SERO-PREVALENCE OF INFECTIOUS LARYNGOTRACHEITIS IN BACKYARD CHICKENS IN SOUTHERN ETHIOPIA

MSc Thesis

DEMEKE HAILU TEWOLDEMEDHIN

HAWASSA UNIVERSITY

Faculty of Veterinary Medicine

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BY

DEMEKE HAILU TEWOLDEMEDHIN

ADVISOR: KASSAHUN ASMARE (DVM, MSc, PhD)

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ADVISOR’S APPROVAL SHEET

This is to certify that the thesis entitled **“Sero-prevalence of Infectious Laryngotracheitis in Backyard Chickens in Southern Ethiopia”** produced by **Demeke Hailu** under my supervision, has fulfilled the requirements of the graduate program of Hawassa University, Faculty of Veterinary Medicine, and hence I recommend the thesis to be submitted to the department as part of the partial fulfillment of Master of Science in Veterinary Epidemiology.

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Name of Advisor Signature Date

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HAWASSA UNIVERSITY

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Name of Chairman Signature Date

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Name of Advisor Signature Date

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Name of Internal Examiner Signature Date

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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the Department of Graduate Council (DGC) of the candidate's major department. I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the thesis requirement.

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DEDICATION

I dedicate this thesis manuscript to the Almighty God, my constant guide, strength and protection during ups and downs.

STATEMENT OF THE AUTHOUR/DECLARATION

I declare that this work is my genuine work and that all sources of material used in this work have been duly acknowledged. I solemnly declare that this work will not be submitted to any other institution for the purpose of obtaining any academic degree, diploma or certificate.

Name: DEMEKE HAILU (demhai2006@yahoo.com)

Signature: ____

Place: Hawassa University, Faculty of Veterinary Medicine, Hawassa

Date of Submission:_______________________________________

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CAM	Chorioallantoic Membrane
CEO	Chicken Embryo Origin
CI	Confidence Interval
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immuno Sorrbent Assay
GaHV	Gallid Herpes Virus
ICP4	Infected Cell Polypeptide 4
ILT	Infectious Laryngotracheitis
ILTV	Infectious Laryngotracheitis Virus
OD	Optical Density
ODNC	Optical Density of the Negative Control
ODPC	Optical Density of the Positive Control
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RFLP	Restricted Fragment Length Polymorphism
ROC curve	Receiver Operating Characteristic curve
TCO	Tissue Culture Origin

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ABSTRACT

Infectious laryngotracheitis is an acute, highly contagious upper-respiratory viral disease of chickens, clinically characterized by inflammation and hemorrhage of the larynx and trachea with a significant economic impact on poultry industry globally. This cross sectional study was conducted between December, 2022 and May, 2023 using multistage cluster sampling technique to estimate the sero-prevalence of ILTV and identify potential risk factors in backyard chickens in selected districts of southern Ethiopia. Sample size was determined using a method recommended by Thrusfield (2018) for estimating the total number of households when the average sample size per household is fixed. A total of 240 serum samples were collected from chickens in 240 households and screened using an indirect ELISA test kit (IDvet, ID Screen® ILT, ILTS ver 0416 GB) to estimate the household-level sero-prevalence of ILTV. To this end, data were generated using a semi-structured questionnaire and serological screening. The overall household level sero-prevalence was 27.9% (95% CI: 22.6% – 34.0%). The highest prevalence was recorded in the midland agro-ecologic zone 32.9% (95% CI: 23.8% - 43.6%) followed by the lowland 28% (95% CI: 19.0% - 39.2%) and the highland 22.5% (95% CI: 14.6% – 33.0%). Among the hypothesized risk factors, namely, location (district/kebele), agro-ecology, age, sex, breed and flock size, only age and flock size were identified as potential risk factors for ILTV prevalence in backyard chickens ($p < 0.05$). In line with this, the prevalence was high in adult birds (OR: 2.6, 95% CI: 1.3 - 5.2) and among birds in larger flock (OR: 4.3, 95% CI: 2.3 - 8.0). The study also indicated that the backyard chicken husbandry practice was highly risky in the transmission and maintenance of ILTV due to poor biosecurity and management practices. Thus, this study suggests the disease is prevalent in the study area and demands control intervention.

Keywords: Backyard Chicken, Infectious Laryngotracheitis, Sero-prevalence, Southern Ethiopia

1. INTRODUCTION

Poultry farming plays an important role in the global food security, however the consequence of globalization, climate change and rapidly expanding poultry population are leading to the emergence of several diseases (Gowthaman *et al*., 2020). Among the emerging diseases, Infectious Laryngotracheitis (ILT) has been identified as a major concern for poultry health and welfare globally (Bagust *et al*., 2000).

Infectious Laryngotracheitis is one of the acute contagious respiratory viral diseases of chickens caused by Gallid Herpes virus-1 (GaHV-1) which belongs to the genus Iltovirus, within the subfamily Alphaherpesvirinae of Herpesviridae family (Davison, 2010). Clinically, the disease is characterized by inflammation and hemorrhage of the larynx and trachea (Craig *et al*., 2017) and the lytic infection of the disease causes increased serous or mucoid or mucopurulent ocular discharges, frequent coughing, gasping, dyspnoea with hand pump type of respiration and mortality in severe cases (Coppo *et al*., 2013).

Infectious Laryngotracheitis was first described by May and Tittsler in the United States in 1925 (as reviewed by Wolfrum, 2020) and was the first avian viral disease against which an effective vaccine was developed (Munuswamy *et al*., 2019). It was first identified as Avian Diphtheria and/or Infectious Bronchitis, however the name ILT was adopted in 1931 by the Special Committee on Poultry Diseases of the American Veterinary Medical Association (Garcia *et al*., 2013) and now it is considered as one of the World Organization for Animal Health (WOAH) mandatorily notifiable disease.

Infectious Laryngotracheitis causes great economic losses due to mortality, decreased egg production, weight loss and susceptibility to infections with other aviary pathogens; however, the overall economic significance of ILT has not been precisely determined (Guy and Garcia, 2008). In acute forms, egg production is significantly impacted and mortality may reach as high as 50% in adult birds infected with virulent strains, or 10-15% in milder strains (Wernery, 2016). Recent studies around the world (USA, Brazil, Norway, Palestine, Australia) showed that ILT is responsible for egg-laying losses that exceed 30% in layer hens, however, milder strains of ILT only confer slight

reductions in egg production and average daily gain, with very low mortality and only minor clinical signs (Parra *et al*., 2016). According to Kirkpatrick *et al.* (2006) average daily gain in affected birds might be reduced by up to 14%.

