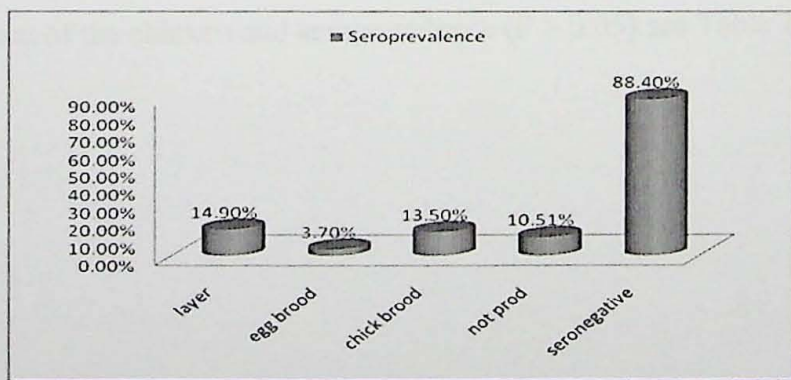


Figure 10: Seroprevalence of ND in different physiological status of chickens

4.4.4. Physical status of chicken

Among the different body condition of chicken, chicken having body condition of score-2 had the highest ND seroprevalence (13.4%), while chicken having body condition-3 and body condition-1 had ND seroprevalence of 11.5% and 10.0%, respectively. Similarly, chicken having body weight of < 1.25kg had the highest ND seroprevalence of 14.7% followed by body weight of 1.26-1.6kg (10.9%) and less seroprevalence was reported in heavier chickens. Newcastle disease seroprevalence between age was not statistically significant ($P > 0.05$) (Table14).

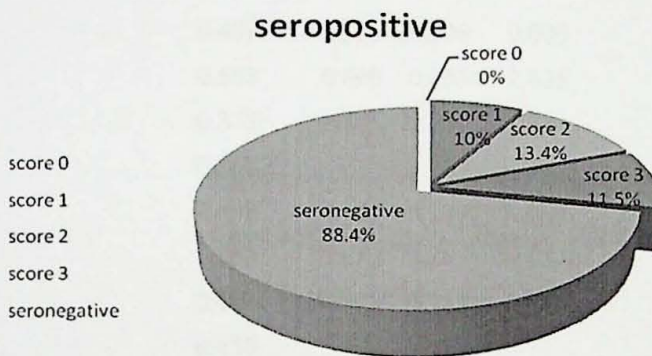


Figure 11: Seroprevalence of ND in different body condition score of chickens

4.4.5. Parasitic burden

More than half of the sampled chickens had either endo-parasitic or ecto-parasitic burden. The current study tried to investigate different kinds of endo-parasites and ecto-parasites from faecal samples even though the difference was not statistically significant between parasitic burden of the chicken and seroprevalence ($P > 0.05$).see Table 14

Table 15: Univariable analysis of bird level seroprevalence in different season in Horo and Jarso,Ethiopia

		Season A (Wet)				Season B (Dry season)			
		Horo		Jarso		Horo		Jarso	
Parameter	level	P	OR	p	OR	p	OR	p	OR
Marketshed	Mrsh2	0.014	4.191	0.097	0.446	0.976	0.986	0.028	3.200
Village	Village1	0.042		0.002		0.759		0.013	
	Village2	0.465	1.741	0.006	0.053	0.972	0.977	0.998	1.405E8
	Village3	0.049	3.917	0.046	0.201	0.455	1.626	0.998	8.077E8
	Village4	0.340	0.326	0.004	0.195	0.455	1.626	0.998	2.126E8
Age	<9month	0.709		0.153				0.099	
	9-18mth	0.409	0.558	0.998	0.000			0.318	0.500
	>18 mth	0.568	0.690	0.053	2.835			0.036	0.304
Sex	male	0.373	1.583	0.280	1.784				
Layingsta	layer	0.568		0.363					
	Eggbrood	0.458	1.583	0.189	1.905				
	Chichbrood	0.563	0.679	0.755	0.714				
	No production	0.497	0.701	0.325	0.580				
Parasiteburden	low	0.426		0.410					
	medium	0.916	1.125	0.229	0.400				
	High burden	0.486	2.118	0.609	0.698				
Birdwght	<1.25kg			0.279					
	1.26-1.6kg			0.641	0.533				
	1.61-2kg			0.219	0.213				
	>2kg			0.189	0.167				
Bodycondition								0.078	0.389

4.5. Characterization of outbreak

The average number of outbreak per household in each season is presented in Table 15. 132 households reported outbreak within these woredas. According to respondents, totally 163 outbreaks were experienced in the current study. However, the highest prevalence (61%) of outbreak was observed during rainy season, mainly from April to September while dry season mainly from October to March accounting for 38.9%.

More outbreaks were reported in the first sampling period compared to October (second) sampling. Significantly higher number of households in Horo (47.2%) reported outbreaks compared to Jarso (19.1%) woreda (Table 15).

Table 16: The average number of outbreaks and household reporting Outbreak

parameter	N	No of HH reporting OB	No of Outbreak	Mean	SD	Variance
First sampling	198	85	115	0.58	0.781	0.610
second sampling	198	47	48	0.24	0.44	0.193
Outbreak in Horo	198	94	123	0.62	0.769	0.592
Outbreak in Jarso	198	38	40	0.20	0.427	0.182
Outbreak in Horo during wet season	198	55	78	0.39	0.601	0.362
Outbreak in Horo during dry season	198	39	46	0.23	0.490	0.240
Outbreak in Jarso during wet season	198	21	27	0.14	0. 423	0.179
Outbreak in Jarso during dry season	198	17	21	0.11	0. 340	0. 116

Similarly, the number of outbreaks reported in each woredas had marked variation during the wet and dry seasons.78, 46, 27 and 21 outbreaks were reported in Horo during wet season, Horo during dry season, Jarso during wet season and Jarso during dry season, respectively.

