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**EPIDEMIOLOGY OF VILLAGE CHICKEN DISEASES: A LONGITUDINAL
STUDY ON THE MAGNITUDE AND DETERMINANTS OF MORBIDITY AND
MORTALITY- THE CASE OF NEWCASTLE AND INFECTIOUS BURSAL
DISEASE**

MSc Thesis



By

Desalegn Jarso Modjo

**Addis Ababa University College of Veterinary Medicine and Agriculture,
Department of Clinical Study**

June, 2015

Bishoftu, Ethiopia

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DISEASE



A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis
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By

Desalegn Jarso Modjo

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Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Study

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: Desalegn Jarso entitled “Epidemiology of Village Chicken Diseases: a Longitudinal Study on the Magnitude and Determinants of Morbidity and Mortality - The Case of Newcastle and Infectious Bursal Disease” and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Epidemiology

<u>Dr. Abebe Fromsa</u>	_____	_____
Chairman	Signature	Date
<u>Dr. Bekele Megersa</u>	_____	_____
External Examiner	Signature	Date
<u>Dr. Fufa Abuna</u>	_____	_____
Internal Examiner	Signature	Date
<u>Dr. Reta Duguma</u>	_____	_____
Major Advisor	Signature	Date
<u>Dr. Barbara Wieland</u>	_____	_____
Co- Advisor	Signature	Date
<u>Dr. Fufa Abuna</u>	_____	_____
Department chairperson	Signature	Date

STATEMENT OF AUTHOR

I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Desalegn Jarso

Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June 15, 2015

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LIST OF ABBREVIATIONS

CSA	Central Statistic Agency
DA	Development Agent
ELISA	Indirect Enzyme Linked Immune Sorbent Assay
HAI	Hemagglutination Inhibition
HAU	Hemagglutinating Units
IBD	Infectious Bursal Diseases
ILRI	International Livestock Research Institute
NAHDIC	National Animal Health Diagnostic and Investigation Center
NCD	Newcastle Diseases
NVI	National Veterinary Institute
PA	Peasant Association
PBS	Phosphate Buffered Saline
RBC	Red Blood Cell

Epidemiology of Village Chicken Diseases: a Longitudinal Study on the Magnitude and Determinants of Morbidity and Mortality - the case of Newcastle and Infectious Bursal Disease

ABSTRACT

A longitudinal study was carried out from September 2014 to May 2015 on village chicken of Lume district for the aim of determining incidence rate of mortality of Newcastle disease (NCD) and infectious bursal disease (IBD) and the associated risk factors. In addition in a retrospective survey past occurrence of these disease was assessed. Simple random sampling method was used to select the peasant associations (PAs) and the households. Owners and veterinary field workers perception on chicken diseases was collected from 120 respondents through structured questionnaire. The majority (75%) of the respondents put diseases as major causes of village chicken mortality, out of which 78.3% of the respondents indicated NCD locally known as “Fangle” as the leading disease that cause mortality of chicken in the village. Of the 1358 registered chicken, 202 (14.9%) survived the entire follow-up period. A total of 843 chickens found dead of NCD outbreak during the follow-up period. The general mortality rate was 62.1% whereas the incidence rate was 113.2 cases per 1000 chicken month. Over the duration of the study, serum samples of 521 chickens were collected to confirm the cause of the outbreak, 242 from sick and 279 from apparently health chicken. Serology using HAI and I-ELISA test were conducted to determine the seroprevalence of NCD and IBD, respectively. In total 28.6% (149/521) and 20.7% (108/521) were positive for NCD and IBD, respectively. Among the 242 sera collected from clinically diseased chicken 61.6% (149/242) and 38.4% (93/242) were positive for NCD and IBD, respectively. Statistically significant ($p < 0.05$) difference in prevalence of NCD was found between highland and lowland; chicken flock size and sampling months. Statistically significant ($p < 0.05$) difference in seroprevalence of IBD was found between different age groups; household flock size and sampling months. This study has shown that NCD and IBD are one of the major infectious diseases threatening the survival and productivity of traditionally managed local chickens in East Showa zone. Thus, routine vaccination program is recommended.

Key Words: *Incidence rate, Survival analysis, Seroprevalence, Lume district*

1. INTRODUCTION

Alarming poverty has been reported in Ethiopia with food and financial crisis. Poultry is an interesting tool to respond rapidly to poverty gaps if included in rural development strategies. It has fast generation interval and high reproductive rate. It is prolific, easy to rear and their output can be generally expanded more rapidly and easily than that of other livestock. Different scales of poultry productions are available in Ethiopia: scavenging, large, small-scale and commercial. The 3 production systems have their own specific chicken breeds, inputs and production properties. Each can sustainably co-exist and contribute to solve the socio-economic problems of different target societies (Duguma, 2009).

Chicken production under backyard system has long been practiced in Ethiopia and almost every rural family owns which has been widely used for egg, meat production, other purposes (Ogle, 2001; Halima, 2007). Village chickens contribute more than 98% of the total meat and egg production in the country (Udo *et al.*, 2006). The total chicken population in Ethiopia is estimated to be 50.38 million out of which 97% is indigenous breed that are well adapted to the local environmental conditions (hot, humid, dry and rainy weather, feed and disease challenges (CSA, 2013). The majority (97%) of these chickens are maintained under this scavenging production system with no inputs for health care (CSA, 2010). In fact, 80% of the total poultry population in the world is in traditional village-based production systems, being “low input_ low output” systems (Permin *et al.*, 2000). They have deep-rooted impact in the socio-cultural and economic profile of the rural community. However, in research, extension and development agenda the village indigenous chickens are poorly considered. The commercial poultry sector which covers only approximately 3% is distributed in a limited urban and pri-urban location in Ethiopia, as it demands electricity, infrastructure and investment for intensification. It is found at an infant stage. It is constrained by high cost of input supplies such as day-old exotic chicks and feed (Duguma, 2009; CSA, 2010).

Some published information on the constraints to backyard chicken production in Ethiopia indicated, it is characterized by high mortality caused by disease, predators, and poor management and nutrition. Out of which, infectious diseases are one of the most important cause of mortality in village chicken (Tadesse, 2005; Selam and Kelay, 2013; Ashenafi, 2000). The most devastating diseases of village chicken in Ethiopia are Newcastle disease (NCD) and Infectious Bursal Disease (IBD) (Chaka *et al.*, 2013, Serkalem *et al.*, 2005; Zeleke *et al.*, 2005; Shiferaw *et al.*, 2012). An overall 32.7% and 50% mortality rates caused by NCD and IBD were reported by Mohamed *et al.* (2014) and Zeleke *et al.* (2005), respectively. The high mortality rate caused by NCD and IBD make the diseases compulsory to get priority over the other diseases. Numbers of works have been published on the seroprevalence of NCD and IBD in village chicken population. Despite the fact that the seroprevalence of NCD and IBD is increasing at an alarming rate all over Ethiopia, no works has been done so far towards estimating incidence of mortality and morbidity and identifying the associated risk factors in East Showa zone via follow up and sero-epidemiology methods. Therefore, the general and specific objectives of this study were the following;

General Objective

- To investigate incidence of morbidity and mortality of NCD and IBD in Village chicken and associated risk factors

Specific objectives

- To determine incidence rate of NCD and IBD in village chicken.
- To determine survival rate of village chicken
- To assess indigenous knowledge of farmers on the major causes of chicken morbidity and mortality in backyard production system

2. LITERATURE REVIEW

2.1. Major Causes of Village Chicken Diseases in Ethiopia

Chicken diseases of various etiologies that have more economical importance in backyard and commercial production system have been diagnosed in Ethiopia. Newcastle Disease (NCD), Infectious Bursal Disease (IBD), Marek's Disease (MD), Mycoplasmosis, Salmonellosis, Colibacillosis, Coccidiosis, Toxoplasmosis and Helminthosis are identified as the major cause of poultry diseases in Ethiopia.

Different works have been conducted in Ethiopia (Nasser, 1998; Ashenafi, 2000; Serkalem *et al.*, 2005; Zeleke *et al.*, 2005 and 2005b; Mazengia *et al.*, 2009; Chaka *et al.*, 2012 and 2013 and Belayheh *et al.*, 2014) to assess the problem of NCD in backyard and commercial production system. It is mentioned as one of the most important disease problems that are related with high morbidity and mortality in commercial and backyard chickens in most parts of Ethiopia. Similarly, from the works of others that have been done in Ethiopia (Aschalew *et al.*, 2005; Zeleke *et al.*, 2005; Woldemariam and Wossene, 2007; Mazengia *et al.* 2009 and 2010; Hailu *et al.*, 2010; Shiferaw *et al.*, 2012; Kassaa and Molla, 2012; Tesfaheywet and Getnet, 2012; Chaka *et al.* 2012), IBD is also regarded as the other most important diseases problem that is related with high morbidity and mortality of backyard and commercial chickens production in Ethiopia. From the works of Bettridge *et al.*, 2014; Duguma *et al.*, 2005; Lobago and Weldemeskel, 2004 who conducted study in backyard and commercial poultry production, MD has been identified as a problem of poultry industry in Ethiopia. Salmonella and other related infections studied by some author in Ethiopia (Endrias and Poppe, 2009; Genet *et al.*, 2014; Kassaye *et al.*, 2010 and Medina *et al.*, 2013) indicated as a problem in backyard and commercial chickens production system. In the same way, little works have been done in Ethiopia (Abadi *et al.*, 2013 and Tesfaheywet and Berhanu, 2013) to identify the problem of colibacillosis in commercial poultry farm. It has been said one of the common bacterial diseases in Ethiopia. One Study conducted in commercial poultry farm (Mersha *et al.*, 2009) identified mycoplasmosis as problem of chicken production in Ethiopia. Studies have been conducted on coccidiosis (Ashenafi *et al.*, 2004; Getachew *et al.*,

2008; Lobago *et al.*, 2005; Luu *et al.*, 2013) to identify its problem in different production system. These works indicated that coccidiosis is a disease impacting Ethiopian chicken's production. On the other hand, studies conducted on Helminthosis (Ashenafi and Eshetu, 2004; Eshetu *et al.*, 2004; Tesfaheywet *et al.*, 2012) indicated that chicken raised under traditional and small scale management system in Ethiopia are invariably infected by diverse species of cestodes and nematodes. *Toxoplasma gondii* which are widely prevalent in humans and free-range chicken in Ethiopia is also identified as the problem of poultry industry in Ethiopia (Gebremedhin *et al.*, 2014 and Tilahun *et al.*, 2013).