Infectious Laryngotracheitis has been reported as an endemic disease worldwide, particularly in areas of large poultry concentration and intensive production, including Asia, North America, Central America, the Caribbean, South America, Europe, Africa and Oceania (Hidalgo, 2003; Chacón & Ferreira, 2009). In Africa, ILT outbreaks, molecular and serological evidences had been mentioned in the last two decades, in Morocco (El Houadfi *et al*., 2005), Egypt (Shehata *et al*., 2013; Abdo *et al*., 2017; Magouz *et al*., 2018; El-saied *et al*., 2021), Nigeria (Owoade *et al*., 2006; Shittu *et al*., 2016), Tunisia (Kaboudi *et al*., 2016), Namibia (Molini *et al.,* 2019), Libya (Kammon *et al*., 2020) and Algeria (Ramdani *et al*., 2021; Salhi *et al*., 2021). As a result of recent ILT outbreaks in Namibia alone, over 200,000 commercial poultry (layers and broilers) were lost and a mortality of 10% reported during the outbreak months of July, August and September, 2018 (Molini *et al.,* 2019).

In Ethiopia, in addition to the challenges posed by endemic diseases with high morbidity and mortality, poor biosecurity has also increased the emergence of different viral diseases including ILT in the poultry industry (Tesfaye *et al*., 2019). Among all others, the circulation of ILTV in the backyard and commercial production system was confirmed through limited studies conducted in the last five years. The accrued serological surveys showed an overall ILTV sero-prevalence of 19.4% in south and central Ethiopia (Tesfaye *et al*., 2019) and 54.7% in Ada'a district (Bishoftu town) (Roba *et al*., 2020). In addition there has been a report of 50.5% in central Ethiopia (Habte *et al*., 2022) and 59.1% in North Western Ethiopia (Birhan *et al*., 2022). Furthermore, Berhanu *et al*. (2019) reported the first ILTV outbreak in exotic layer chickens managed under semi-intensive production system between the outbreak months of June and July, 2018 in Hawassa zuria district, Sidama region, through postmortem and histopathologic examinations. Above all, very recent virus isolation and molecular investigation conducted by Galana, *et al*. (2023) indicated the circulation of three ILTV strains namely ICP4, TCO low and TCO high in small and medium scale intensive poultry farms in and around Bishoftu town and Liban Chuqala districts. Therefore, the information gap noted due to the limited report in the country substantiates the need for additional evidence in backyard chickens. The production system in question become the focus of attention from an epidemiological point of view due to high prevalence, prevailing poor biosecurity and carrier nature of infection survivors (Langeroudi *et al*., 2020; Roba, *et al*., 2020; Birhan, *et al*., 2022; Habte, *et al*., 2022). Following the

central part of the country, southern Ethiopia is one of the growing poultry producing regions of the nation. Therefore, this study conducted in selected districts in southern Ethiopia could provide further epidemiological evidence to fill gaps in the existing national data.

1.1. Significance of the Study

The outcome of the study may provide additional evidence on ILT in Ethiopian backyard poultry production and possibly initiate the control intervention. The study may also help field veterinarians to anticipate ILTV independently or as co-infection in any of chicken respiratory syndrome in the study area.

1.2. General Objective

The general objective of this study was to estimate the sero-prevalence of ILTV in backyard chickens in southern Ethiopia and identify the potential risk factors for the occurrence and distribution of the disease.

1.3. Specific Objective

The specific objective of this study was to estimate the sero-prevalence of ILTV in backyard chickens in different agro-ecological zones in selected districts of southern Ethiopia and identify potential risk factors for the occurrence of ILTV in the study area.

1.4. Scope of the Study

This study was limited to focus on the sero-prevalence and associated risk factors of ILTV in backyard chicken in southern Ethiopia due to the recently rising evidences on emerging ILTV in different regions of the country.

2. LITERATURE REVIEW

2.1. Etiology

The etiological agent of ILT is a pneumotropic virus of the family Herpesviridae, genus Iltovirus, taxonomically classified as Gallid Herpesvirus-1 (King *et al*., 2012). Based on DNA sequence analysis, the virus has been shown to be genetically distinct from other Alpha-herpes viruses that led to its recent classification as a single member of the genus Iltovirus (McGeoch *et al*., 2000).

The GaHV-1 virion has a hexagonal nucleocapsid with icosahedral symmetry surrounded by a protein tegument layer and encapsulated by an outer envelope containing virus encoded glyco-proteins (Garcia and Spatz, 2020). Several envelope proteins protrude from the surface of the virus, consisting of the specific glycoprotein B(gB), gC, gD, gH, gJ, gM, and gN which are glycosylated and constitute the primary virus antigens responsible for stimulating humoral and cell-mediated immune responses (Fuchs *et al*., 2007).

The size of the virion varies between 200 and 350 nm in diameter with a constant capsid diameter of 100 nm (Granzow *et al*., 2001). The ILTV genome is a linear double stranded DNA approximately 150 killobase pair that encodes 80 viral proteins (Menendez, *et al*., 2014) and is organized in four distinct regions, unique long (UL) and unique short (US) which were also flanked by two inverted repeats, internal repeat (IR) and terminal repeat (TR), typical of the genomic arrangement of type D herpesvirus (Guy and Garcia, 2008).

2.2. Epidemiology

Infectious Laryngotracheitis virus has a narrow host range, with the chicken being the only major host for which no other reservoir species recognized, even though pheasants and peafowl can sometimes be naturally infected by contact with chickens actively shedding ILTV (Guy and Bagust, 2003). Although chickens older than three weeks of age are reported to be highly susceptible to this disease (Dufor-Zavala, 2008), the most characteristic symptoms and lesions are observed in adult chickens (Munuswamy *et al*., 2019).

Infectious Laryngotracheitis virus strains are antigenically homogenous regardless of variation in virulence in chickens (Kirpatrick *et al*., 2006). In conformity to early findings of antigenic homogeneity of GaHV-1 vaccine strains and field isolates, latest studies showed the antigenic

homogeneity to be endured towards the newly evolving field isolates because of the use and effective protection of the same attenuated GaHV-1 vaccines (as reviewed by Garcia and Spatz, 2020). Hence, despite differences in the virus strains and virulence, the existence of ILTV as a single serotype is an advantage for control through vaccination and diagnosis serologically (Jones, 2010).

Even though, there is no evidence for trans-ovarian or vertical transmission in chickens (Bagust *et al*., 2000), natural horizontal transmission occurs by direct contact via the upper respiratory and ocular routes and/or by oral route mechanically through contaminated equipments and exposure to improperly disposed litter, manure and carcasses (Dufour-Zavala, 2008). Sources of ILTV infection include chickens clinically affected or latent carriers, contaminated dust, litter, beetles, drinking water, fomites and poultry farm personnel contaminated with ILTV (Hidalgo, 2003; Ou and Giambrone, 2012).