Table 17: Univariable analysis of outbreak in Horo and Jarso,Ethiopia

Parameter	level	B	S.E.	df	p	OR	95.0% C.I.	
wet season		.896	.220	1	.000	2.449	[1.591	3.769]
Region	Jarso	1.333	.230	1	.000	3.793	[2.419	5.948]
Markets	Marketsed			3	.000			
	Marketsed()	.957	.306	1	.002	2.604	[1.428	4.748]
	Marketsed(2)	1.019	.306	1	.001	2.769	[1.521	5.041]
	Marketsed(3)	-.791	.377	1	.036	.453	[.217	.949]
Village	Afgug			7	.000			
	Bedasa	1.087	.431	1	.012	2.965	[1.274	6.898]
	Lafin-Fedo	.723	.431	1	.094	2.061	[.885	4.800]
	Aman	1.126	.429	1	.009	3.083	[1.330	7.149]
	Didibe-Kistana	.805	.430	1	.061	2.236	[.962	5.197]
	Doyo-Beriso	-.220	.470	1	.640	.803	[.320	2.015]
	Haro-Aga	-2.111	.791	1	.008	.121	[.026	.570]
	Bone-Abuna	-.107	.462	1	.817	.899	[.363	2.224]
Housing	separate			3	.008			
	mainhome	-1.404	.688	1	.041	.246	[.064	.946]
	kitchen	-1.147	.521	1	.028	.317	[.114	.882]
	veranda	-.434	.553	1	.433	.648	[.219	1.918]

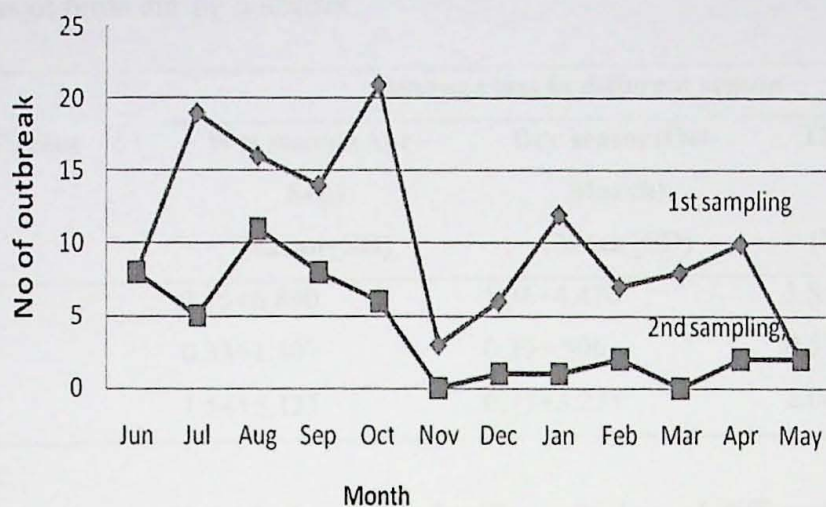


Figure 12 Outbreak reports in various months in Horo and Jarso woredas of Ethiopia

According to respondents, fewer outbreaks were observed in Ahmed-Imam market-shed than Ejersa-Goro. However, the number of outbreak was highest in sekela market-shed.

Table 18: Outbreak report in different market shed

No of Outbreak	1 st sampling		2 nd sampling	
	Wet season (Mean±SD)	Dry season (Mean±SD)	Wet season (Mean±SD)	Dry season (Mean±SD)
Horo MS-1	0.54+0.734	0.38+0.635	0.24+0.431	0.06+0.240
Horo MS-2	0.50+0.678	0.36+0.563	0.50+0.678	0.36+0.563
Horo	0.52+0.703	0.37+0.597	0.26+0.441	0.09+0.288
Jarso MS-1	0.18+0.560	0.10+0.364	0.18+0.560	0.10+0.364
Jarso MS-2	0.16+0.422	0.30+0.505	0.16+0.422	0.00+0.00
Jarso	0.17+0.493	0.20+0.449	0.10+0.333	0.01+0.100
Both region	0.35+0.631	0.29+0.534	0.18+0.398	0.05+0.218

Loss of birds during outbreak

Farmers also acknowledged that diseases (outbreak) were the major cause for the loss of chicken, accounting for 66% and 23% of the losses in Horo and Jarso woredas, respectively.

Table 18: Loss of birds during outbreak

Loss/hh by different region	Average loss in different season		
	Wet season(Apr-Sep)	Dry season(Oct-March)	Throughtout year
	(Mean±SD)	(Mean±SD)	(Mean±SD)
Horo	2.75+6.890	1.36+4.470	3.55+7.897
Jarso	0.33+1.507	0.20+.906	0.57+1.761
Both region	1.54+5.125	0.77+3.273	2.06+5.912

They also reported that although the outbreak affects chicken of different age, sex and ecotypes indiscriminately, layers and brooding hens being the most vulnerable and affected groups, accounting for 43%,21,and 16 of chick, grower and Adult, respectively.

Table 19: Age group affected by the outbreak

Age group affected by the outbreak /HH	Woredas		
	Horo	Jarso	H+J
	(N=230) (Mean±SD)	(N=230) (Mean±SD)	(N=230) (Mean±SD)
Chick	21.7%	4.0%	12.8%
Grower	19.2%	4.5%	11.8%
Adult	35.9%	15.6%	25.7%

4.6. Immune response

Table 20: Averaging of HI Titer and age of chicken by different Treatment groups

	N	Haemagglutination Inhibition titer				Age	
		Min	Max	Mean	SD	Variance	(mean±SD)
Group 1(Improved and parasitic)	17	32	128	50.82	32.117	1.032	15.12±7.84
Group 2(Improved and de-wormed)	26	32	128	87.38	36.829	1.356	17.04±5.95
Group 3(Indigenous and parasitic)	16	32	128	76.00	32.790	1.075	18.69±3.99
Group 4(Indigenous and de-wormed)	11	32	128	90.18	37.368	1.396	20.27±6.06
Total	70	32	128	76.34	37.466	1.404	

Table21: Percentage of HI-titer in different treatment group

Treatment Group	HI titer			
	n	≤32	64	≥128
Improved chicken	27	11.1%	55.6%	33.3%
Indigenous chicken	43	32.6%	37.2%	30.2%
De-wormed chicken	37	10.8%	45.9%	40.5%
Un-dewormed	33	39.4%	42.4%	18.2%
Total	70	24.2%	44.3%	31.4%