Although all the above mentioned diseases are the problem of chicken production in Ethiopia, NCD and IBD are identified as the major cause of chicken diseases that related with high morbidity and mortality in Ethiopia.

2.1.1. *Newcastle Disease (NCD)*

Definition: ND is caused by avian paramyxovirus serotype 1 belonging to the family Paramyxoviridae, genus Avula virus (Mayo 2002). NCD is a highly contagious and the most dreaded disease of chickens, turkeys and many other birds and can be categorized in to highly pathogenic (velogenic), intermediate (mesogenic), and less pathogenic (lentogenic) strains based on pathogenicity in chickens. The Velogenic strains of NCD virus are widely distributed throughout the world and divided in to two classes (class I and class II). Class I contains, almost exclusively, low virulence strains recovered from wild waterfowl worldwide. Class II includes strains of low and high virulence isolated from poultry and wild birds (Czegledi *et al.*, 2006).

Clinical symptom: the frequent clinical symptom of virulent NCD; chicken fluffs its feathers and appears to 'have its coat dragging on the ground, lethargy and inappetance, respiratory signs such as mild rales and snick can be detected by careful observation, severe respiratory distress and gasping, swelling of the head and neck, pink eye and swollen eyelids with abnormal accumulation of liquid, foamy discharge from respiratory tract, greenish diarrhea. When the disease is advanced nervous signs of tremor, torticollis, convulsions and paralysis of wings and legs will be seen (Czegledi *et al.*, 2006).

Transmission: the transmission of NDV occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment and clothing. The usual source of virus is an infected chicken, and spread is usually attributed to the movement of chickens through chicken markets and traders.

Diagnosis: is made by virus isolation from tracheal or cloacal swabs together with blood testing to demonstrate high antibody levels. Infectious bronchitis or infectious laryngotracheitis can give similar clinical signs, but lesions, blood tests, and virus isolation tests are decisive. In chickens NCD is characterized by lesions in the brain or gastrointestinal tract. More specific serological techniques most notably monoclonal antibody based serology, have shown the existence of considerable antigenic variation between the different strains of NCD (Ouandaogo, 1990).

Prevention and control of ND: there are three general approaches to the control of NCD: **Hygiene:** this is always important, especially in the control of NCD in semi-intensive systems where birds are confined within a fenced yard or house. Hygiene includes measures such as cleaning, disinfection, limiting access to wild birds, and personal hygiene of the farm staff. **Slaughter of infected flocks:** this is a drastic measure, which has been successfully employed in isolated regions or islands that are essentially free of the disease.

Vaccination in combination with appropriate hygiene measures: this remains the most effective way of controlling NCD (Moerad, 1987). Vaccination campaign is the only form of prevention. A proper vaccination campaign can rapidly and significantly minimize losses due to disease. In Indonesia, after a NCD vaccination campaign, mortality in village flocks dropped from 50 to 8 percent and the population of chickens increased from 900 to 3 500, representing a 250 percent increase. NCD vaccines are available in either “live” or “dead” forms: **Live vaccines** are fragile and have very precise rules for use, requiring a cold chain up to the point of application to the bird. Their effectiveness is reduced if there are residual antibodies in the chickens. **Killed vaccines** give good immunity but require priming with a live vaccine for best results, unless a natural infection has already served this purpose. They have been used successfully in Burkina Faso (Verger, 1986, and Ouandaogo, 1990). In Ethiopia, vaccination has been

reported as the only safeguard against endemic NCD. However, vaccines currently in use are mainly of benefit to commercial poultry producers whose chickens are kept in large, single-age, confined flocks. Manufacturers produce heat-labile NCD vaccines in multi-dose vials, often containing 1,000 or 2,500 doses, which must be kept cold (within 19a 'cold chain') from manufacture until administration to the chickens. In contrast, village chickens are raised in small, multi-age, free-range flocks and large multi-dose vials of vaccine are inappropriate. The cold chain is difficult to maintain under village conditions and purchase of commercial vaccines is a drain on foreign exchange (Usman, 2002).

Epidemiology of Newcastle Disease in Ethiopia

ND is endemic in the village chicken population in Ethiopia. A number of studies have been conducted to determine the prevalence of ND in different agro-ecology and season of Ethiopia (Nasser, 1998; Ashenafi, 2000; Serkalem *et al.*, 2005; Zeleke *et al.*, 2005 and 2005b; Mazengia *et al.*, 2010; Chaka *et al.*, 2012 and 2013 and Belayheh *et al.*, 2014). It was mentioned as one of the most important disease problems in backyard chickens in most parts of Ethiopia. Mortality may be very high, often reaching 50 to 100 %. The prevalence of NCD varies among years in Ethiopia. In this line, starting from 1998 the prevalence of NCD is slightly increasing from year to year as shown on figure 1.

Nasser (1998) reported 2.3% and 58.5% from state poultry farms and Dembi, respectively; Ashenafi (2000) reported 22.5% and 47.3% from Rift valley and central highland of Ethiopia; Zeleke *et al.* (2005) reported 0%, 0%, 12.9%, 16.7%, 35.9% and 47.6% from Arbegona, Shebedino, Hawassa, Butajira, Alage, Hossana, respectively; Serkalem *et al.* (2005) recorded 28.6%, 29.7% and 38.% at Debreberhan, Sebeta and Adama, respectively; Mazengia *et al.* (2010) recorded 21.7% at Bahir Dar; Chaka *et al.* (2012) reported 5.14% and 7.12% from ATGK and Adea, respectively; Nega *et al.* (2012) reported 83.8%, 90.9%, 91.7% and 95.1% from Mecha, Tillili, Ferta and Melohamusit, respectively; Chaka *et al.* (2013) reported 16.73% and 32.2% from Adamitulu gidokombolcha and Adea, respectively; Belayheh *et al.* (2014) recorded 5.6% at Kersna Kondality.

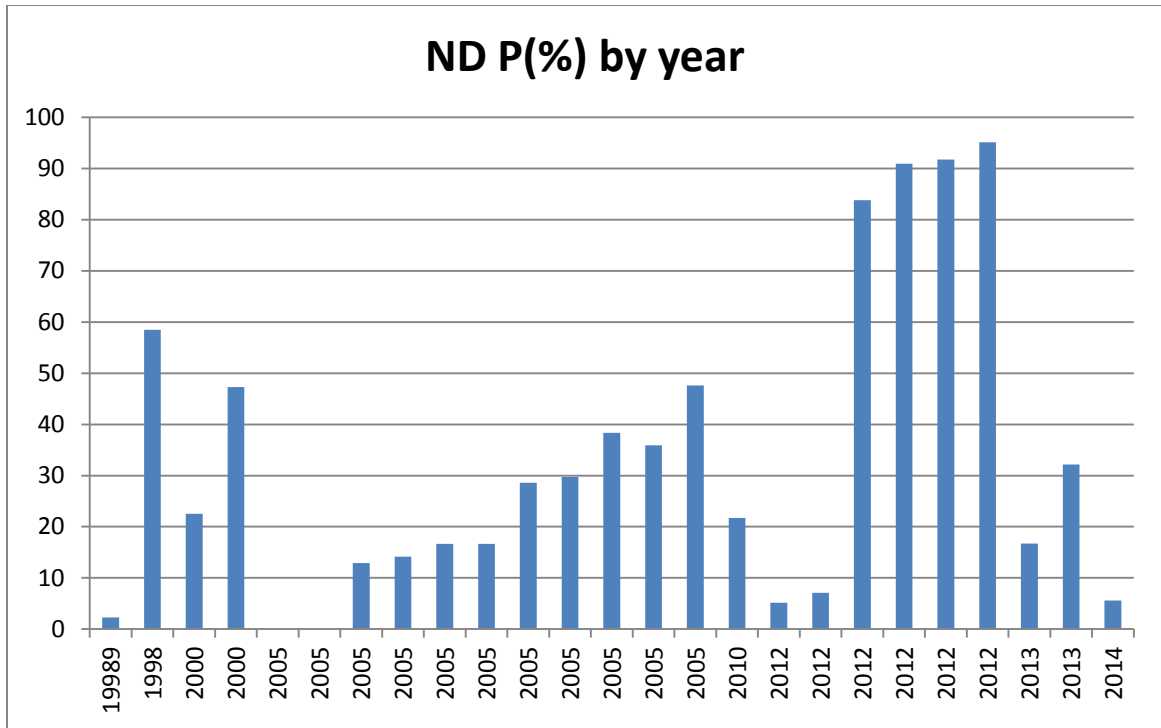


Figure1: Yearly increasing of NCD prevalence in Ethiopia (Source: synthesized by the author)

2.1.2. *Infectious Bursal Disease(IBD)*

Definition: IBD is a highly contagious, acute viral disease of poultry caused by IBD virus (IBDV). IBD is caused by the genus *Avibirna virus* of the family Birnaviridae. It is very pathogenic to chicks, although it may affect other avian species (Van den berg, 2000). Infectious bursal disease virus replicates in lymphocytes of the Bursa of Fabricius, causing the immunosuppressive and often fatal condition called infectious bursal disease (IBD) or Gumboro (Muller et al., 2003; Sapats and Ignjatovic, 2000). It is double stranded RNA virus in the genus *Avibirnavirus*. This virus may exacerbate infection with other etiologic agents and reduce the chicken's ability to respond to vaccination. Two serotypes of IBD virus strains are described: 1 and 2. Serotype 1 strain, pathogenic to chickens, is classified into several pathotypes, from mild to hypervirulent, according to their virulence. Serotype 2 strains are classified as pathogenic.

One of the most interesting features of IBDV is its ability to remain infectious for a very long period of time and its resistance to commonly used disinfectants.

Clinical symptom: Clinical IBD occurs usually between 4 and 8 weeks of age. clinical sign of include white watery droppings, ruffled feathers, loss of appetite, and a tendency to sit when forced to move and have an unsteady gait, accumulation of urate in the urinary structures, and severe depression and finally may die. The subclinical form caused by the immunosuppressive effect of the IBD virus is now of more economic importance in that the immune system of the bird is damaged. Gumboro disease related diseases such as inclusion body hepatitis are more frequent in these birds. In broilers this form of the disease results in bad performance with lower weight gains and higher feed conversion ratios (Saif and Barnes, 2003).