To date, there is no evidence for the transmission of GaHV-1 to human beings and other mammals, however, birds exposed to live virus or vaccine remains latently infected and shed the virus asymptomatically when reactivated under stress–conditions such as onset of laying or transfer and mixing of flocks (Garcia and Spatz, 2020). The virus establishes a latent state in the trigeminal ganglion of the nervous system after 7 days of acute infection to evade the hosts immune defense, leading to the persistence of ILTV in infected or recovered chicken flocks (Munuswamy *et al.,* 2019) and are recognized as potential sources of infection for their lifetime (Bagust *et al*., 2000).

The incidence of ILT is increasing in many parts of the world, which may be due to the use of modified live ILTV vaccines (Tamilmaran *et al*., 2020). At present, there are two main types of modified live vaccines extensively used for controlling ILT outbreaks worldwide, that are produced through sequential passages in embryonated chicken eggs (Chicken embryo origin, CEO) and in tissue culture (tissue culture origin, TCO), in which most ILTV outbreaks are believed to be caused by CEO vaccine isolates that had the tendency to revert to virulence following bird to bird passages (Gowthaman *et al*., 2020) and that persist in long-lived bird operations and spill-over into poultry populations (Blacker *et al*., 2011). Recently, ILTV vaccines based on recombinant DNA technology have also been developed that cause no latent infection, not reverted to virulence and are safer than live attenuated vaccines, despite their use being limited by their high cost and the need for injection (Ou & Giambrone, 2012) as well as their limited usefulness in mass vaccination and the likelihood of outbreaks due to its failure in limiting shedding of the challenge virus (Munuswamy *et al*., 2019).

Inactivated ILTV vaccines are also not considered suitable for immunization of large chicken flocks due to the high costs associated with their production as well as the high costs and inconvenience of parenteral administration (Fuchs *et al*., 2007). Therefore, it is common to use live attenuated ILTV vaccines only in endemic areas (Garcia & Zavala, 2019). Some of the other risk factors that have contributed to the increase in ILT incidence globally include the trend towards producing denser poultry populations in shorter cycles and rearing different types and ages of poultry in the same area combined with lax biosecurity (Garcia and Spatz, 2020). Guy and Bagust (2003) also mentioned that concentration of farms in a given geographic site as a predisposing factor for the spread of the virus through wind. The greatest incidence of the disease however is generally seen in areas of highly intensive poultry production and serious outbreaks occur periodically whenever ILTV spreads from persistently infected flocks into flocks of unvaccinated chickens (Bagust *et al*., 2000).

Although, the outcome of infection depends on the virulence of the strain and co-infection with other respiratory pathogens (Gowthaman *et al.,* 2020), the severe epizootic forms caused high morbidity (90 to 100%) and variable mortality (5% to 70%); and the mild form resulted in a mortality rate of less than 2% (Guy and Garcia, 2008). The incubation period is usually 6 to 14 days following natural infection and 3 to 7 days post inoculation under experimental condition (Oldoni *et al*., 2009). However, most chickens generally recover within 10 to 14 days (Guy and Bagust, 2003).

Different ILTV strains have different heat resistance and at lower temperatures the virus maintains infectivity for a longer period of time. In addition, the virus survives for several days or months at temperatures between 13-23^{0}C in tracheal exudates and chicken carcasses or for months and years when stored at temperatures between -20 ^{0}C to -60 ^{0}C (Ou and Giambrone, 2012) and can also remain detectable in swabs left at different temperatures (room temperature, 0°C and 5°C) for 3 to 14 days and in contaminated litter for 3 to 20 days (Dufour-Zavala, 2008). On the other hand, ILTV can easily be inactivated by low heat or by freeze-thawing (in a medium which is free of organic materials or protein) and lipolytic agents such as ether, chloroform and chemical disinfectants derived from coal tar, formaldehyde, hypochlorite and iodophor (as reviewed by Bagust *et al*., 2000). Besides, heating the litter to 38 ^{0}C for 24 hours and composting it for 5 days reduces the virus concentration below the detection limit (Ou and Giambrone, 2012)

2.3. Pathogenesis

Infectious Laryngotracheitis virus shows tissue tropism towards the epithelia of the respiratory tract and the eyes. In the respiratory system, the epithelial cells lining the larynx and trachea are always affected, while other mucous membranes, respiratory sinuses, air sacs and lung tissue may or may not be affected depending upon the route, infective dose and sequelae of infection. (Munuswamy *et al.,* 2019).

The ILTV enters the upper respiratory tract and conjunctival mucosa via aerosol or ocular or ingestion route and establishes initial infection. Primarily, ILTV multiplies in the epithelial cells lining the upper respiratory tract including the nasal sinuses, naso-lacrimal duct, larynx, trachea and epithelium of conjunctival mucous membrane resulting in severe mucosal epithelial damage and necrosis due to cytolytic infection with associated local acute inflammatory reaction. The viral load at the primary multiplication peaks between 4 and 6 days post-infection and the virus can be detected in the trigeminal ganglion as early as 2 days during acute phase of cytolytic infection (Oldoni *et al*., 2009). However, no clear evidence exists for a viremic phase of infections (Hidalgo, 2003).

Envelope glycoproteins mediate entry of the virus into host cells by attaching to cell receptors and fusing the viral envelope to the cell membrane. After fusion of the viral particle to the cell membrane, the viral nucleocapsid is released into the cytoplasm and transported to the nuclear membrane, the nucleocapsid then released viral DNA and migrates into the nucleus where transcription and replication of viral DNA occurs.(Guy and Garcia, 2008).

2.4. Clinical Signs

Two main forms of ILT have been described in chicken under field conditions, the severe acute or epizootic form characterized by significant respiratory distress, nasal discharge, depression, sneezing, gasping, marked dyspnea, expectoration of bloody mucous, severe haemorrhagic tracheitis and conjunctivitis with closed eyes accompanied by high mortality ranging from 5% to 70%; and a milder form characterized by mild to moderate catarrhal tracheitis, sinusitis, persistent conjunctivitis, watery eyes, swelling of infraorbital sinuses, decreased egg production, weight loss, relatively low morbidity and occasional mortality which usually ranges between 0.1 and 2% (Ou and Giambrone, 2012).