Table 22: Hypotesized risk factors for vaccine immune response

Treatment Group		HI Titer		
		≤32	64	≥128
Sex	Male	46.2%	30.8%	23.1%
	Female	19.3%	47.4%	33.3%
Supplemented	Yes	17.4	41.3	41.3
	No	37.5	50.0	12.5
Restocking	Home breed	21.1	47.4	31.6
	Out side	38.5	30.8	30.8
Housing	Separate shelter	12.8	46.2	41.0
	House/kitten perch	38.1	47.6	14.3
	Veranda	40.0	30.0	30.0

Table 22: Multivariant analysis of HI titer with breed and parasitic burden

Titre	B	SE	T	P	OR	95% CI	
						LCI	UCI
64 v's 32							
Intercept	-2.80	1.18	-2.38	0.02			
Breed	2.01	0.79	2.53	0.01	7.5	1.6	35.5
Dewormed	1.89	0.74	2.56	0.01	6.7	1.6	28.4
128 v's 32							
Intercept	-3.53	1.35	-2.62	0.009			
Breed	1.94	0.87	2.23	0.03	7.0	1.3	38.4
Dewormed	2.66	0.82	3.25	0.001	14.3	2.9	70.9

5. DISCUSSION

5.1. Seroprevalence and incidence of Newcastle disease

The main objectives of the current study were to assess seroprevalence and incidence of Newcastle disease, to identify potential risk factors that might be associated with Newcastle disease, to characterize the outbreak in backyard chickens during wet and dry season and to investigate avian immune response towards ND vaccine (lasota) between parasitic and de-wormed chickens as well as between indigenous and improved breed.

Haemagglutination Inhibition (HI) test was used to analyze chicken sera for ND virus antibodies and the overall seroprevalence was 19.4%, (for household level) and 11.1 % (bird level seroprevalence).

However, according to recent serological surveys conducted in non-vaccinated birds in the southern and rift valley districts of Ethiopia (Zelege *et al.*, 2005b) and in central Ethiopia by Taddess *et al.*, (2005), a bird level seroprevalence varying from 22.51% to 43.68 %, respectively. Similarly, Hailu (2008) reported that 64.10% of the chickens had antibodies against Newcastle disease.

The least seroprevalence of ND in this study may be due to the fact that no intensification and dissemination of improved breed (either cross or pure exotic) since they are susceptible to the major viral disease in general and ND in particular.

From the available literature, Alexander (1993) reported that when non-immune domestic chickens and highly pathogenic strains of Newcastle disease virus meet, the common sequel is an acute disease with mortality close to 100%. This severe disease, attributed to strains of Newcastle disease virus that we call *velogenic*, which is the only form of the disease that is of consequence in village chickens. The syndromes attributable to viral strains of lesser virulence would not be noticed in village flocks. Therefore; Most of ND infected chicken had been lost due to the acute disease before serum collected. Only surviving (recovered) chicken had been included in the current serological study.

Furthermore, chicken sera were collected from only indigenous village chickens raised by randomly selected households from two completely different woredas, market-sheds and, villages. According to Tadesse *et al.* (2005) chickens were bought from local open-air markets. Sera from market had relatively high antibodies for Newcastle disease since farmers had common practice of selling of apparently healthy/ sick chicken as a measure to minimize economic loss due to the disease. Similarly, Hailu (2008) included in his study improved breeds rearing in the backyard system besides local breeds.

However, the bird level seroprevalence found in the current study concurs well with the reported mean seroprevalence of 13.8% in village indigenous chickens in varied agro-ecological zones in Kenya (Njagi *et al.*, 2010).

The other most important epidemiological occurrence of Newcastle disease in this study was household level seroprevalence, estimated using seropositivity of at least one birds in the household. The current household seroprevalence concurs well with the recent reported mean household seroprevalence of 22.7% (Chaka *et al.*, 2010) in backyard chicken flocks in Ethiopia.

The seroprevalence and incidence of ND differed across the two areas, with Jarso having a higher seroprevalence and incidence than Horo. Overall incidence of ND was 4.8% with a coefficient of variation of 7.87, indicating significantly higher incidence in Jarso than in Horo.

Similar reports by previous workers in Ethiopia (Zelege *et al.*, 2005b, Tadesse *et al.*, 2005), in Brazil (Orsi *et al.*, 2010) and in Kenya (Njagi *et al.*, 2010) supported this variation in seroprevalence. Tadesse *et al.* (2005) reported that seroprevalence rates for ND were different in the high, mid-range, and low altitudes, despite statistically insignificant difference ($P > 0.05$) in his report.

Yongolo (1996) reported a seroprevalence varying from 25 % to 81.5% in Tanzania, which had variation in different months and localities. There was a significant difference in the percentage of seropositive samples between regions in Orsi *et al.* (2010) and Njagi

et al. (2010) reports. However, the data in the current study and yongolo's report had not been aggregated to reflect occurrence in specific agro-ecological zones.

Similarly, higher incidence of ND in Jarso may be attributed to the diversity in the agro-climate and management practices such as frequency of contact with other chickens during rainy season. The current study indicated that overall incidence of ND throughout the households having chicken regularly mixing with others was 5.26% with a coefficient of variation of 21.00, indicating significantly higher incidence in chickens having contact with other chickens for more than 1 hour per day in every week comparing to those never mix at all.

In the current findings, the differences between seasons in seroprevalence were also marked. Dry season had higher seroprevalence compared with wet season.

Previous studies had associated ND incidence with change of seasons, some reportedly associated with the start of the wet season (Thitisak *et al.*, 1988; Jintana 1987), while others with hot weather. According to Chaka *et al.* (2010), more households were seropositive during dry season than wet season. Tadesse *et al.* (2005) also cited that, Newcastle was more severe in the dry season (Sonaiya, 1990), although Dessie and Ogle had stated that the disease was more widespread in the rainy season in the central highlands of Ethiopia.