Transmission: the disease is transmitted through water, feed, droppings and through fomites (Sun Ming *et al.*, 2001). Some of the factors that have been associated with the maintenance of IBDV include carrier chickens, village poultry population dynamics, other poultry species, including wild birds, and heterogeneity of IBDV (Wei *et al.*, 2006; Kasanga *et al.*, 2007 and Wu *et al.*, 2007). One of the most interesting features of IBDV is its ability to remain infectious for a very long period of time and its resistance to commonly used disinfectants. Poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies, the absence of vaccination in traditionally managed chickens, and contact of chickens of 1 village with those in other villages may facilitate the spread of IBDV. The ease of contact at local open-air markets between chickens from different areas, which are then taken back to various localities, can undoubtedly facilitate the rapid spread and persistence of IBD among indigenous chickens.

Diagnosis: in acute cases the bursa of Fabricius is enlarged and gelatinous, sometimes even bloody. Muscle haemorrhages and pale kidneys can be seen. Infection by variant strains is usually accompanied by a fast bursal atrophy (in 24-48 hours) without the typical signs of Gumboro disease. Also in chronic cases the bursa is smaller than normal (atrophy). The bursa destruction is apparent on histologic examination. The lack of white blood cells (lymphocytes) results in a reduction in the development of immunity and decreased resistance of the birds to other infections. Typical signs and lesions are diagnostic of IBD. Histopathological examination, serology (ELISA) and/or virus

isolation are helpful tools. IBD can be confused with sulfonamide poisoning, aflatoxicosis, and pale bird syndrome (Vitamin E deficiency)

Prevention and control: vaccination of parent breeders and/or young chicks is the best means of control. The induction of a high maternal immunity in the progeny of vaccinated breeders, together with the vaccination of the offspring is the most effective approach to successful IBD control.

Vaccination: In-ovo injection for embryonated broiler chicken eggs at 18 days of incubation: the dose of rehydrated vaccine is 0.05 ml (i.e. 2,000 doses reconstituted with 100 ml of diluent or 5,000 doses reconstituted with 250 ml of diluent). Subcutaneous injection for day-old chicks: the dose of rehydrated vaccine is 0.1 ml (i.e. 1,000 doses reconstituted with 100 ml of diluents or 2,500 doses reconstituted with 250 ml of diluents). Dissolve completely the freeze-dried contents of the vial in a volume of diluents that complies with the size of the packaging and the route of administration (Saif and Barnes, 2003).

Epidemiology of Infectious Bursal Diseases

Currently, IBDV has a worldwide distribution, occurring in all major poultry producing areas (Tesfaheywet *et al.*, 2012). It was estimated that IBD has considerable socio-economic importance at the international level, as the disease is present in more than 95% of the OIE member countries (Etteradossi, 1995). Infectious bursal disease is a viral disease regarded as the second most important diseases of village chickens in Africa (Abdu *et al.*, 1992) following NCD. The disease imposes threat through mortality, reduced weight gain, and condemnation of carcasses due to marked hemorrhage in the skeletal muscle. It represents one of the most severe chicken diseases and is responsible for marked economic losses (Van den berg, 2000; Tesfaheywet *et al.*, 2012). Losses due to very virulent strains of the virus in Europe have reached approximately 30-40% mortality in broilers and 50-70% in commercial layers (Contreras *et al.*, 2000). IBDV infection also lowers the egg production, leads to deterioration of egg shell and internal egg quality (Moody *et al.*, 2000). The first report of IBD in Ethiopia was in 2005 involving 20–45 day old broiler and layer chickens from commercial farms (Zelege *et al.*,

2005). Since its inception, prevalence of IBD is increasing from year to year and has become a priority problem in backyard poultry production system in Ethiopia, shown in figure 2.

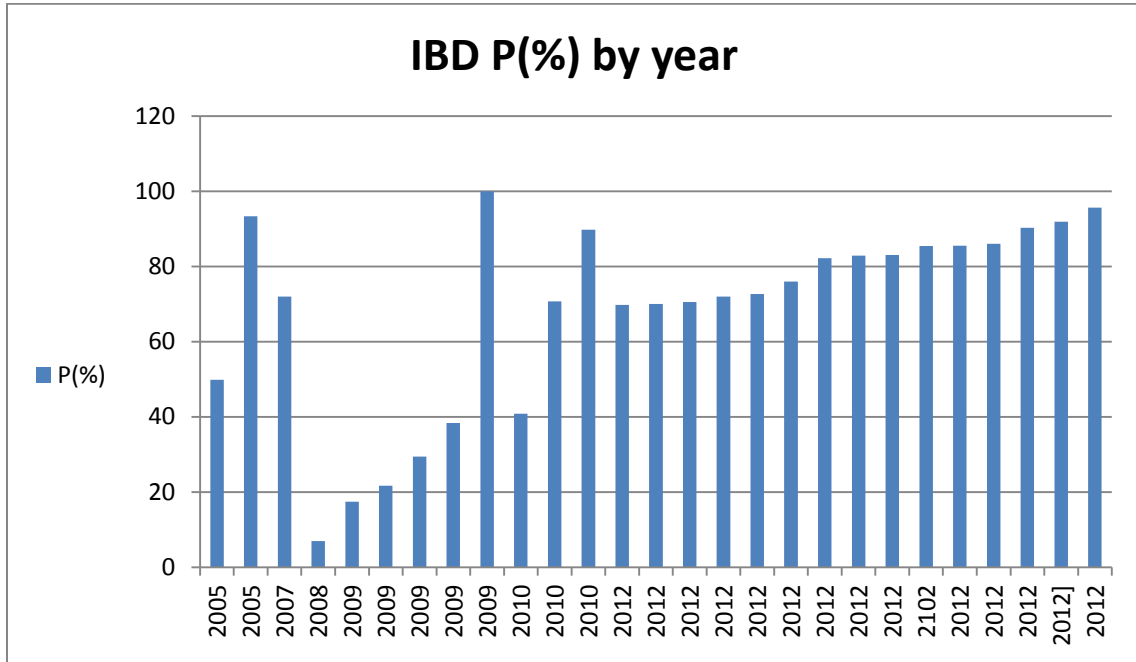


Figure2: Yearly increasing of IBD prevalence in Ethiopia (Source: synthesized by the author)

Aschalew *et al.* (2005) and Zeleke *et al.* (2005) reported 49.9% and 93.3% from debrezeit, respectively; Woldemariam and Wossene (2007) recorded 72% at Andasa poultry farm; Hailu *et al.* (2010) reported 17.4% and 38.4% from Farta and Bahir Dar, respectively; Mazengia *et al.* (2009 and 2010) reported 21.7% and 29.4% from Farta and Bahir Dar, respectively; Hailu *et al.* (2010) reported 40.8%, 70.7% and 89.8% from Welmera, Ambo and Waliso, respectively; Shiferaw *et al.* (2012) recorded 69.8%, 70.5%, 82.9%, 83%, 85.4%, 85.5%, 86% and 90.3% at Gonder, Bahir Dar, Hawassa, Adama, Addis Ababa, Kombolch, Adea and Mekele, respectively; Kassaa and Molla (2012) reported 70%, 72%, 72.7%, 76% from Dembya, Mecha, Gonder and Bahir Dar, respectively; Tesfaheywet and Getnet (2012) reported 82.2% from Debreziet; Chaka *et al.* (2012) recorded 91.9% and 95.7% from Adea and Adami Tull Gido Kombolcha (ATGK), respectively.

2.2. Determinants of NCD and IBD in Village Chicken in Ethiopia

2.2.1. Area-wide variation in prevalence of Newcastle Disease

According to existing knowledge in literature, NCD is the most dominant infectious disease which is widely distributed throughout the country as shown in Table 1.

Table 1: Prevalence of Newcastle Diseases by Area

Newcastle Disease				
Areas	No. Sampled	No. Positive	Prevalence (%)	References
Hawassa	31	4	12.9	Zelege <i>et al.</i> (2005)
Alagae	64	23	35.9	Zelege <i>et al.</i> (2005)
Adami Tulu	96	16	16.7	Zelege <i>et al.</i> (2005)
ATGK	502	84	16.7	Chaka <i>et al.</i> (2012)
Hossana	21	10	47.6	Zelege <i>et al.</i> (2005)
Butajira	18	3	16.7	Zelege <i>et al.</i> (2005)
Arbegona	43	0	0	Zelege <i>et al.</i> (2005)
Shebedino	10	0	0	Zelege <i>et al.</i> (2005)
Debreberhan	56	16	28.6	Serkalem <i>et al.</i> (2005)
Sebeta	64	19	29.7	Serkalem <i>et al.</i> (2005)
Adama	60	23	38.3	Serkalem <i>et al.</i> (2005)
Tillili	55	50	90.9	Nega <i>et al.</i> (2012)
Mecha	74	62	83.8	Nega <i>et al.</i> (2012)
Farta	72	66	91.7	Nega <i>et al.</i> (2012)
Melohamusit	81	77	95.1	Nega <i>et al.</i> (2012)
Adea	367	118	32.2	Chaka <i>et al.</i> (2013)
Kersana-Kondalaity	355	20	5.6	Belayheh <i>et al.</i> (2014)

ND is the first most endemic and prevalent chicken disease in most parts of Ethiopia. These diseases are determined as the most important causes of morbidity and mortality that resulted in periodic outbreaks with subsequent destruction of large proportion of chickens. It is possible to say that currently all areas in Ethiopia are at risk to ND.

2.2.2. Area-wide variation in prevalence of Infectious Bursal Disease

IBD is found as the second most important diseases of village and commercial chickens in Ethiopia. Continuous presence of these diseases in village poultry populations has been reported elsewhere (Aschalew *et al.*, 2005; Mazengia *et al.*, 2009; Hailu *et al.*, 2010; Kassaa and Molla, 2012; Shiferaw *et al.*, 2012; Tesfaheywet and Getnet, 2012), shown in Table 2. The distribution of improved breed of chickens from infected poultry breeding

and multiplication centers to the village is suspected of disseminating diseases to village chicken.