2.5. Pathology

Gross pathological lesions are most consistently observed in the larynx and trachea, though the conjunctiva and other respiratory tissues could also be affected (Guy and Garcia, 2008). Edema and congestion of the epithelium of the conjunctiva & infraorbital sinuses may be the only gross lesions observed in mild forms, while in severe forms mucoid inflammation, degeneration and necrosis of the trachea, diphtheritic changes that may be seen as mucoid casts that extend the entire length of the trachea may be present (Hidalgo, 2003).

Early microscopic changes in tracheal mucosa include the loss of goblet cells and infiltration of inflammatory cells into the mucosa. As the viral infection progresses, multinucleated cells (syncytia) are formed and after 2-3 days lymphocytes, histiocytes and plasma cells migrate into the mucosa and sub-mucosa. Subsequent destruction and desquamation of cells result in a mucosal surface either covered by a thin layer of basal cells or lack any epithelial covering (Guy and Garcia, 2008). Edema and infiltration of inflammatory cells (lymphocytes, histiocytes and plasma cells) can be observed in the lamina propria of the affected mucosa (Coppo *et al*., 2013).

Microscopic lesions characteristic of Alphaherpes virus infections are the formation of syncytia and intra-nuclear inclusion bodies in the mucosal epithelium (Ou and Giambrone, 2012). Inclusion bodies are usually present in the early stages of infection (1 to 5 days post infection) and disappear as infection progresses as a result of necrosis and desquamation of epithelial cells (Guy and Bagust, 2003).

2.6. Diagnosis

Presumptive diagnosis of ILT can reliably be made in severe acute disease based on the high mortality, accompanied by typical symptoms of the disease such as expectoration of bloody mucus. Otherwise, diagnosis should be based on confirmatory laboratory procedures (Craig *et al*., 2017) such as histo-pathological examination of the trachea, detection of anti-ILTV antibodies, detection of ILTV antigens in tracheal tissues or respiratory mucus and detection of the virus (Bagust *et al*., 2000).

2.6.1. Histo-pathological examination

Infectious Laryngotracheitis virus infection can be diagnosed on the basis of the development of pathognomonic histo-pathological changes in the respiratory and conjunctival epithelial cells. The

detection of multinucleated syncytial cells with intra-nuclear eosinophilic inclusion bodies and the presence of inflammatory cells consisting of heterophils, lymphocytes and macrophages in the larynx, trachea and conjunctival mucosa are diagnostic features of ILTV infection (Guy and Garcia, 2008).

Histo-pathological examination of fixed tissues is the most commonly used rapid test for the diagnosis of ILTV, however visualization of inclusion bodies in tissues has been shown to be less sensitive than virus isolation, as intra-nuclear inclusion bodies only appear in the early stages of infection (Guy and Bagust, 2003)

2.6.2. Detection of anti-ILTV Antibodies

A variety of techniques have been described to detect the presence of specific anti-ILTV antibodies in serum including serum-virus neutralization assays, agar gel immuno-diffusion techniques, indirect fluorescent antibody tests and enzyme-linked immunosorbent assay (ELISA) (Bagust *et al*., 2000). The ILTV-specific neutralizing antibodies can be detected in serum within 5 to 7 days of tracheal infection, however the antibody titer peaks 21 days post-infection and decline over the following months before remaining low for a year or longer (Munuswamy *et al*., 2019).

Even though, other than detecting infected chickens, serology cannot differentiate between carrier and infected chickens or vaccine and field strains, agar gel immuno-diffusion was found to be the least, while ELISA and virus neutralization to be equally sensitive (Garcia and Spatz, 2020). ELISA test was the most commonly used method because it is simple to perform, can test large number of sera quickly and easily and requires very little serum (Hidalgo, 2003). Recently, an ELISA that utilized a recombinant Escherichia coli was developed to detect ILTV-specific antibodies expressing ILTV glycoproteins, gE and gp60 (Chang *et al*., 2002). This recombinant-based ELISA has been shown to distinguish between ILTV vaccinated and unvaccinated or unexposed chickens, but sensitivity and specificity have not been reported (Guy and Garcia, 2008).

The diagnosis of ILT is also confirmed by detecting viral antigens in infected epithelium, syncytial cells and macrophages using Immunohistochemistry (Fuchs *et al*., 2007).

2.6.3. Isolation and Identification of ILTV

Embryonic chicken eggs and avian cell lines that includes chicken embryo lung cells, chicken embryo liver cells, chicken embryo kidney cells, adult chicken kidney cells as well as chicken

embryo fibroblast cell lines are commonly used to propagate and isolate ILTV. Inoculation of virus-infected suspensions of respiratory and conjunctival exudates or homogenates of appropriate tissue suspension on to the dropped chorioallantoic membrane (CAM) of the embryonating chicken eggs induces opaque plaques with a depressed centre to variably sized pock-like lesions in ILTV-infected CAM two days after inoculation and death of embryos between 2 and 8 days depending upon the virulence and level of passages in homologous host (Munuswamy *et al*., 2019).

Although virus isolation is a sensitive technique, definitive identification of ILTV is required and PCR or quantitative real-time PCR is the preferred molecular test for confirmation and quantification of viral load in biological samples (Fuchs *et al*., 2007). Molecular epidemiological tools such as PCR-RFLP and PCR-sequencing of specific genes can be used to distinguish between ILT field isolates and vaccine strains (Menendez *et al*., 2014).

2.7. Differential Diagnosis

Respiratory disease associated with ILTV must be distinguished from other respiratory diseases with similar clinical signs and lesions in poultry including diphtheritic form of Avian Poxvirus, Newcastle disease virus, Avian Influenza virus, Infectious Bronchitis virus, Fowl Adenovirus and Aspergillus species (Guy and Garcia, 2008).

2.8. Control and Prevention

Coordinated stakeholder efforts to prevent the spread of the virus as well as rapid diagnosis and culling of infected flocks in the event of an outbreak are required to prevent and control the virus (Wolfrum, 2020),

Due to the lack of treatment for the disease (Guy and Bagust, 2003), ILT control strategies are generally based on preventing contact between the agent and the host through biosecurity and/or vaccination (Dufour-zavala, 2008). Compliance with strict biosecurity measures is a prerequisite to prevent the introduction of ILTV into poultry production facilities. Besides, on-site quarantine and disinfection procedures should be followed to prevent the introduction of the virus through fomites (Menendez *et al*., 2014).

In general, the poultry housing area should be maintained to prevent wild birds, rodents and dogs from entering. Adequate record must also be kept to prevent mixing of vaccinated and non-

vaccinated birds and in the event of an outbreak the carcasses should be disposed of immediately by burning or burying for prevention and control of the disease (Munuswamy *et al*., 2019).