5.2. Risk factors for seroprevalence and incidence of Newcastle disease

As cited by Otim *et al.* (2007), Newcastle disease is the most important disease afflicting free-range poultry but its epidemiology in village poultry is not well documented (Spradbrow, 1990; Martin, 1992; Mukiibi, 1992; Awan *et al.*, 1994). To generate information on the epidemiology of ND in indigenous free-range chickens and further support previous studies on Newcastle disease, a prospective epidemiological study on risk factors was undertaken. The prospective nature of this study makes it less susceptible to bias, and use of both questionnaires and laboratory analyses further enhances its validity.

Although a higher seasonal seroprevalence, incidence and severity of ND have been reported in the dry season in Ethiopia (Chaka *et al.*, 2011), Zambia (Sharma *et al.*, 1986) and Uganda (Mukiibi, 1992) and in winter in Bangladesh (Asadullah, 1992) and Vietnam (Nguyen, 1992), the current study gave some clue that the seasons may indirectly affect the epidemiology of ND in that there is an increase in the movement and the number of chickens in the markets during the dry season.

This made season a confounding factor that influenced the magnitude of OR of previous studies for the effects of some of the various exposure variables considered in the study. Season may not be a risk factor for Newcastle disease. Our study did not detect any significant difference in either seroprevalence or incidence of ND in the dry and rainy seasons, although there was an observed tendency for higher number of outbreaks for flocks during the rainy season compared with the dry season.

The higher percentage of seroprevalence was mainly attributed to the wave of ND epidemic at the onset of the dry season in November/December as a result of sales of chickens and increased socio-cultural activities among flock owners. These activities are reduced at the onset of the rainy season, thus limiting the Newcastle disease outbreaks that are associated with them. Spread of ND during rainy season was thus mainly due to management factors, especially proximity of households, which permits the mixing of infected and non-infected flocks.

Other factors such as flock size, feeding system, confinement, and contact with other chicken and parasitic burden may have their own contributions.

5.2.1 Housing, flock structure, feeding, contact with other chickens and confinement as house hold risk factors

Despite the limited number of households keeping chicken on veranda compared to perch on main house, the seroprevalence of ND was, surprisingly, high. This might be due to the fact that veranda is much exposed to faecal contamination excreted from migratory

wild birds. From the previous studies, migratory wild birds had been suspected as a possible source of infection in ND outbreaks under village chickens

Contrary to commercial flock, the present study showed higher seroprevalence of ND in households having less flock size compared to more flock size in household.

Similarly, recent serological survey conducted in Ethiopia by Chaka et al. (2010) reported that seroprevalence in households having less flock size was significantly higher compare to having more flock size($p<0.05$). This may be due to the fact that the major mode of transmission of ND in village chicken appeared to be by the faecal-oral route than respiratory route.

The nutritional management practiced in the study woredas is predominantly scavenging with some sort of supplementary feeding. Significantly higher seroprevalence of Newcastle disease was found for households stop to give supplement feeds during rainy season (later we called it “rain feed”) than offering more rain feed ($p<0.05$). Chickens would go far from their home to find feed; in such a way that they would have chance to contact with other chicken and migratory wild birds.

Moreover; various nutrients also have a significant influence on 'the immune system of poultry. For example, the shortage of valine (it makes 7% of poultry gammaglobuline) reduces the HI titre (Bhargava *et al.*, 1970). The total amount of proteins in feed (Mazija *et al.*, 1981) all have significant effects on the immune response to ND.

In the current study, frequently mixing of chickens was significantly associated with sero prevalence of Newcastle disease ($p<0.05$). According to Spradbrow (2000), rural poultry commonly eat faeces and other material likely to be contaminated with faeces. This is the consequence of frequently mixing with neighboring chickens or wild birds.

Confinement can be a risk factor of infection for domestic poultry, either via direct contact, or by the high density of birds favoring stress.

5.2.2. Physiological status, sex, source of restocking and parasitic burden of chicken as bird level risk factors

Some of the risk factors identified in this study may be responsible in creating uninterrupted cycles of infections whereby the virus passes from one chicken to another throughout the year. The different climatic environments and the management practices further enhance the differences in disease outbreaks within different region.

The current result was in agreement with previous studies by Kutubuddin (1973) indicated that male chickens were more affected by ND than female chicken. However, in Njagi et al. (2010) study the females had higher viral load than male chicken in all age groups. Despite higher seroprevalence in layers than chickens in brooding, the actual cause of this apparent sex related differences in NDV carriage is not yet understood.

The current study did not detect any significant difference in seroprevalence of ND, although statistically significant association had been reported by Chaka et al. (2010) between methods of restocking. However; Otim et al. (2007) identified restocking chickens from the market and the neighborhood as the most important risk factors for ND outbreaks.

There is also report that some of the ectoparasites, especially tick and mites, are vectors of other poultry diseases such as Newcastle disease, (Ruff, 1999; Denmark and Cromroy, 2006), even though the difference was not statistically significant between parasitic burden and seroprevalence ($P > 0.05$).

5.3. Characterization of outbreak

Unlike Otim *et al.* (2007); our study suggested that flock owners may not know definitely the clinical signs associated with ND, even if they considered ND as the number one chicken destructive disease. The study indicated that the flock owners called almost all poultry diseases in local name “kofis” or “fengil”. Therefore; the current study characterized the outbreaks in general other than specific ND outbreak.

The overall number of outbreaks report in this study was 162. The current study confirmed that the prevalence of poultry disease outbreak in general, was significantly higher at the main rainy season and Horo.

However, all studies reported as higher seroprevalence of ND outbreak in dry season than wet season. Even Chaka *et al.* (2010) and the current study reported higher ND seroprevalence in dry season. This seasonal variation between different studies on prevalence of the disease may be due to the fact that studies were focused on ND in particular, which is more prevalent in dry season, but all poultry disease outbreaks were included in outbreak characterization studies of the current study.

Moreover; management is the most common factor for almost all poultry diseases than other factors such as increase movement of chickens which permits only for a limited number of diseases like ND. Spread of poultry disease during rainy season is thus mainly due to management factors.

Outbreak occurs year round in most rural poultry populations, but is most common and severe at times of climatic stress and cold stress had been known to worsen the outcome of the outbreak.