Table 2: Prevalence of IBD by Area

Infectious Bursal Diseases				
Areas	No. Sampled	No. Positive	Prevalence (%)	References
Farta	1316	229	17.4	Mezengia <i>et al.</i> (2009)
Bahir Dar	2593	995	38.39	Mezengia <i>et al.</i> (2009)
Waliso	186	167	89.78	Hailu <i>et al.</i> (2010)
Ambo	116	82	70.69	Hailu <i>et al.</i> (2010)
Welmera	49	20	40.81	Hailu <i>et al.</i> (2010)
Hawassa	304	252	82.9	Shiferawet <i>et al.</i> (2012)
ATGK	253	242	95.7	Chaka <i>et al.</i> (2012)
Adea	350	301	86	Shiferaw <i>et al.</i> (2012)
Mecha	50	36	72	Kassaa & Molla (2012)
Adama	430	357	83	Shiferaw <i>et al.</i> (2012)
Bahir Dar	200	141	70.5	Shiferaw <i>et al.</i> (2012)
Bahir Dar	150	114	76	Kassaa & Molla (2012)
Kombolcha	387	331	85.5	Shiferaw <i>et al.</i> (2012)
Addis Ababa	377	322	85.4	Shiferaw <i>et al.</i> (2012)
Mekele	350	316	90.3	Shiferawet <i>et al.</i> (2012)
Gonder	199	139	69.8	Shiferaw <i>et al.</i> (2012)
Gonder	150	109	72.7	Kassaa & Molla (2012)
Dembya	50	35	70	Kassaa & Molla (2012)
Debrezeit	276	227	82.2	Tesfaheywet & Getnet (2012)

2.2.3. Age-wise variation in prevalence of Infectious Bursal Disease

The magnitude of IBD is found high in young age group while the lowest prevalence's are seen in adults as shown in Figure 3. The prevalence of these diseases reaches a peak for some weeks of age group and then decline as the age increase. Tesfaheywet and Getnet (2012) reported 80.8% and 83.9% seroprevalence in chicken less than 3 weeks and greater than 3 weeks age, respectively. Hailu *et at.* (2010) recorded 87.6% seroprevalence in chicken aged 3-6 weeks. Shiferaw *et al.* (2012) recorded a prevalence of 86.6% and 72% in chicken less than 8 and greater than 8 weeks, respectively. Hailu *et at.* (2010) also reported 74.4% and 55.4% seroprevalence in chicken aged 13-24 and 25-36 weeks, respectivel. This difference could be due to the immune status of the chicken; young chickens have less immunity as compared to adult chicken.

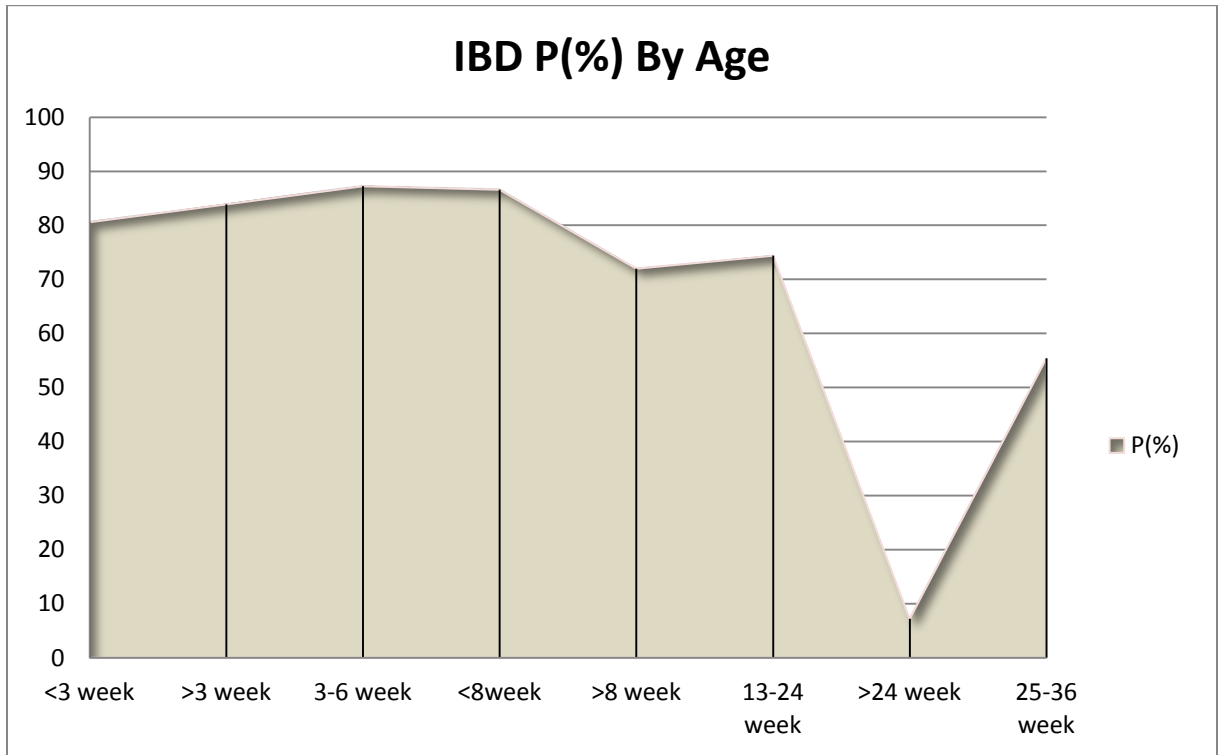


Figure 3: Prevalence of IBD in Different age category (Source: synthesized by the author)

2.2.4. Sex-wise variation in prevalence of Newcastle and Infectious Bursal Disease

Different studies have been conducted on ND and IBD to see whether the susceptibility of chickens to these diseases is influenced by the sex of chickens (Serkalem *et al.*, 2005; Zeleke *et al.*, 2005; Kassaa and Molla, 2012; Shiferaw *et al.*, 2012; Tesfaheywet and Getnet, 2012 and Belayhehet *et al.*, 2014). But no significance differences in magnitudes were observed between male and female, although some diseases are more prevalent in male than female.

2.2.5. Season-wise variation in prevalence of Newcastle Disease

In general, higher prevalence of ND is during dry season than wet season. However, rare higher prevalence of ND is also seen during wet season that may be related to Ethiopian Holidays (Filseta, Enkutatesh etc) celebrated during wet season. Human activity and

increased turnover in the chicken markets during dry season could leads to outbreaks of NCD that have been attributed to high prevalence during dry season. In many areas the villagers recognize the season when NCD will occur, or they recognize the early cases, and they dispose of their chickens by sale, thus initiating or sustaining outbreaks (Zelege *et al.*, 2005; Chaka *et al.*, 2012 and 2013 and Nega *et al.*, 2012). **Table 3** shows the prevalence of chicken diseases in different season.

Table 3: Prevalence of ND in relation to Season

Newcastle Disease					
Area	Seaso	No. Sampled	No. Positive	Prevalence (%)	References
Hawassa	Dry	31	4	12.9	Zelege <i>et al.</i> (2005)
Alagae	Dry	64	23	35.93	Zelege <i>et al.</i> (2005)
A.Tulu	Dry	95	16	16.66	Zelege <i>et al.</i> (2005)
Hossana	Wet	21	10	47.61	Zelege <i>et al.</i> (2005)
Butajira	Wet	18	3	16.66	Zelege <i>et al.</i> (2005)
Arbegona	Wet	43]	0	0	Zelege <i>et al.</i> (2005)
Shebedin	Wet	10	0	0	Zelege <i>et al.</i> (2005)
Adea	Dry	100	6	6	Chaka <i>et al.</i> (2012)
Adea	Wet	97	7	8	Chaka <i>et al.</i> (2012)
ATGK	Dry	127	27	21.5	Chaka <i>et al.</i> (2013)
ATGK	Wet	130	20	15.2	Chaka <i>et al.</i> (2013)
Adea	Dry	122	42	34.5	Chaka <i>et al.</i> (2013)

2.2.6. Breed and Altitude wise variation in prevalence of Newcastle Disease

Breed based studies conducted on NCD indicated high significant difference in NCD prevalence between local and cross breeds of chickens. Highest prevalence's (20.3%) are recorded in cross breeds of chickens than local breed (2.7%) Belayheh *et al.* (2014). Similarly, altitude influences the prevalence of NCD; the low altitudes do have higher prevalence than the mid and high altitude. Zelege *et al.* (2005) reported 22.51% and 14.13% prevalence at low altitude and High altitude, respectively. Serkalem *et al.* (2005) reported 38.33%and 28.57% prevalence at low altitude and High altitude, respectively. Similarly, 7.8%and 0.9% NCD prevalence was recorded at low altitude and high altitude, respectively by Belayheh *et al.* (2014). The possible explanation they indicated was few

chickens in the highland area of the country and chicken population number is a factor for the transmission of the disease. Another explanation may also be because of ecological variations in NCD activity and may perhaps be a reflection of the impact of environment on the speed of transmission and viability of NDV and epidemiology.

2.2.7. Production system as a factor for variation in prevalence IBD

The result of studies indicates that challenge of free ranging village poultry production and intensive poultry production system in Ethiopia. Difference prevalence's of IBD under different production system were reported. Higher magnitude (85.9%) and 81.6% was recorded in intensive and backyard production system, respectively (Shiferaw *et al.*, 2012). It has been said that this difference might be due to the fact that local breeds have better resistance to IBD as compared to exotic breeds (Aschalew *et al.*, 2005 and Shiferaw *et al.*, 2012).

2.2.8. Prevalence's of IBD in relation to different hygiene condition

The prevalence of IBD is found to be very high (83.3%) in village chicken kept under poor hygienic condition as compared to chicken kept under good hygienic condition (54.2%) . This may be due to poor management of the village chickens and high contact to the stressful external environment as compared to moderate and good management of chicken. High prevalence IBD in poor management of village chicken might also be due to frequent exposure of local backyard chickens to immunosuppression causing factors such as heat stress during scavenging seeds, water deprivation, and poor nutrition (Hailu *et al.*, 2010 and Tesfaheywet and Getnet, 2012).