2.9. Status of Infectious Laryngotracheitis in Ethiopia

The poultry industry in Ethiopia is hindered by diseases with high morbidity & mortality, and poor biosecurity. Specifically, the scavenging production system is at high risk of disease transmission due to high chance of contact with wild birds, neighboring chickens and disease vectors with little or no biosecurity or control over the ranging area (Tesfaye *et al*., 2019). Besides, the vast majority of chickens, 93.4% in the scavenging and 94.8% in the intensive production systems in Ethiopia were sero-positive for at least one of the pathogens of Avian Metapenumovirus, ILTV, Infectious Bronchitis and Mycoplasma Gallisepticum, as well as, there is a strong evidence for the involvement of multiple pathogens including ILTV in the causation of "*Fengil*", a disease usually recognized as Newcastle disease in Ethiopia (Habte *et al*., 2022). Nevertheless, ILT has long been considered nonexistent and a disease of no concern in Ethiopia (Birhan *et al*., 2022).

Based on this review, limited histo-pathological, serological and molecular studies conducted so far in different regions of the country demonstrated the circulation of ILTV. Berhanu *et al*. (2019) confirmed the incidence of a single ILT outbreak through gross and histopathological technique in three small scale poultry farms in exotic layer flocks managed under semi-intensive production system in Hawassa zuria district, Sidama region and the virus was assumed to have entered the country through contaminated crates along with the importation of day old chicks. Furthermore, most recently Galana, *et al*. (2023) isolated and identified ILTV from suggestive samples with an overall PCR detection rate of 25% and molecularly characterize three strains of ILTV in Ethiopia namely ICP4, TCO low and TCO high in small and medium scale intensive poultry farms in and around Bishoftu town and Liban Chuqala district.

Additional serological evidences that revealed the circulation of ILTV in different regions of the country since 2019 is summarized in table 1.

Table 1: Serological evidences of Infectious Laryngotracheitis virus in chickens in Ethiopia

S/N	Study area	Study year	Production type	Prevalence (%)	Type of test	Source
1.	Central & south Ethiopia	2017/18	Backyard	34.30%	indirect ELISA	Tesfaye *et al.* (2019)
			Commercial	13.30%		
2.	Ada'a district	2019	Backyard	54.70%	indirect ELISA	Roba *et al*. (2020)
3.	Central Ethiopia	2019/20	Backyard	56%	indirect ELISA	Habte *et al.* (2022)
			Commercial	30.20%		
4.	North west Ethiopia	2020/21	Backyard	45.50%	indirect ELISA	Birhan *et al.* (2022)
			Commercial	61.90%		

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This study was conducted in three districts of southern Ethiopia namely Arbaminch zuria, Sodo zuria and Doyogena representing the lowland, midland and highland agro-ecologic zones respectively (Figure 1). The region has diverse climatic agro-ecological zones with various landscapes.

Arbaminch zuria district is situated at 6° 00' 0.00" N latitude and 37° 34' 59.99" E longitude. It has an altitude ranging from 1100 to 1320 meter above sea level (m.a.s.l.) with mean annual temperature of 22.5 to 29.9^{0}C and mean annual rainfall of 830.7 mm. The total chicken population in the district was estimated to be 182,592. On the other hand, Sodo zuria district is located between 6° 49' 59.99" N latitude and 37° 44' 59.99" E longitude at an altitude ranging from 1,500 to 2,900 m.a.s.l. The mean seasonal temperature and annual rainfall is 20°C and 1,296.6 mm respectively. The total chicken flock in the district was estimated to be 139,145.

Doyogena district is located between 7° 18' 25" N - 7° 21' 49" N latitude and 37° 45' 33° E - 37° 48' 51" E longitude. It has an altitude ranging from 2,100 to 2,748 m.a.s.l., annual rainfall of 1,400 mm and temperatures of 10°C to 16°C. The district has two major agro-ecologies, Dega (70%) and Woyina dega (30%). The total chicken flock in the district was estimated to be 116,206 (Respective Woreda Offices of Agriculture baseline data). Study area maps were created using Arc GIS version 10.3.

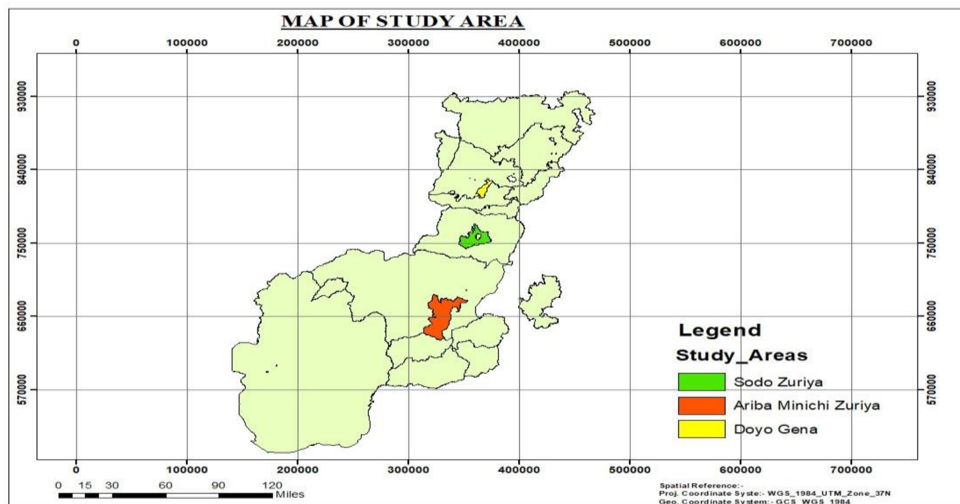

Figure 1**:** Map of the study area

3.2. Study Animals

The target population was all breed, sex and age groups of chickens in southern Ethiopia kept under the extensive management system with little and occasional supplement. The study population was comprised of all breed, sex and age groups of above three weeks in selected districts of southern Ethiopia namely Arbamich zuria, Sodo zuria and Doyogena.

In the selected study sites backyard chicken production was composed of local and exotic breeds (Table 2). All chickens examined were apparently healthy, of varied breeds, sexes and ages that include, young (3-21 weeks) and adult (above 21 weeks). These chickens were owned by different individuals and largely scavenging outdoors, in the course birds from neighboring households were mixed and spend the day together.

Table 2: Flock structure in the study area

			Total chicken Population		
Agro-ecologic zones	Selected Districts	Total kebeles	Local	Exotic	Total
Lowland	Arbaminch zuria	18	64,310	118,282	182,592
Midland	Sodo zuria	23	74,452	64,693	139,145
Highland	Doyogena	14	42,997	73,209	116,206
		55	181,759	256,184	437,943

Source: Unpublished data from the respective district offices of agriculture

3.3. Study Design

A cross sectional study design was implemented from December, 2022 to May, 2023.