Farmers also acknowledged that diseases (outbreak) were the major cause for the loss of chicken. Although the outbreaks affected chicken of different age, sex and ecotypes indiscriminately; layers and brooding hens being the most vulnerable and affected groups. This might be resulted from the stress.

5.4. Immune response

This study was designed as a multi-factorial longitudinal study, with main objective to investigate the effect of concurrent parasitic burden and breed/ecotype difference on the immune response. This study was further extended to include multi-factorial variance analysis models describing the connections between the parasite burden, the HI-titer, and the breed or ecotype.

In the present study significant association was found between the parasite burden, breed/ecotype, the anthelmintic treatment given pre-experimentally and the HI-titer measured during the experimental period.

From previous study conducted by Sofus (2003), there was a clear tendency that chickens with a high helminth infection level had a lower antibody response compared to chickens with a lower infection level and at the peak level at week two the difference in the antibody response between the different helminth infection levels was significant ($p < 0,05$).

Similarly, in the current study, when chicken were de-wormed, 6.7 times more likely to have a titre of 64 than 32, compared to chicken not de-wormed, and were 14.3 times more likely to have a titre of 128.

Helminths and viral infections are known to induce a Th2 immune response in murine. At the same time helminths also do seem to have an immunosuppressive effect on the immune response against viral infections in chickens which could indicate an immune modulation induced by the parasites or due to another kind of interaction in the immune mechanisms.

Moreover; literatures showed that individuals with concurrent infections (NDV and helmenths) had significantly lower number of lymphocytes (Dahl *et al.*, 2002), and indicates a suppression of the immune response in which the lymphocytes are a key factor (Agger *et al.*, 1995).

Breed comparative to ND antibody response profile, have not yet been isolated in chickens, and only few papers deal with concurrent infections in chickens. However, some studies tried to highlight indigenous chicken were known to possess desirable characteristics such as thermo-tolerance, resistance to some diseases, despite their low productivity,

The current study showed that indigenous chickens were about 7 times more likely to have high titres (64 or 128) compared to exotic breeds. It is clear that the breed difference had a pronounced effect on weight gain, white blood cell differential counts. Even if, the hypotheses may not have been the underlying explanation requires further investigation, weight gain and white blood cell differential counts may associate with the difference.

6. CONCLUSION AND RECOMMENDATION

Based on the available records of prospective, retrospective data, laboratory investigations, and from the questionnaire results of the poultry farmers, the current study convinced that ND was endemic in non-vaccinated, indigenous ecotype chickens in the studied areas, with minor variations in the numbers affected during the dry and rainy seasons.

More household and chickens were seropositive in dry season than wet season. Higher incidence and seroprevalence of ND was found in Jarso than Horo. Similarly, Shambu and Ejersa Goro market-sheds which are relatively accessible to transportation, marketing and increased movement of chickens from/to other areas; had significantly higher ND seroprevalence and incidence indicating that local open markets where huge numbers of chickens are gathered served as continuous foci of infection.

The current study provides an understanding of the household-level and bird-level risk factors for ND seroprevalence and incidence under different scenarios. The models suggested that the occurrence of ND in backyard household flocks is associated with different market-sheds, villages, offering of rain feed, frequency of contact with neighbouring chickens during wet season, mode of waste disposal and housing. Contrary to expectations, management practices, including confinement, flock size and supplementation, all which form circumstances favouring the maintenance of the virus in the village poultry populations, were difficult to appreciate.

Similarly; each bird level risk factors such as laying status, body weight, and body condition, source of restocking, sex, age and parasitic burden of the chicken ($P > 0.05$) did not agree with the incidence of ND.

Moreover, the current study demonstrated that the recurring wide spread mass death of chicken in Ethiopia could be attributed to disease outbreaks in the rainy season.

From the present experiment study, we concluded that there was a significant reduction in NDV specific antibody production post vaccination in non treated individuals compared to de-wormed chicken. Moreover; the findings confirmed that there is a significant dramatic reduction in NDV specific antibody production post vaccination in indigenous ecotype compared to improved breeds.

The following recommendations were made based on the results:

- Continuous sampling with retrospective study should be undertaken in the main rainy season before sudden death in order to have clear image on either season was confounding factor or not.
- It is recommended that vaccination programmes should be initiated strategically to protect chickens before the active period of NDV infections.
- It should be emphasized that in no circumstances can vaccination be regarded as an alternative to good management practice, bio-security, or good hygiene in rearing domestic poultry.
- We recommend that, besides using vaccination, extension services should educate flock owners to purchase restocking chickens not from the market but only from vaccinated flocks. Flock owners should also be discouraged from salvage sales during ND outbreaks.
- To understand the role of other domestic poultry species and migratory wild birds in the epidemiology of ND in rural free-range chickens, more prospective studies are needed, including cohort studies with larger sample sizes and similarly long periods of follow-up as in our study.
- However, although the two breeds of chickens seem to differ genetically in their immune responsiveness to the antigen/s of NDV following challenge, difference in their susceptibility to the virulent challenge virus needs further studies.

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8. Appendix (ANNEX)

Annex 1: Preparation of Phosphate buffered saline (PBS)

Reagents

- Sodium chloride NaCl 40.0g
- Potassium chloride KCl 1.0g
- Potassium dihydrogen phosphate anhydrous KH_2PO_4 1.0g
- Disodium hydrogen phosphate anhydrous Na_2HPO_4 4.6g
OR Disodium hydrogen phosphate dihydrous $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 5.75g
OR Disodium hydrogen phosphate dodecahydrous $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 11.6g
- Distilled water to make up to 5L

Method

1. Weigh out the reagents and place in a 5 L conical flask.
2. Add distilled water to make 5 L. Mix well.
3. Check pH. Adjust to pH 7.3 to 7.4.
4. Pour into storage bottles.
5. Autoclave at 121°C for 15 minutes. Use a slow exhaust.
6. Allow to cool, then tighten the lids and label the bottles.