3. MATERIAL AND METHODS

3.1. Description of the Study Area

The study was conducted in East Showa zone of Oromia regional state from September 2014 to May 2015. East Showa zone is located at about 98 km east of Addis Ababa that covers the total area of approximately 10241 Km² and Adama town is the capital of the zone. The Zone extends between 70°33'50"N – 90°08'56"N and from 380°24'10"E – 400°05'34"E. The temperature of the area ranges from 10⁰c in uplands to over 30⁰c in rift valley depressions with the mean temperature of 20⁰C. Since the large portion of the zone is located along the rift valley system, rainfall varies from 600mm to 1000mm with mean annual rainfall of 816 mm. The livestock population of East Showa zone is estimated to be 1,090,091cattle, 319,598 sheep, 568,761 goat, 10644 horse, 7039 mule, 284, 583 donkey, 6818 camels, 14627 beehives and 1,250, 059 poultry (CSA, 2013). Out of a total 1,250,059 poultry, around 94% (1,169,710) are indigenous poultry whereas only 6% (69,562) are hybrids (CSA, 2013).

The study was conducted in Lume District, which is one of the districts in East Showa Zone. The District covers an area of 92, 751.33 ha. Modjo is the town of the study district, located at 70 km South East of Addis Ababa with a human population of about 95,000. The average altitude is about 1880 meter above sea level. The average annual rainfall is about 839 mm and the average temperature is 24⁰C. The District has a village poultry population of 24,045 (ILRI, 2013). The soil and climate are similar to many highlands in Ethiopia. Poultry keeping is widely practiced in most rural and urban of Lume district. The area is assumed to be suitable which gave a characteristic climatic condition that is conducive for the production of chicken. Furthermore, due to the geographical proximity of the zone to capital city Addis Ababa, it has a great advantage for market access for poultry products.

3.2. Study Design

A prospective study was carried out from September 2014 to May 2015 on village chicken of Lume district for the aim of determining incidence of NCD and IBD and their associated morbidity and mortality. In addition a retrospective survey past occurrence of these disease was assessed by recall methods. Random sampling technique was used to select 6 out of 35 PA found in the district. Then, a list of farm households was prepared jointly with the community representatives, village leaders, village elders and the development agents working in the selected PA's. Finally, simple random sampling technique was employed to select 20 households from each PA, which made a total of 120 households. All chicken in a farm household was sampled as a cluster. A total of 1358 chickens from these 120 household were included in the study. The average flock size per house hold was 11.3. The sample population was unvaccinated apparently health and sick backyard chickens population of all age and sex group found in different PAs of the district.

3.3. Methods of Data Collection and Procedures

3.3.1. Questionnaire Survey

Questionnaire survey was conducted to gather owner's and veterinary field professional's knowledge of chicken diseases. In all study PAs veterinary personnel and poultry owners were interviewed with a structured questionnaire. Emphasis was given on the frequent clinical symptoms manifested; possible source of the disease; season of the year the disease commonly occurs; more affected chicken groups and history of vaccination, whenever outbreaks of poultry diseases occurred in the study PAs. Tentative diagnosis was made based on the classical disease manifestation and the epidemiological information available. A total of 120 respondents were set for the interview and to follow up their chicken throughout the study period. The respondents were provided with variables such as flock size change, major causes of chicken mortality, date of outbreaks,

major disease responsible for the mortality of chicken, seasonality of the diseases, and relation of diseases occurrence with chicken market turnover.

3.3.2. Follow up Data collection

A prospective study was conducted to determine the incidence rate, survival rate and predictors of NCD in village chicken death during the nine months (September 2014 to May 2015) of follow-up period. Chicken were visited every week and also visits were made upon argent telephone call. Records were made on chicken flock size dynamics, disease outbreaks, clinical findings and serum sample collection. Formats was prepared for recording of monthly chicken population dynamics and health status of local chickens enable to determine aspects like the incidence of diseases, mortality and morbidity rate, symptoms of the disease and season of occurrence. Data were extracted from the chicken follow up records by investigator and animal health professionals working in the PA clinics of the district. To ensure quality of the collected data one day orientation was given by the investigator to the animal health professionals, Development agents (DAs) and chicken owner. Regular visit and telephone call by the owner of the chicken, animal health professionals and DAs was the main means of communication whenever any morbidity and mortality of chicken were occurred.

3.3.3. Laboratory Investigation

Based on congregated epidemiological information, laboratory investigation of causes and determinants of morbidity and mortality of village chicken was made. Apparently health and sick chicken were observed and sample was collected. Then, determining prevalence of ND and IBD virus was done.

Blood Sample Collection: blood sample was collected from the brachial vein in 3-mL disposable syringes, left horizontally for 3hr, and then vertically for the serum to ooze out. Serum was collected in labeled 2-mL cryovial tubes and kept cool for transportation to National Animal Health Diagnostic and and Investigation Center (NAHDIC), Sebata and National Veterinary Institute (NVI). The serum in the cryovial tubes was stored at

-20°C until testing. Serum samples were analyzed using Indirect ELISA for IBD and HAI test for ND.

Serology test: Serum samples were analyzed at NAHDIC and NVI, using Hemagglutination Inhibition (HAI) test for ND and Indirect ELISA for IBD.

Indirect ELISA: IDvet innovative diagnostic indirect ELISA kit (Louis Pasteure-Grabels, France) was used to detect the presence of anti-IBD antibodies in the chicken serum following the kit manufacturers' recommended protocol. The test sera were pre-diluted by dilution buffer 14 in a pre-dilution plate according to the established protocol or kit instructions, and each was dispensed into the requested number of micro wells. In the ELISA plate pre-diluted samples and dilution buffer 14 were added and incubated for 30min \pm 3min at 21⁰C. After incubation, the sera were discarded from the plates, and each well was washed 3 times by 300 μ l of washing solution. About 100 μ l anti-chicken immunoglobulins peroxidase conjugate was dispensed into the wells and the plates were incubated for 30min \pm 3min at 21⁰C. After incubation, again the sera were discarded from the plates, and each well was washed 3 times by 300 μ l of washing solution. About 100 μ l substrate solutions were dispensed into each test well and again incubated for 15 min \pm 2min at 21⁰C in the dark place. After a final incubation, the substrate chromogen reaction was stopped by adding about 100 μ l stop solution and the color reactions were quantified by measuring the optical density of each well at 450 nm.

To check the validity of IBD ELISA result, validity test was done. In valid IBD ELISA result, the mean Optical Density (OD) value of positive control serum is greater than 0.250, and the ratio of the mean value of the positive and negative control (OD_{PC} and OD_{NC}) is greater than 3. For the interpretation of the result, serum sample positive (SP) control ratio was required. Accordingly, the following equation was applied.

$$S/P = \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{\text{PC}} - OD_{\text{NC}}}$$

If SP value was ≥ 0.3 , the IBD antibody status was considered to be positive but < 0.3 was taken as negative.

HAI test: was done according to the procedures of NAHDIC (2009). The test was carried out by running two fold dilutions of equal volumes (25 μ l) of PBS and test serum (25 μ l) in a V-bottomed micro titer plates. Four HAU of virus/antigen were added to each well

and the plate was left at room temperature for a minimum of 30 minutes. Finally 25µl of 1% (v/v) chicken RBCs was added to each well and, after gentle mixing, the RBCs were allowed to settle for about 30 minutes at room temperature. The HI titer was read from the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 25µl RBCs and 50µl PBS only) were considered to show inhibition. A titer greater than or equal to 2^3 or 3 (log to base 2) was taken as positive.

3.4. Data Analysis

Data obtained from Questionnaire, follow up and Laboratory test (HAI and Indirect ELISA) were inserted into Microsoft® Excel for Windows 2007. Analyzes were performed using SPSS statistics 20 software (2011). Survival curve over the follow up time was calculated using the Kaplan Meier and Cox Proportional Hazard Analysis method. Descriptive statistical methods were used to summarize prevalence of IBD and NCD during outbreaks, and to summarize the population characteristics of the study animals. Chi-square was used to test the presence of significant variation among the different risk factors. Univariate and multivariate logistic regression was conducted to examine the association of the risk factors with occurrence of NCD and IBD. A 95 % confidence intervals were calculated and alpha value of <0.05 was used as cut-off or for significance.

4. RESULTS

4.1. Questionnaire Survey

4.1.1. Flock size change and major causes of chicken mortality

As to chicken flock size, 95.8% (115/120) of the respondents indicated that flock size of chicken was decreased during the last 5 months while 2.5% (3/120) of the respondents indicated that flock size of chicken was increased during the last 5 months. The rest 1.7% (2/120) respondents indicated that flock size of chicken was constant during the last 5 months (Table 4).

In this study, 75% (90/120), 20% (24/120) and 5% (6/120) of the respondents indicated that the higher death of their chicken was due to diseases, predation and unknown cases, respectively. Specific chicken diseases that lead to high mortality were also mentioned by the respondents. Most of the respondents were familiar with NCD locally known as “Fengel” which was manifested by frequent clinical symptoms (greenish dropping, swelling of eyelid with abnormal accumulation of liquid, black comb and brachial vein, lowering the head down, paralysis and sudden death) during disease outbreak. Overall, 78.3% (94/120) of the respondents indicated that “Fengel” was the leading disease to cause mortality of their chicken in the village. While, 15.8% (19/120) of the respondents indicated that Fowl pox locally known as “Fentata” which has frequent clinical symptoms (nodules on the wattles comb and face) was the leading disease to cause chicken mortality. The other 5.8% (7/120) of the respondents indicated that Marek’s disease which was manifested by symptoms like paralysis of wing, dropping of limb and twisted neck was the leading cause of mortality. In relation to season of the year when the diseases frequently occur, most of the respondents (80.8%) indicated, disease occurrence was higher at dry season. However, 10% of the respondents experienced high rate of disease occurrence at the wet season. There were also respondents (9.2%) who experienced disease occurrence at any time in a year. Most of the respondents (95%) indicated that the occurrence of village chicken disease was highly related with high market turnover, especially during holyday celebration. And the rest 5% of the

respondents indicated disease occurrence was not related with market turnover, shown in Table 4.