3.4. Sample Size Determination

Sample size was determined using a method recommended by Thrusfield (2018) for multistage cluster sampling. The relevant formula for estimating the total number of clusters (households) to be sampled when the average sample size per cluster, n, is fixed for a 95% level of confidence is given by,

$$g = \frac{1.96^2\{(n-1)Vc + P_{exp}(1-P_{exp})\}}{nd^2}$$

Where,

g= number of households (clusters) to be sampled; n= predicted average number of chickens per household; $\mathbf{P_{exp}}$ =expected prevalence; d= desired absolute precision; Vc= between-cluster variance.

The between-cluster variance can be deduced by guessing the standard deviation (i.e., the square of the average difference expected between an individual cluster prevalence and the overall mean cluster prevalence) and then squaring it to give the between-cluster variance component.

For this purpose, a 95% confidence level, 5% absolute precision, predicted average number of chickens per cluster was estimated to be 6 and an overall optimum flock level prevalence of 50% (0.5) was anticipated. The standard deviation was then guessed to be $(0.5^2) = 0.25$ which also gives the between-cluster variance component to be 0.25^2=0.0625. Accordingly, the minimum number of clusters (households) to be sampled for the study was 173; however, 240 chickens were actually sampled from 240 households in the selected districts. For each agro-ecology proportional allocation of households were made based on respective poultry population (Table 3).

Table 3: Total number of chickens sampled in the study areas

Agro-ecologic zones	Selected districts	Number of sampled Kebeles	Total households sampled	Number of sampled chickens		
				Local	Exotic	Total
Lowland	Arbaminch zuria	3	75	22	53	75
Midland	Sodo zuria	3	85	35	50	85
Highland	Doyogena	3	80	22	58	80
Total		**9**	**240**	**79**	**161**	**240**

3.5. Sampling Methodology

Multistage cluster sampling technique was applied in selecting the study units (districts, kebeles, households and individual chickens) (Figure 2). The districts considered were purposely selected based on poultry population, road accessibility and the traditional agro-ecologic classification. Accordingly, Arbaminch zuria, Sodo zuria and Doyogena districts were selected representing the

lowland, midland and highland respectively. List of kebeles were taken from each selected district administrative offices and 3 kebeles from each district were selected by a simple random sampling technique using a lottery system. Totally nine kebeles were selected for this study.

In each selected kebele, a list of households with chicken flock size of approximately 4 or more in the lowlands, 8 or more in the midlands and 7 or more in the highlands were compiled in collaboration with the respective development agents and proportional number of households were selected by a simple random sampling technique.

Finally in each selected household (cluster), 10% of the chickens were picked for blood sampling and a targeted cluster of 173 was determined using Thrusfield (2018) for multistage cluster sampling when the average sample size per cluster is fixed. In households with chicken flock size of less than 10, one chicken was picked for blood sampling. However, due to the limited number of chickens per each household, the number of individual chicken sampled per each cluster was only one which makes the total chickens sampled and the total household (cluster) size to be equal. The total number of chickens sampled was 240 from 240 households. Information such as flock structure, management and environmental factors was also recorded using questionnaire interview from 181 households' selected using simple random sampling technique. Participants were eligible for the study if they lived in Arbaminch zuria, Sodo zuria and Doyogena districts and had the minimum chicken flock size set above during sampling. Besides, unwilling randomly selected eligible participants were replaced by their neighbors.

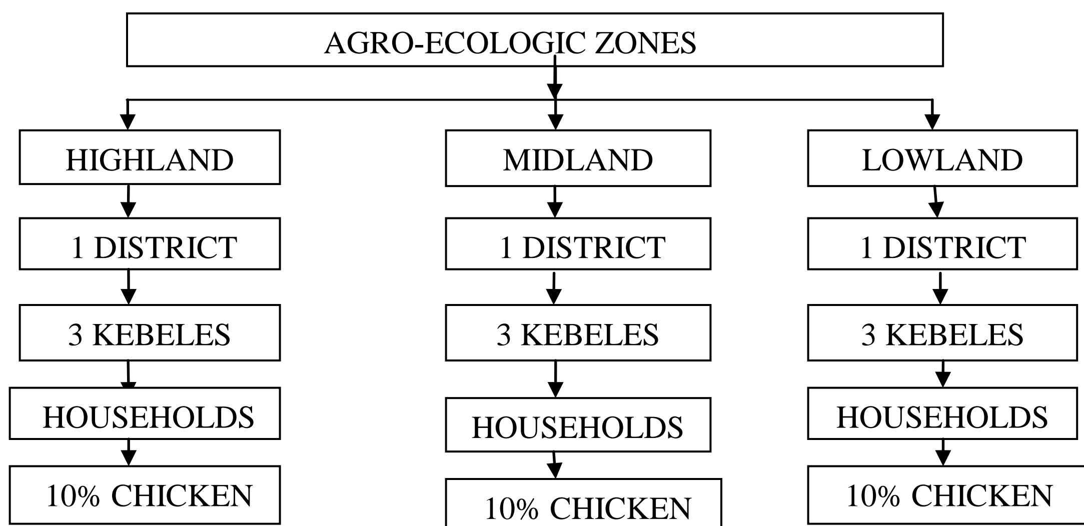

Figure 2: Schematic diagram showing sampling technique

3.6. Data Types, Sources and Collection Procedures

Both qualitative and quantitative data types were collected. The qualitative data include district, kebele, breed, sex, age, agro-ecology, flock size, management and environmental factors while the quantitative data comprised of total chicken population and indirect ELISA test results. Primary and secondary data were considered as the sources of information for this study. The primary data includes those data associated with the serological screening and the semi-structured questionnaire that was obtained through blood sample collection and administration of interview directly to chicken owners respectively whereas the secondary data were collected from the respective district agricultural office records, published journals and articles.

3.6.1. Serological screening

Blood collection procedure

After each selected chicken was controlled safely by the assistant, a feather covering the wing vein was clipped, the site was disinfected using 70% alcohol and 1.5 milliliters (ml) of whole blood was collected by puncturing using a 3 ml disposable sterile syringe fitted with 23 gauge hypodermic needle aseptically. Each blood sample collected using a syringe was kept in a shade in a slant position until it coagulates after labeled with sample number, breed & location and then transported to Wolaita Sodo Regional Animal Health Laboratory in an icebox on the same day of collection.

Serum preparation procedure

In the laboratory, the samples in each syringe were kept overnight at room temperature to allow for complete separation of the serum from the red and white blood cells. Subsequently, the sera from the syringe were poured off into a 1.5 ml sterile plastic snub cup micro-centrifuge tubes that were labeled similarly and stored at -20°C until serum analysis were performed.