Ten times (10 x) stock solution

If you require large volumes of PBS in your laboratory, you may find it useful to prepare a stock solution that is ten times the strength of the working solution used in the laboratory procedures. This is called a ten times stock solution, usually labeled by the abbreviation “10 x stock solution”. Before use, prepare the “working solution” by making a one in ten dilution of the stock solution.

To prepare 1 L of working solution, mix 100 mL of the stock solution with 900 mL of distilled water.

Annex 2: Two-fold serial dilutions

Step1. Use the micropipette to dispense 25 μ L of the PBS diluent to all the wells across a row of a microwell plate.

Step2. Use the micropipette to transfer 25 μ L of the test solution to the first well and mix. This is the first two-fold dilution.

Step3. Use the micropipette with the same tip to carry out a second two-fold dilution.

Step4. Continue the series of two-fold dilutions until the second last well of the microwell plate. The last well is used as a control well in the haemagglutination and haemagglutination inhibition tests.

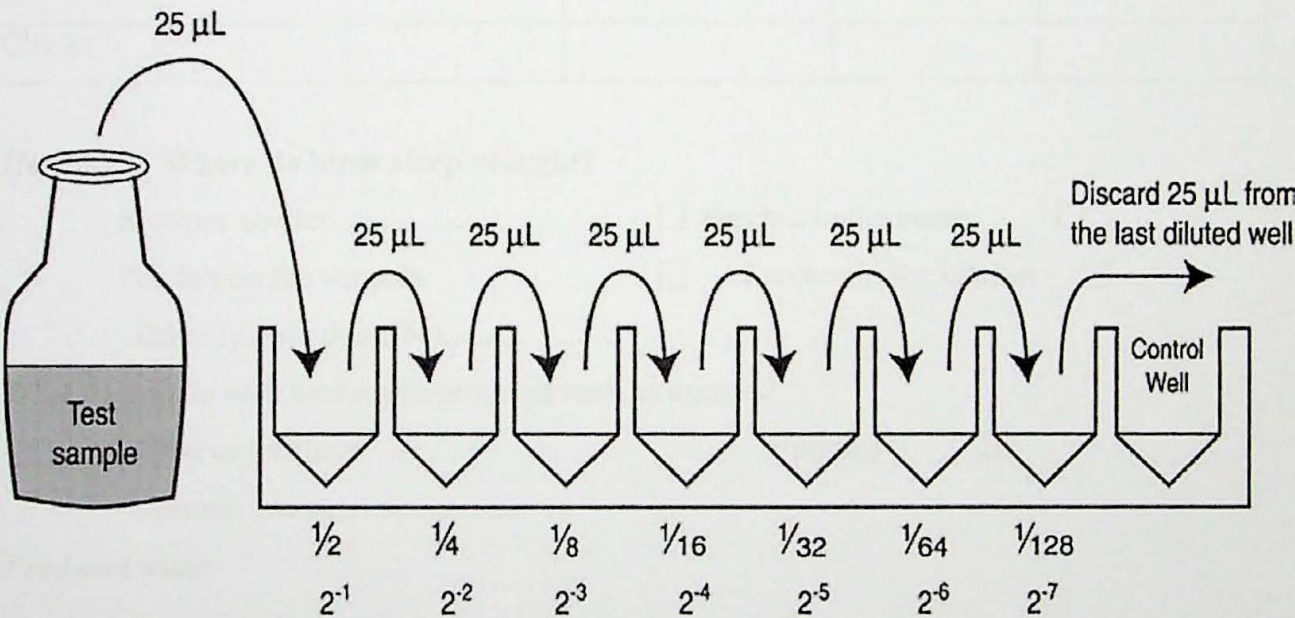


Figure 3: Two-fold serial dilutions

Annex 3: Management and Health Questionnaire

Poultry ownership, loss and selling

Poultry by Age and sex group	Number currently owned	No. lost during last 12 months due to diseases	No. lost during last 12 months due to predator/accidents	Number sold during last 12 months	Number consumed during last 12 months
Hens					
Cocks					
Pullets					
Cockerels					
Chicks					

Housing Where do birds sleep at night?

Separate shelter.....

☐ Perches in the house... ☐

Perches on the veranda

☐ Perches in the kitchen ☐

Other (please describe).....

What do you do with bird waste products such as excreta?

Used as fertilizer

Burning ☐

Thrown away.....

Feed and water

1. Do you ever provide any supplementary feed for your birds? Yes ☐ No ☐

2. Do you provide separate supplementary feed just for chicks? Yes ☐ No ☐

3. During the rainy season, do you provide: More feed..... ☐

The same amount of feed..... ☐

Less feed..... ☐

No feed..... ☐

4. Are there periods during the year when birds are confined within their housing eg planting, harvesting? Yes ☐ No ☐

If _____ yes, _____ which
months.....

Source of new birds

When was the last time you bought or were given a new bird?

Within the last 12 ☐ months..... If yes, which
month?.....

Between 1 and 2 years ago..... ☐ Between 2 and 3 years ago.....

More than 3 years ago..... ☐

Was this bird: From family, or a friend or acquaintance in your village?..... ☐

From family, or a friend or acquaintance in a different village?..... ☐

From the market?..... ☐

From another source (please describe).....

Poultry and egg selling place, target season, and reason by households

Type	Selling place (A)	Main season (month?) at which you sell them (B)
Chicken		

(A) 1=Neighbors, 2=Local collectors, 3=Near market, 4=Take to far markets 5= to
restaurants 6= others

(B) 1= Christmas, 2= Easter, 3= Ramadan, 4= "Meskel", 5= new year, 6= any time
need arise or the product available

Contact with other birds

For each season, can you grade the amount of time you estimate your birds spend in areas
where they can mix with other peoples' birds? Only consider adult birds without chicks
for this exercise.

	Not at all	Rarely	A few times a week, for	A few times a week for	Every day for less than one	Every day for more than one	Don't know

			<i>less than one hour</i>	<i>more than one hour</i>	<i>hour</i>	<i>hour</i>	
<i>Dry season</i>	<i>0</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>99</i>
<i>Rainy season</i>	<i>0</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>99</i>

Loss of birds to diseases

In the last 12 months, how many outbreaks have you had, where you lost several of your birds to the same disease?