Table 4: Answers of 120 respondents on chicken flock size and causes of chicken mortality, major diseases and seasonality of the diseases

Variables	Level	No. of respondents	Percent (%)
Chicken flock size change	Decreased	115	95.8
	Increased	3	2.5
	Constant	2	1.7
Causes of mortality	Diseases	90	75
	Predation	24	20
	Unknown	6	5
Major diseases	ND(Fangle)	94	78.3
	Fowl Pox(Fentata)	19	15.8
	MD	7	5.8
Seasonal Loss	Dry Season	97	80.8
	Wet Season	12	10
	Both Season	11	9.2
Chicken offtake	High	114	95
	Low	6	5
History of Vaccination	Not vaccinated	119	99.17
	Vaccinated	1	0.83

4.1.2. Monthly average number of chicken ownership dynamics

Male chickens suffered from population reduction in different months than the female counterparts, because, male chicken were slaughtered for home consumption during holyday and other purpose, given as a gift, sold for household income ahead of diseases outbreak occurrence. Female population reduced in only December within 5 months of follow up. Similarly, chicken population reduced in November, December and January within 5 month follow up. Female and chick population reduction was more related with diseases outbreak occurrence. NCD Disease outbreak was occurred in November, December and January during follow up period (Table 5).

Table 5: Monthly Average household chicken flock size dynamic

Chicken category	September	October	November	December	January
Male	2.46	2.21	2.33	1.83	1.21
Female	4.62	4.73	4.87	3.84	4.74
Chick	6.71	6.93	6.13	2.98	2.90
Pullet	2.05	2.08	2.13	1.70	5.19
Layer	2.57	2.66	2.76	2.17	1.60
Cockerel	1.99	1.76	1.90	1.49	0.26
Cock	0.38	.43	.43	.36	.35
Total	13.70	13.85	13.33	8.65	4.53

4.2. Survival Analysis of ND in Village Chicken

A Longitudinal study was conducted to determine the Incidence rate, survival rate and predictors of ND in village chicken death during the nine months of follow-up period. Weekly and urgent telephone call visits were made during follow up period. All of the studied sick chickens reported were new cases. Of the 1358 registered chicken, 202 (14.9%) survived the entire follow-up period. During the study period 843 chickens, which belonged to different age and sex categories, were found dead as a result of ND occurrence based on clinical signs. The general mortality was 62.1%. Of the 843 chicken died during the nine months interval, 85.5% (680/843) died within the third (November) and fourth (December) months of the start of follow up. The highest death 40.5% and 40.2% occurred in the December and November, respectively. The probabilities of chicken to die in these months were 44% and 29%, respectively. The 1358 chickens were followed for a total of 7448 chicken-months. The nine months (September 2014 to May 2015) incidence rate of NCD was 113.2 cases per 1000 chicken months. An overview of the duration of active surveillance and total chicken entered and lost to the follow up each month was presented in Table 6.

Table 6: Kaplan Meier survival analysis of ND in village chicken

Month	No. Entering Interval	No. withdrawn *	No. Exposed to Risk	Death due to ND	Probability of death in the month	Probability of Survival in the month	Cumulative Survival probability
0	1358	0	1358	0	0.00	1.00	1.00
1	1358	88	1314	9	0.01	0.99	0.99
2	1261	23	1249.5	32	0.03	0.97	0.97
3	1206	42	1185	339	0.29	0.71	0.69
4	825	106	772	341	0.44	0.56	0.39
5	378	0	378	0	0.00	1.00	0.39
6	378	10	373	10	0.03	0.97	0.38
7	358	25	345.5	36	0.10	0.90	0.34
8	297	19	287.5	59	0.21	0.79	0.27
9	219	12	118	17	0.14	0.86	0.23

*Withdrawn for sale, gift, slaughter, Predation

Area wise incidence of Newcastle Disease

In the case of different PAs the highest incidence of NCD was found at Biyo Bisike, which is a midland PA, (141.3 cases per 1000 chicken month) while the lowest incidence was recorded at Tulu Rea, which is the highland PA, (53.4 cases per 1000 chicken month), shown in Table 7.

Table 7: Incidence of NCD in different PAs of the district

Diseases	Study PA	Total chicken month	Number of Cases	Incidence rate per 1000
ND	T/Rea	1310	70	53.4
	Biyo	1401	198	141.3
	Dibandiba	1169	140	119.8
	Tade	1400	158	112.9
	K/Fatole	1065	130	122.1
	Bika	1103	147	133.3
Total		7448	843	113.2

Cox Proportional Hazard Analysis of NCD

Among the risk factors assessed in this study, flock size was significantly associated with NCD in chicken death ($p < 0.05$). However, the risk factors like age category and sexes were not statistically significant ($P > 0.05$) with NCD in village chicken death (Table 8).

Table 8: Cox proportional hazards regression analysis of NCD in village chicken

Covariates	B	SE	Wald	HR	95% CI	df	P-value
Age ≤ 4 month			1.325			2	0.515
≥ 5 to ≤ 9 month	-0.085	0.091	.863	1.0305	0.769-1.098	1	0.353
≥ 10 month	0.005	0.090	.003	1.0618	0.843-1.197	1	0.959
Sex	-0.007	.075	.009	0.8798	0.857-1.150	1	0.924
Flock size ≤ 4			38.312			2	0.000
≥ 5 to ≤ 9	-0.910	0.267	11.619	1.0632	0.239-0.679	1	0.001
≥ 10	-0.504	0.093	29.494	1.7371	0.504-0.725	1	0.002

Village chicken kept in household flock size ≥ 5 to ≤ 9 and flock size ≥ 10 were significantly (log rank = 54.958, $p < 0.05$) more likely to die as compared with those chicken kept in flock size ≤ 4 . The survival function of flock size was shown in Figure 4.

4.3. Laboratory (serology)

Over the duration of the study, serum samples of 521 chicken were collected, 242 from sick and 279 from apparently health chicken. In total 28.6% (149/521) and 20.7% (108/521) tested positive for NCD and IBD, respectively. Among the 242 sera collected from clinically diseased chicken 61.6% (149/242) and 38.4% (93/242) were positive for ND and IBD, respectively.

Seroprevalence of NCD and IBD in different PAs during disease outbreak

In the case of different PAs the highest prevalence of NCD was found at Biyo (36.2 %) while the lowest was recorded at Tulu Rea (12.5%). On the other hand the highest seroprevalence of IBD was found at Bika (24.2 %) and the lowest was recorded at Tade

(18.3%). The differences in the seroprevalence of NCD among the study PAs were statistically significant ($p < 0.05$) while the differences in the seroprevalence of IBD among the study PAs were not statistically significant ($p > 0.05$) as shown in Table 9.

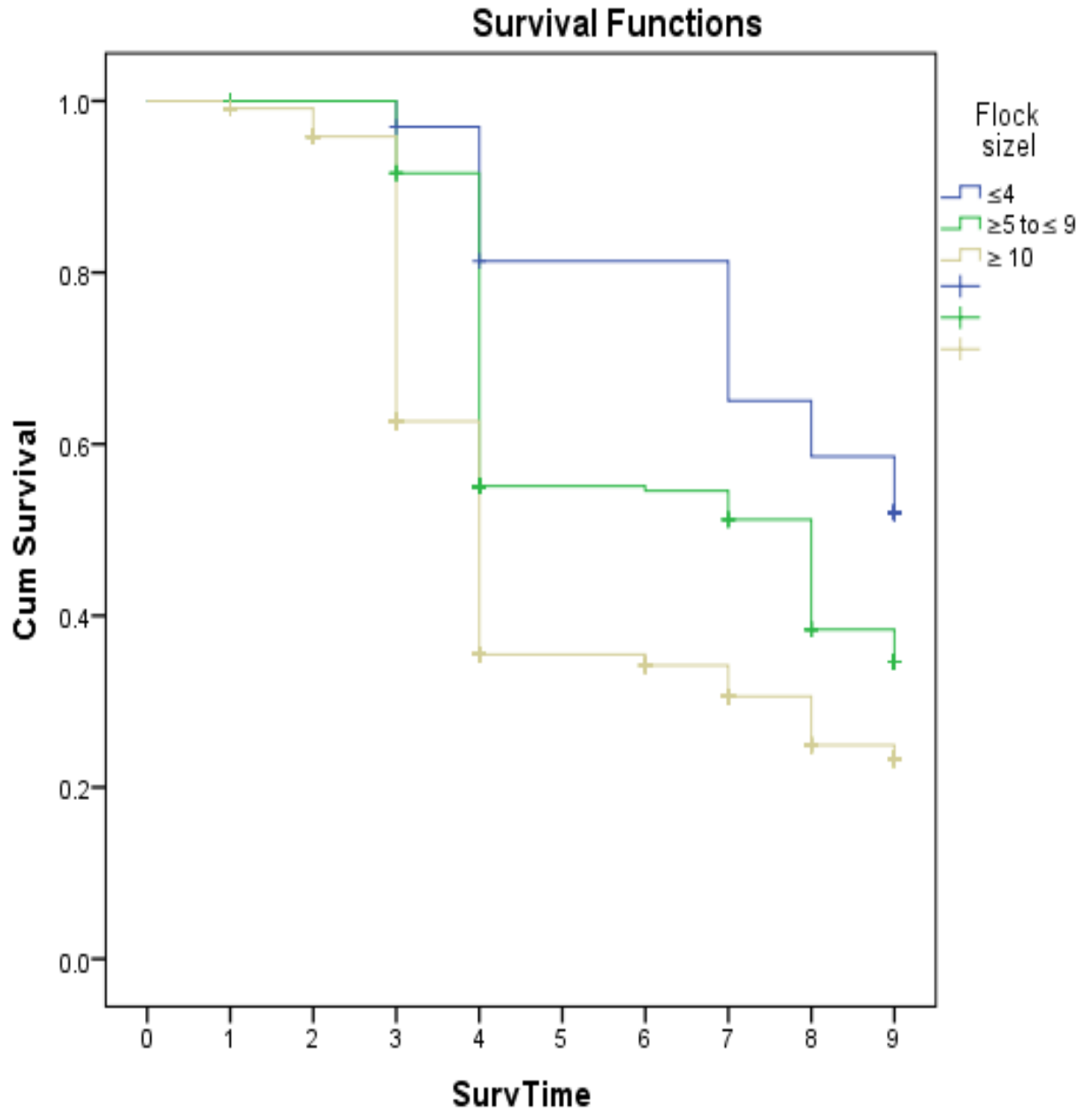


Figure 4: Survival function of ND in village chicken flock size

Table 9: Seroprevalence of NCD and IBD in different PAs of the district

Study PAs	Newcastle Disease				Infectious Bursal Disease			
	n	+ve	95 % CI	P-V	n	+ve	95 % CI	P-V
T/Rea	88	11(12.5%)	26.4-46.0	0.007	88	21(23.9%)	12.9 – 29.61	0.892
Biyo	94	34(36.2%)	5.5-19.45		94	20(23.3%)	14.89- 32.84	
Dibandiba	93	28(30.1%)	20.7-39.5		93	18(19.4%)	11.26- 27.45	
Tade	104	33(31.7%)	22.7-40.7		104	19(18.3%)	10.70- 25.75	
K/Fatole	80	21(26.2%)	16.5-36.0		80	15(18.8%)	10.12-27.38	
Bika	62	22(35.5%)	23.5-47.5		62	15(24.2%)	13.42-34.97	
Total	521	149(28.6%)	24.7-32.49		521	108(20.7%)	17.24-24.22	

n= number sampled, +ve= Number positive, CI= confident interval, P-V= P-value

Association of ND and IBD prevalence with Age, Sex, flock size, calendar month and agro-ecology during disease outbreak