Serum analysis

Serum samples were tested using commercially available indirect ELISA kit (IDvet, ID Screen® ILT Indirect, 310, rue Louis Pasteur-Grables-France, ILTS ver 0416 GB) with a sensitivity and specificity of 100% for the detection of antibodies against ILTV at Wolaita Sodo Regional Animal Health Laboratory as per the manufacturer's protocol (Appendix 4). Each serum sample after being diluted at the final ratio of 1 micro liter sample to 500 micro liter of diluents, the optical density value of

tested samples were determined using an E-Max plus micro-plate reader (Molecular Devices, LLC MA) at 450 nm and results were expressed in titers.

Sample positivity or negativity was determined by calculating the sample (diluted sera) to positive control (S/P) ratio according to the methods provided by the manufacturer as follows:

$$\frac{S}{P} = \frac{ODS - ODNC}{\mathrm{ODPC} - ODNC}$$

Where,

"S/P": sample to positive control ratio, **"ODS"**: optical density of a given sample, **"ODNC":** mean optical density of the negative control, **"ODPC":** mean optical density of the positive control.

Accordingly, sample to positive (S/P) ratio of ≤ 0.3 (titer ≤ 611) and > 0.3 (titer >611) were interpreted as negative and positive samples, respectively.

3.6.2. Questionnaire Survey

A semi-structured questionnaire was applied (Appendix 1) and 181 chicken owners were interviewed to identify potential risk factors for ILTV infection transmission in village chicken flocks. The questionnaire was pre-tested in the field, adjusted as required to characterize the management system and to identify risk factors associated with ILTV. In the questionnaire survey flock structure, management and environmental factors that were believed to influence the spread and maintenance of ILTV were considered.

3.7. Operational Definitions

- **Backyard chicken production** is a system of chicken production characterized by small flock size maintained in an extensive system under low or no input venture mainly scavenging with little or no supplementary feeding, rudimentary housing that provides night shelter and minimal health care.
- **Clusters** are households with 4, 8 and 7 or more chickens in the lowland, midland and highlands of the study area respectively.
- Household chicken flocks were considered **large or small flock** if the household contained chicken population of ten or more and fewer than ten respectively in the extensive system in the study area.

❖ **Traditional agro-ecologic classification** of Ethiopia includes lowland (kola) with an altitude ranging from 500 to 1300 m.a.s.l., midland (woyinadega), 1301 to 2300 m.a.s.l. and highland (dega) 2,301 to 3,000 m.a.s.l.

3.8. Data Management and Analysis

Data was stored in Microsoft (MS) Excel Spread Sheet 2010 program and were filtered, coded, statistically analyzed using STATA version 15 (Stata Corp. College Station, Tx, USA) to estimate the sero-prevalence and analyze the association with risk factors. The statistical methods applied were both descriptive and analytical statistics. In order to assess the unconditional association of predictors a univariable logistic regression analysis was used. As the number of potential predictors was few, all of the predictors were checked for multi-collinearity using Kruskal gamma statistics. Non collinear predictors with gamma value between -0.6 and +0.6 were included in the multiple logistic regression analysis and odds ratio with a 95% confidence interval was used to express the strength of association. Confounding effect was also assessed using delta beta of the OR. Variables with delta beta exceeding 20% were considered confounder and excluded in the model (Dohho *et al.*, 2009). The final model was built in backward stepwise elimination procedure using Wald and log likelihood ratio statistics. The final model was evaluated for goodness of fit using the Hosmer and Lemeshow statistic and the Receiver Operating Characteristic curve (ROC curve) for assessing final model performance (predictive accuracy). Anti-body titer was also compared using Analysis of Variance (ANOVA). In this study, differences were considered statistically significant at $p < 0.05$.

4. RESULTS

4.1. Sero-prevalence of ILTV in Chicken Flocks

In surveyed households (clusters) the flock size was seen to range between 4 and 14 birds. From each flock a single bird was sampled. Accordingly, 240 chickens were considered from 240 households. The overall household level prevalence of ILTV in backyard chicken in this study was 27.9 % (95% CI: 22.6% – 34.0%). The highest prevalence was recorded in the midland agro-ecologic zone in Sodo zuria district 32.9% (95% CI: 23.8% - 43.6%) followed by lowland in Arbaminch zuria district 28.0% (95% CI: 19.0% - 39.2%) and highland in Doyogena district 22.5% (95% CI: 14.6% - 33.0%) (Table 4)

Table 4: The sero-prevalence of ILTV in backyard chicken flocks in the study area

Agro-ecologic zones	Selected districts	Name of kebeles	Total examined	positive chickens	Prevalence % (95% CI)
Lowland	Arbaminch-zuria	Lante	20	6	30.0 (14.1 - 52.8)
		Dorga	25	5	20.0 (8.5 - 40.1)
		Shellemella	30	10	33.3 (18.9 - 51.7)
		Sub total	**75**	**21**	**28.0 (19.0 - 39.2)**
Midland	Sodo zuria	Wajakero	31	12	38.7 (23.4 - 56.6)
		Worezalasho	36	9	25.0 (13.5 - 41.6)
		Gurmuwoyidae	18	7	38.9 (19.7 - 62.3)
		sub total	**85**	**28**	**32.9 (23.8 - 43.6)**
Highland	Doyogena	Hawora	20	1	5.0 (0.7 - 28.4)
		Zeraro	21	4	19.1 (7.3 - 41.3)
		Lemi suticho	39	13	33.3 (20.4 - 49.4)
		Sub total	**80**	**18**	**22.5 (14.6 – 33.0)**
		overall	**240**	**67**	**27.9 (22.6 – 34.0)**

In an attempt to identify potential risk factors six predictors were considered that include location (district/kebele), breed, age, sex, agro ecology and flock size as shown in the table(5).