For each outbreak, can you **write how many** birds you lost in the month the outbreak occurred and **tick which age groups** were affected for each outbreak?

For example:

	Sept		Mar
Total lost in month	4		3
Chicks	√		√
Growers			√
Adults	√		

	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	Jun	Jul	Aug
Total lost in month												
Chicks												
Growers												
Adults												

Treatment of sick birds and treatment of healthy birds to prevent disease

If you had an outbreak in the last 12 months, did you attempt any treatment of sick birds?

Yes ☐ No ☐

If yes, what did you try?

Was your treatment successful at curing the sick birds?

Completely ☐ Partly ☐ Not at all ☐

Did you give the same treatment to healthy birds to prevent them from becoming sick?

Yes ☐ No ☐

Was your treatment successful at preventing birds from becoming sick?

Completely ☐ Partly ☐ Not at all ☐

Do you use any other measures to try and reduce losses from disease outbreaks?

Sold birds before your flock was affected by disease..... ☐

Sold healthy birds after you had seen disease in your flock..... ☐

Isolated sick birds from healthy birds..... ☐

Slaughtered birds showing signs of disease..... ☐

Slaughtered healthy birds..... ☐

Use ethno-veterinary treatment(s) to prevent disease..... ☐

Other. (Please describe).....

What do you do with carcasses of birds which have died from disease?

Throw away in or close to your yard..... ☐

Bury..... ☐

Throw away far away from your yard..... ☐

Burn..... ☐

Other (please describe)..... ☐

Do you have any birds with any disease problem at the moment? Yes ☐ No ☐

If Yes, how many?.....

Have you observed any variation in disease resistance among your chickens?

☐ Yes ☐ No

Thank you very much for taking part in this survey.

Annex 4: Definitions of terminologies

The basic epidemiological unit of interest for the study is the indigenous chicken rearing in free-ranging traditional poultry production system. For the purposes of this study, some terminologies are often confusing as they are referred to as “*indigenous*”, “*native*”, “*local*” or even a “*village chicken*”.

Indigenous: Occurring naturally in an area; not introduced; native.

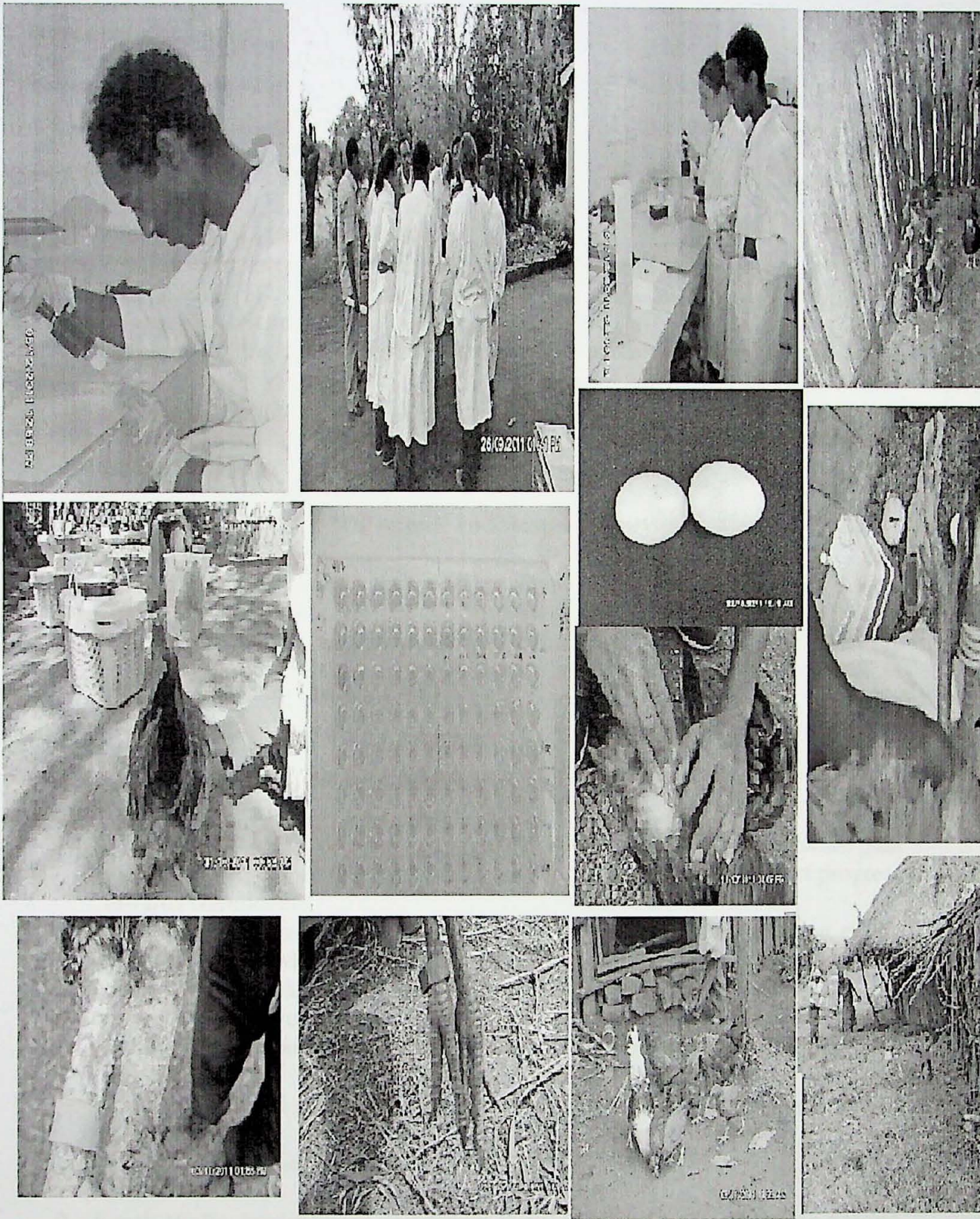
Native: belonging by birth or origin to a specified country or place; inborn; indigenous as opposed to foreign or exotic; unaffected by artificial influences.

Local: native inhabitant; of or pertaining to a place.