The highest (29.6%) and the lowest (22.4%) prevalence of NCD was found in age groups of greater than or equal to 10 months and less than or equal to 4 months, respectively while, the highest (77.6%) and the lowest (8.4%) prevalence of IBD was found in age groups of less than or equal to 4 months and greater than or equal to 10 months, respectively. The differences in the prevalence of NCD among different age group were not statistically significant ($p > 0.05$). But statistically significant ($p < 0.05$) seroprevalence of IBD was found in different age groups as shown in Table 9. The prevalence of NCD was found high (33.3%) in flock size level greater than or equal to ten and lower (25.8%) in flock size less than or equal to four. In the case of IBD also, higher prevalence (26.6%) in flock size greater than or equal to 10 and lower prevalence (11.3%) in the flock size less than or equal to four. The differences in the seroprevalence of NCD and IBD in relation to different flock size were statistically significant ($p < 0.05$).

Different sampling months were compared to see the variation of prevalence of NCD and IBD. Accordingly, the highest prevalence (50.8%) and the lowest prevalence (0%) of NCD were recorded in April and February, respectively while the highest prevalence (69.2%) and the lowest prevalence (10.8%) of IBD were recorded in January and March,

respectively. These differences in the prevalence of NCD and IBD in relation to different sample collection months were statistically significant ($p < 0.05$).

The seroprevalence of chicken kept in lowland agro-ecology was higher (35.5%) than that of kept in highland (12.5%) in the case of NCD. The difference in the prevalence of NCD in different agro-ecology was statistically significant ($p < 0.05$).

Table 10: Prevalence of ND and IBD in relation to different determinants

Variables	Newcastle Disease				Infectious Bursal Disease			
	n	+ve	95 % CI	P-V	n	+ve	95 % CI	P-V
Sex								
Female	378	108(28.6%)	24.0-33.1	0.818	378	67(17.7%)	13.86-21.59	0.652
Male	143	41(28.7%)	21.2-36.1		143	41(28.7%)	21.22-36.13	
Age category								
≤ 4 month	49	11(22.4%)	10.6-4.2	0.588	49	38(77.6%)	65.72- 89.38	0.000
≥ 5 to ≤9month	175	50(28.6%)	21.8-35.3		175	45(25.7%)	19.21- 32.22	
≥ 10month	297	88(29%)	24.4-34.8		297	25(8.4%)	5.25- 11.59	
Flock size								
≤ 4	186	48(25.8%)	19.5-32.1	0.048	186	21(11.3%)	6.72-15.86	0.000
≥ 5 to ≤ 9	278	82(29.5%)	24.1-34.9		278	73(26.3%)	21.06-31.45	
≥ 10	57	19(33.3%)	21.0-45.7		57	14(24.6%)	13.26-35.86	
Months								
December	77	37(48.1%)	36.8-59.3	0.000	77	14(18.2%)	9.49 -26.87	0.000
January	13	3(23.1%)	0.82-47.0		13	9(69.2%)	43.06-95.41	
March	120	45(37.5%)	28.8-46.2		120	13(10.8%)	5.24-16.43	
April	118	60(50.8%)	41.8-59.9		118	28(23.7%)	16.00-31.46	
May	110	4(3.6%)	0.11-07.2		110	14(12.7%)	6.46-19.00	
Agro-ecology								
Highland	88	11(12.5%)	5.53-19.5	0.001	88	21(23.9%)	14.89-32.84	0.892
Midland	371	116(31.3%)	26.5-36.0		371	72(19.4%)	15.37- 23.45	
Lowland	62	22(35.5%)	23.5-47.5		62	15(24.2%)	13.42-34.97	

n= number sampled, +ve= Number positive, CI= confident interval, P-V= P-value

Association of risk factors with occurrence of NCD by multivariate logistic regression

Overall age category was associated with the occurrence of NCD when univariate analysis was carried out (chi square = 13.9). But up on multivariable logistic regression analysis there was no significant different in the occurrence of NCD among various age categories (Table 10). Flock size had significant effect on the seroprevalence of NCD in the study area with overall chi square of 92.46. Flocks of chicken with size greater than or equal to 10 animals per flock had an odd of having NCD that is 2.54 times higher than flocks with size less than or equal to 4 animals per flock. This difference was statistically significant. Flock size of 5 - 9 had odds of 1.14 times higher than that of those with flock size less than 4 but this difference was not significant. Months of sampling had significant effect on the prevalence of NCD in the study area. Sampling during the months of December, January, April and May gave significantly higher odds of being positive to NCD than sampling during the month of March.

Table 11: Association of risk factors with NCD by multivariate logistic regression

Determinants	B	S.E.	Wald	df	Sig.	OR
Age category ≤ 4 months			3.727	2	0.155	
≥ 5 to ≤ 9 month	-0.701	0.442	2.515	1	0.113	0.496
≥ 10 month	0.180	0.274	0.435	1	0.509	1.198
Flock size ≤ 4			9.616	2	0.008	
≥ 5 to ≤ 9	0.135	0.415	0.105	1	0.746	1.144
≥ 10	0.933	0.378	6.102	1	0.014	2.542
Sex(male)	0.066	0.272	0.059	1	0.808	1.068
Samp. Month- December			47.521	5	0.000	
January	3.796	0.590	41.349	1	0.000	44.532
March	-17.860	4184.885	0.000	1	0.997	.000
April	2.803	0.552	25.799	1	0.000	16.500
May	3.259	0.548	35.407	1	0.000	26.020
Agro-ecology Lowland			21.860	2	0.000	
Midland	-2.668	0.607	19.318	1	0.000	0.069
Highland	-0.884	0.420	4.435	1	0.035	0.413

There was significant difference in the occurrence of NCD among agro-ecology. Chicken kept in lowland and midland agro-ecology had odds of having NCD higher than chickens kept at highland

Association of risk factors with occurrence of IBD by multivariate logistic regression

Age category was significantly associated with the occurrence of IBD when multivariable logistic regression analysis was carried out (Table 12). Household flock size had significant effect on the seroprevalence of IBD in the study area. Flocks of chicken with size of 5 - 9 animals per flock had an odd of having IBD higher seropositivity than flocks with size less than or equal to 4 animals per flock. This difference was statistically significant. Flocks of chicken with size greater than or equal to 10 animals per flock had an odd of having IBD higher than that of those with flock size less than 4 but this difference was not significant.

Months of sampling had also significant effect on the prevalence of IBD in the study area. Sampling during the months of December, February and March gave significantly higher odds of being positive to IBD than sampling during the months of January and April. There was no significant difference in the occurrence of IBD among different agro-ecology. Chicken kept in all agro-ecology had similar odds of having IBD.

Table 12: Association of risk factors with IBD by multivariate logistic regression

Determinants	B	S.E.	Wald	df	Sig.	OR
Age category ≤ 4 months			65.602	2	.000	
≥ 5 to ≤ 9 month	3.653	.452	65.359	1	.000	38.596
≥ 10 month	1.029	.298	11.929	1	.001	2.799
Flock size ≤ 4			7.855	2	.020	
≥ 5 to ≤ 9	-1.375	.508	7.345	1	.007	.253
≥ 10	-.539	.425	1.611	1	.204	.583
Sex(male)	-.358	.287	1.553	1	.213	.699
Cal. Month- December			25.579	5	.000	
January	-.578	.535	1.165	1	.280	.561
February	2.188	.812	7.257	1	.007	8.916
March	1.130	.466	5.872	1	.015	3.097
April	-.608	.497	1.499	1	.221	.544
May	.658	.418	2.477	1	.115	1.932
Agro-ecology Highland			1.745	2	.418	
Midland	-.685	.570	1.441	1	.230	.504
Lowland	-.614	.485	1.600	1	.206	.541
Constant	-1.049	.774	1.835	1	.176	.350

5. DISCUSSION

A questionnaire survey on the occurrence of NCD disease was assessed via farmers' interview. From the interview, none of the chicken owner had ever vaccinated their chicken. An average household chicken flock size was found decreased in December and January during the study period. Except male chicken which suffered from population reduction in different months, the female chicken population reduced in December during the follow up period. Similarly, high population reduction of chick was also seen in December and January. The chicken population reduction during December and January was due to NCD outbreak occurred during this time. This result is in agreement with the finding of Chaka *et al.* (2013) who reported households was found their flock size reduced mainly due to diseases during dry season. On the other hand, 75%, 20% and 5% of the respondents indicated that the decreased flock size was due to diseases, predation and unknown case, respectively. This finding is in agreement with Chaka (2012) and Nega (2012) who reported 71.7% and 77.5 of the respondents indicated their flock size decreased due to diseases. These indicated that households had lost their chickens, possibly due to incidence of diseases in their flocks, among other factors. Chicken off take due to sell following occurrence of diseases outbreak, slaughter and gift also contributed to reduce flock size. The majority of the respondents (78.3%) indicated ND locally known as "Fengel" was the leading disease to cause mortality of chicken in the village. This was corroborated, in many cases, by the farmer's report of frequent diseases symptom in their flocks, and sero-positive during the sampling period. Different authors also confirmed this result; Nega (2012) and Selam and Kelay (2013) and Chaka (2012) reported 93%, 86% and 60.5% of the respondents, respectively indicated diseases, mostly ND, were the important causes for chicken mortality in village. But, in contrary with the current study, Selam and Kelay (2013) and Nega *et al.* (2012) reported 91.9% and 80.6% of the respondents, respectively indicated predator was the major cause for chicken loss in the village. In this study low percent of respondent (20%) indicated predation was a major cause of village chicken loss. This difference could be due to increased awareness of farmers to use different techniques that reduced exposure of chicken to predation. This result agrees with the findings of Taddelle and Ogle (2001) who reported disease as

the most important factor in the death of chicks. On the other hand, 80.8% of the respondents indicated, disease occurrence, specifically NCD, was higher at dry season. This result disagrees with the findings of Selam (2013) who reported 77.8% of the respondents indicated disease occurrence was higher at short and long rainy season. But it is supported by Chaka *et al.* (2012 and 2013); Nega *et al.* (2012) and Zeleke *et al.* (2005) who identified human activity and increased in the chicken market turnover during dry season could lead to outbreaks of chicken diseases particularly NCD have been attributed to high prevalence during dry season. In the current study area, the villagers recognize the season when diseases will occur and they dispose of their chickens by sale, thus initiating or sustaining outbreaks.