Household: A domestic unit comprises either one person living alone or a group of persons (not necessarily related) living at the same address with common housekeeping – that is, sharing at least one meal a day or sharing a living room or sitting room. People staying temporarily with the household are included.

Free-ranging traditional poultry production system: is a rural poultry production system which is characterized by minimal human involvement, with birds scavenging in the backyard for food, and no investments beyond the cost of the foundation stock, a few handfuls of local grain, and possibly simple night shades, but no veterinary medical attention (Tadesse *et al.*, 2005).

Appende5. Activitie during research work



9. CURRICULUM VITAE

I. Personal Information

- ❖ Name Berhanu Abera
- ❖ Sex male
- ❖ Date of birth 19/June/1985
- ❖ Marital status single
- ❖ Telephone 0911816031
- ❖ Contact address emusha84@yahoo.com

II. EDUCATIONAL BACK GROUND

- ❖ DVM degree in veterinary medicine from Haramaya University 2008
- ❖ ESLCE CERTIFICATE

III. Professional Experience

I have been working on different governmental and nongovernmental organization since 2008 G.C

1. CONCERN-Ethiopia (WOLLO-PROGRAMME)

Position: Animal health supervisor for Tis Abalima Rural Community Development Project

Duration: 14th August 2008-23rd November 2008 G.C

Major task and Responsibility

Main Purpose

- The main purpose of the post is to coordinate & implement the RCD Project Wollo Program me Livestock Health/Veterinary Service related activities in Tis Abalima and Anbasel Woredas and ensure that the project is successfully implemented according to approved donor and government agreements in close cooperation with relevant Woreda government sector offices and the community.

Key responsibility

- Jointly with the PM, develop and regularly update a detailed project work plan guiding the project implementation in the Woreda and monitor project progress towards completion.
- Closely follow up and regularly report the Livestock Health situation of the Woredas to the PM & the Area office
- Facilitate livestock health assessment and veterinary service provision in the project targeted woredas
- Maintain good coordination and networking with existing SC UK Projects in the Woreda
- Ensure goods and stocks are properly distributed and utilized for the intended purpose.
- Perform other duties as assigned by the PM

2. South Wollo Agricultural and Rural Development Organization on Strong support need woredas(main site Kutaber woreda; minor assistance Dessie zuria Woreda)

Position: chief expert in all veterinary clinics throughout the Woredas

Duration: 1st December 2008-1st November 2009

Major task and Responsibility

Main Purpose

- The main purpose of the post is facilitating effective participation of the community and government stakeholders to coordinate & implement Livestock Health/Veterinary Service related activities in such a way that it improves the community's life in favor of animals productivity.

Key responsibility

- Maintain, strengthen and promote a firm commitment community based design, planning, budget controlling, implementation, monitoring and evaluation of overall physical and financial animal health activities.
- Leads, guides, manage and motivate assistance veterinarians to ensure clear work plan and objectives; and receive quarterly supervisions and reviews.
- Prepare quality programmatic and financial reports for donor and government of zonal and regional office.
- Performing disease surveillance which is useful in measuring the efficacy of disease.
- Investigation of disease outbreak.
- Ensuring feedback to the field.

3. Amhara regional state Kombolcha Regional veterinary laboratory institution

Position: expert in epidemiology department

Duration: 12 November 2009 – Feb. 13 2010

- Maintain; strengthen serology, parasitology, and microbiology laboratory after sample is taken either from Amhara region or Afar region.
- Performing disease surveillance which is useful in measuring the efficacy of disease
- Investigation of disease outbreak
- Facilitate livestock health assessment and veterinary service provision in the region.

4. Researches

- DVM thesis for the fulfillment of Doctor of veterinary medicine Faculty of veterinary medicine, HARAMAYA University ,Duration: 2007GC

“Sero-prevalence of maedi-visna of sheep/ovine prognosis pneumonia/ in and around Debrebrihan” using AGID/Agar Gel Immuno diffusion/ technique

- DVM seminar, Duration: 2007GC
“Concept of Transgenic animal and their Medical Application”

- **Research On governmental institution**

Prevalence of lung worm and faciollosis of sheep in and around kutaber woreda financed by SIDA

5. **ON JOB TRAINING**

TOT/training of trainer/

- Training of trainer of community animal health workers(CAHWS/PARA Vet) to enable bureau of agriculture and rural development(BOARD)
- Practical training on Gumboro disease prevention from Amhara regional state kombolcha center poultry farm.
- Training on trainer organized by Amhara region bureau of Agriculture in jan 2010 by SPS-LMM/USAID funded project/and SPINAP project on Avian influenza.
- Training on small ruminant skin degrading disease to veterinarian and skin and hide industry financed by USAID./Mange ; sheep and goat pox/
- Short term training on quarantine.

TRAINER

- Give training to 46 Para vet/CAHWS/ for five woredas with in south wollo zone for two months
- Prophylactic measures and serum collection for serosurveillance of gumboro disease submitted to regional laboratory.
- Awareness creation and techniques about reporting, noticeable, and transmission of the disease to human in different region of Amhara.
- Give Practical training to assistance veterinarian and CAHWS to the eradication of mange by dipping sheep and goats with diazinol and highly participated to the program me financed by USAID.
- Mass vaccination with sheep and goat pox which is financed by USAID.
- Teaching in yardstick international collage of Distance and open learning in par time.

IV. LANGUAGE				
	reading	speaking	listening	writing
• English	excellent	excellent	excellent	excellent
• Amharic	excellent	excellent	excellent	excellent

V. **Personal skill**

Social skill:

- High level of interaction in multicultural environment, positions where communication is important and situations where teamwork is essential.

Technical skills and expertise

- Basic computer application Microsoft office (Ms-word, excels, access and power point.)

- Internet service
- Open coding
- Basic statically analysis such as SPSS
- Sun system
- Pistry accounting

VI. Reference

- Dr.zewdu belay(DVM,Msc)
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Amhara regional state Agricultural and rural development bureau
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- Pro. ElpidioG. chamizo, DVM, MSc, PHD
Haramaya university
Email:chamizopestana@yahoo.com

10. Signed declaration