Longitudinal study was conducted to determine the incidence rate and predictors of NCD in village chicken death during the nine months of follow-up period. Out of 1358 chickens were registered, only 14.9% (202/1358) chicken survived the entire follow-up period. During the study period 843 birds, which belonged to different age and sex categories, were found dead as a result of NCD occurrence. Out of which, 680 chicken died within the third (November) and the fourth (December) months of the observation period. During these months, human activity and chicken market turnover was high because of holiday known as ‘‘X-mass’’. The incidence rate of mortality was 113.2 cases per 1000 chicken months. The general mortality rate was 62.1%. A comparable study conducted by Mohammed *et al.* (2014) from North Western Amhara, Biswas *et al.* (2007) and Barmon (2002) from Bangladesh reported 32.7%, 15.81%, and 21.6% general mortality rate of NCD, respectively. The mortality rate reported by Biswasin was lower than the present finding. This could be due to different climatic condition which was favorable for the transmission and occurrence of NCD in Ethiopia than Bangladesh.

Serological study was conducted to evaluate the prevalence of NCD and IBD in active clinical case and apparently health chicken during disease outbreak. Overall, 28.6% seroprevalence of NCD was recorded over the duration of the study. This is in concurrence with Zeleke *et al.* (2005) and Tadesse *et al.* (2005) who reported seroprevalence of 19.78% and 32.2%, from Southern and Rift valley districts of Ethiopia

and central Ethiopia, respectively. Similarly, Chaka *et al.* (2013) reported prevalence of 21.5% and 34.5% from Adami Tulu Gido Kombolch and Ade'a wereda, respectively. Serkalem *et al.* (2005) also reported prevalence of 28.57%, 29.69% and 38.33% from Debreberhan, Sebeta and Nazaret, respectively. This study showed NCD is one of the major infectious diseases that reduces the number and productivity of traditionally managed chickens in the study area. The data clearly indicate that local chickens kept under free-range traditional management systems in which chickens literally scavenge their own feed and water were easily exposed to NCD virus from the simply throw away of dead body of the birds in the field that might create a good ground for disease transmission.

Overall, 20.7% prevalence of IBD was found in this study. This record in agreement with Hailu *et al.* (2009) and Mezengia *et al.* (2009) who reported seroprevalence of 17.4% and 29.4% from Farta and Bahirdar, respectively. But the seroprevalence of IBD found in this study was higher than Bettridge *et al.* (2014) who reported 3.6% prevalence from Horro and Jarso, and Woldemariam and Wossene (2008) who reported 7% prevalence from Andasa poultry farm. While, it was found lower than Shiferaw *et al.* (2013) overall report of 83.1% prevalence from eight districts of Ethiopia, Hailu *et al.* (2010) reports of 76.64% seroprevalence from three districts of West and South West Showa, Tesfaheywet (2012) reports of 82.2% from Central Ethiopia. The variation in reports of IBD seroprevalence by different author in different area could be related with the dissemination of IBD through distribution of improved breed of chickens from infected poultry breeding and multiplication centers to the village chick. Related to the above rationale, lower prevalence of IBD recorded in the current study area was because, most of the households decline to rear the improved chicken breeds, and rather they entirely depended on the indigenous local breeds. Shiferaw *et al.* (2013) reported the highest seroprevalence of IBD in cross breed of chicken and the lowest in indigenous local breed of chicken.

In this study relatively higher prevalence of NCD and IBD was recorded in male chickens (28.7% for both diseases) than female (28.6 and 17.7%, respectively), however the difference was not statistically significant ($P>0.05$). This finding was similar with that of

Serkalem *et al.* (2005), Zeleke *et al.* (2005), Reta (2008), Shiferaw *et al.* (2013), Kassa and molla (2012), Tesfaheywet and Getnet (2012) who reported the absence of influence of sex on the prevalence of ND and IBD.

The seroprevalence of the IBD was found high (77.6%) in age group ≤ 4 months, 25.7% prevalence in age groups ≥ 5 to ≤ 9 months, while the lowest (8.4%) prevalence was recorded in age groups ≥ 10 months. Statistically significant difference ($p < 0.05$) was observed in the seroprevalence between different age groups. A comparable seroprevalence (86.6% and 87.26%) of IBD in young chicken was reported by Shiferaw *et al.* (2013) and Hailu *et al.* (2010), respectively. Singh and Dhawedkar (1992) reported that the prevalence was highest (61.82 %) in chickens between 7 and 11 weeks old and lowest (3.92 %) in those above 22 weeks of age. The susceptibility of chickens to IBDV is influenced by their age. The maximum susceptibility was observed between 2 and 7 weeks of age (Hitchner 1978).

A statistical significant difference prevalence of NCD and IBD was observed in different chicken flock size. The highest seroprevalence of NCD and IBD (33.3% and 26.6%, respectively) were found in chicken flock size ≥ 10 and the lowest prevalence of NCD and IBD (25.8% and 11.3%, respectively) were found in chicken flock size ≤ 4 . This difference might be due to the fact that increased chicken population number is a factor for the transmission and widely occurring of the diseases.

Different sampling months were compared to see the variation in prevalence of NCD and IBD. Statistical significant difference prevalence was found; the highest (50.8%) and the lowest (0%) seroprevalence of NCD were recorded in April and February, respectively while, the highest (69.2%) and the lowest (10.8%) prevalence of IBD were recorded in January and March, respectively. This was substantiated with the farmer's report of high diseases occurrence in the months when Ethiopian holydays celebrated (Easter in April and X-mass at the end of December). Because, during this period diseased chickens were brought from different areas and sold by traders, that could facilitated transmission and widely occurrence of diseases during this months.

The seropositivity of chicken kept in lowland agro-ecology was higher (35.5%) than that of kept in highland (12.5%) in the case of NCD. This difference in the prevalence of

NCD in different agro-ecology was statistically significant ($p < 0.05$) but no statistical significant was seen in different agro-ecology in the case of IBD. This records was agree with findings of Zeleke *et al.* (2005), Tadesse *et al.* (2005) and Belayheh *et al.* (2014) who reported a higher prevalence of NCD in the lowland than highland. Serkalem *et al.* (2005) reported a comparable results indicated, although there was no statistically significant difference between different agricultural-climatic zones in NCD virus seroprevalence rates, a relatively higher seroprevalence was observed in Lowland (38.33%) followed by midland (29.69%) than in Highland (28.57%). According to Zeleke *et al.* (2005) and Serkalem *et al.* (2005), the possible reason for this could be there are few chickens in the highland area of the country and chicken population number is a factor for the transmission of the disease. Another explanation may also be because of ecological variations in NCD activity and may perhaps be a reflection of the impact of environment on the speed of transmission and viability of NDV and epidemiology.

6. CONCLUSION AND RECOMMENDATION

A prospective study coupled with seroepidemiology could be a useful tool to assess the status of major village chicken diseases in an area and provide insight for further investigations. This study results showed that the village chicken population is endemically infected with NDV and IBD, with a high proportion of household flocks experiencing new cases. The data clearly indicated that, local chickens kept under free-range traditional management systems in which chickens literally scavenge their own feed and water in the six PAs were exposed to NCD and IBD. Massive mortality in November and December during the follow up and higher infection rate in February and April from serology indicated that there is a tendency towards higher incidence and periodic outbreaks of the disease in different seasons. NDV in household chickens pose a significant threat to the development of traditional poultry production sector in Ethiopia and IBD is also appearing as a significant threat. Based on the above conclusion, the following recommendations were forwarded;

- Improvement of village chicken production and management which is at least partly has a role on successful control of these diseases.
- Programmed vaccination at the household level could be considered to reduce the seasonal incidence and mortality of both diseases,
- Further study is warranted to better understand to characterize virus strains circulating in the study area in order to properly aid control of ND and IBD.
- Further study is necessary to understand the interactions of these infectious poultry diseases and to estimate their impact on the backyard poultry production system.

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8. APPENDIXES

Appendix 1: Questionnaire format for respondent's interview

Part I: General Information

Date _____

Region Oromia, Zone East Shewa, Woreda Lume, Peasant Association _____

Name of the respondent _____

Age of the respondent _____

Sex of the respondent _____

Level of education _____

Part II: Information related to the research purpose

1. How long have you been working with chicken keeping? _____
2. What is your source of replacement flock? _____
3. If you buy from other source, which source? _____
4. How many, which breeds and types of chicken do you have currently?

Chicken Type	Number of chicken by breed		
	Exotic	Hybrid	Local
Layer			
Pullets			
Chickens			
Cockerels			
Cocks			

5. Who is responsible for the attendance of the chicken? _____
6. Where do your chickens spend the day time (Housed/Scavenging)?
7. How far do your chickens move for scavenging and water? _____
8. Is there any interaction of the chicken with other species of wild birds? Yes/No

9. Do you encounter health problem in your chicken/Farm? _____
10. What are the major health problem affecting your chicken?

Name of Major diseases (Local Name)	Affected Age	Affected Sex	Affected Breed	Affecting Season	No of Sick	Clinical sign	No of Dead

11. Which disease causing high losses in your chicken/Farm? _____
12. What are the major losses of the disease (Production status/Morbidity/Mortality)?
13. In which types of diseases and chicken the mortality is more serious? _____
14. Do you vaccinate your chicken for these major diseases in your area? (Yes/No). If yes which one? Source of vaccine? _____
15. What do you think about other predisposing factor that facilitate for the occurrence of major disease? _____?