Table 5: Univariable logistic regression analysis of potential risk factors on ILTV

Variable	Total examined	No of positives	Prevalence % (95% CI)	OR (95% CI)	p-value
District					
Arbaminch zuria	75	21	28.0(19.0 - 39.2)		
Sodo zuria	85	28	32.9(23.8 - 43.6)	1.3 (0.6 - 2.5)	0.499
Doyogena	80	18	22.5(14.6 - 33.0)	0.7 (0.4 - 1.5)	0.431
Breed					
local	79	19	24.1 (15.9 – 34.7)		
Exotic	161	48	29.8 (23.2 – 37.4)	1.3 (0.7 - 2.5)	0.351
Sex					
Female	192	55	28.6 (232.7 – 35.5)		
Male	48	12	25.0 (14.7 – 39.1)	0.8 (0.4 - 1.7)	0.615
Age					
young	85	15	17.6 (10.9 – 27.3)		
Adult	155	52	33.5 (26.5 – 41.4)	2.4 (1.2 - 4.5)	0.01
Agro-ecologic zones					
Lowland	75	21	28.0(19.0 - 39.2)		
Midland	85	28	32.9(23.8 - 43.6)	1.3 (0.6 - 2.5)	0.499
Highland	80	18	22.5(14.6 - 33.0)	0.7 (0.4 - 1.5)	0.431
Flock size					
<10	168	32	19.0(13.8 - 25.7)		
>=10	72	35	48.6(37.3 - 60.1)	4.0 (2.2 - 7.3)	< 0.001

As the number of predictors was few all were checked for multi-collinearity and a multivariable logistic regression analysis was made on non collinear predictors. In the final logistic regression model, age and flock size were found to associate with ILT prevalence significantly ($p < 0.05$) (Table 6).

Table 6: Multivariable logistic regression analysis of potential risk factors on ILTV

Variable	Total examined	Tested positive chickens	Prevalence % (95% CI)	univariable COR (95% CI)	univariable P-value	Multivariable AOR (95% CI)	Multivariable P - value
Age							
young	85	15	17.6 (10.9 - 27.3)				
Adult	155	52	33.5 (26.5 - 41.4)	2.4 (1.2 - 4.5)	0.01	2.6(1.3 - 5.2)	0.006
Flock size							
Small flock	168	32	19.0 (13.8-25.7)				
Large flock	72	35	48.6 (37.3 - 60.1)	4.0 (2.2 - 7.3)	<0.001	4.3 (2.3 - 8.0)	<0.001

Hosmer & Lemeshow X^2 =0.03, p-value = 0.9857; ROC curve = 0.746; AOR= Adjusted Odds Ratio

The model exhibits a very good specificity since among those tested negative 88.4% were correctly predicted where as the model exhibits poor sensitivity since among those tested positive only 38.8% were correctly predicted. However, the overall predictive accuracy rate was good at 74.6%.

4.2. Comparison of Antibody Titer among Different Agro-ecologic Zones

One way ANOVA analysis revealed the mean antibody titer had no significant difference among the different agro-ecologic zones (F= 1.65, p >0.05). The mean antibody titers for ILTV in the lowland, midland and highland agro-ecologic zones were recorded as 1027.2, 788.4 and 577.1 respectively (Table 7).

Table 7: Descriptions and comparison of antibody titers

Agro-ecologic zones	Anti-ILTV Antibody titers Mean (95%CI)	Median	Minimum	Maximum	F-test	*p*-value
lowland	1027.2 (589.8 -1464.7)	304.4	5.769	12,013.60	1.65	0.1937
Midland	788.4 (514 -1062.8)	271.9	1.985	7,728.55		
Highland	577.1 (261.5 - 892.8)	145.4	2.019	11,500.94		
Overall	**792.6 (596.2 - 988)**	**212.3**	**1.985**	**12,013.60**		

4.3. Results of the Questionnaire Interview

All selected flock owners responded to the questionnaire. The semi-structured questionnaire was focusing on environmental and management factors where health and biosecurity queries were incorporated. The potential risk factors recorded from the study sites and their respective frequencies were summarized as follows.

In the study area the communities were found to keep both local and exotic breed chickens. The highest proportion of households had both local and exotic breeds 83.4% (151/181) while there are also some households that owned exotic 12.2% (22/181) and local 4.4%(8/181) chickens exclusively. During the day, 69.1% (125/181) of the chicken scavenging outdoor in the backyard and the remaining partially housed. Besides, only 18.2% (33/181) of the visited households provided simple type of separate night shelters, however the majority of the chickens 58.6% (106/181) reside in a perch that is fixed inside the main house and the remaining 23.2% (42/182) sheltered with other animals. The major feed source was scavenging only 47.5% (86/181) followed by scavenging with occasional supplement of household scarps or crop by products 37.6% (68/181) and scavenging with supplement of industrial byproducts 14.9% (27/181)(Table 8).

Table 8: Housing and feed sources of backyard chickens in the study area

Descriptions	Categories	Frequency (%)
Chicken population	Local and exotic chicken	151 (83.4%)
	Exotic chicken only	22 (12.2%)
	Local chicken only	8 (4.4%)
Chicken day time reside	Scavenging	125 (69.1%)
	Partially housed	56 (30.9%)
Availability of night shelter	in the main house	106 (58.6%)
	separate house	33 (18.2%)
	with other animals	42 (23.2%)
Major feed source	Scavenging only	86 (47.5%)
	Scavenging with household scraps or crop-byproducts	68 (37.6%)
	Scavenging with industrial byproducts	27 (14.9%)

High rate of mortality in chicks 61.9% (112/181) and loss of weight or egg 31.5% (57/181) were mentioned as the major loss while disease 50.3% (91/181), predation 37.6% (68/181) and feed scarcity 12.1% (22/181) was reported as the major concern in backyard poultry production. Respiratory difficulty, coughing, nasal or ocular discharges 42% (76/181), diarrhea 29.8 % (54/181), dropping of the head or neck 18.8% (34/181) and circling or paralysis 9.4% (17/181) were also reported as major clinical syndromes of the diseases observed in the area. Diseases were seen to break out between June and August commonly (54.7%) followed by March to May (21%) and September to November (17.1%) (Table 9).

Table 9: Major challenges and their effects on poultry production in the study area

Potential factors	Categories	Frequency (%)
Major concern in chicken production	Diseases	106 (58.6%)
	Predators	56 (30.9%)
	Feed shortage	19 (10.5%)
Major loss in poultry production	Mortality of chicks	112 (61.9%)
	Egg or weight loss	57 (31.5%)
	treatment cost	12 (6.6%)
Major syndromes of diseases	Respiratory symptoms	76(42%)
	Diarrhea	54(29.8%)
	Dropping of the wing or head	34(18.8%)
	Circling or paralysis	17(9.4%)
Season of frequent disease occurrence	June to August	99 (54.7%)
	March to May	38 (21%)
	September to November	31(17.1%)
	December to February	13 (7.2%)

The chicken husbandry practices was also shown to be poor due to common practices of mixing of chickens with wild birds 74.6% (135/181) and chickens of different breeds 96.1% (174/181), absence of cleaning practice 60.8% (110/181), random disposal of dead birds 87.3% (158/181), absence of practice of isolation of sick/healthy chickens 90.6% (164/81), careless handling of sick chicken 87.9% (150/181) and absence of culling practices 98.9% (179/181) (Figure 3).

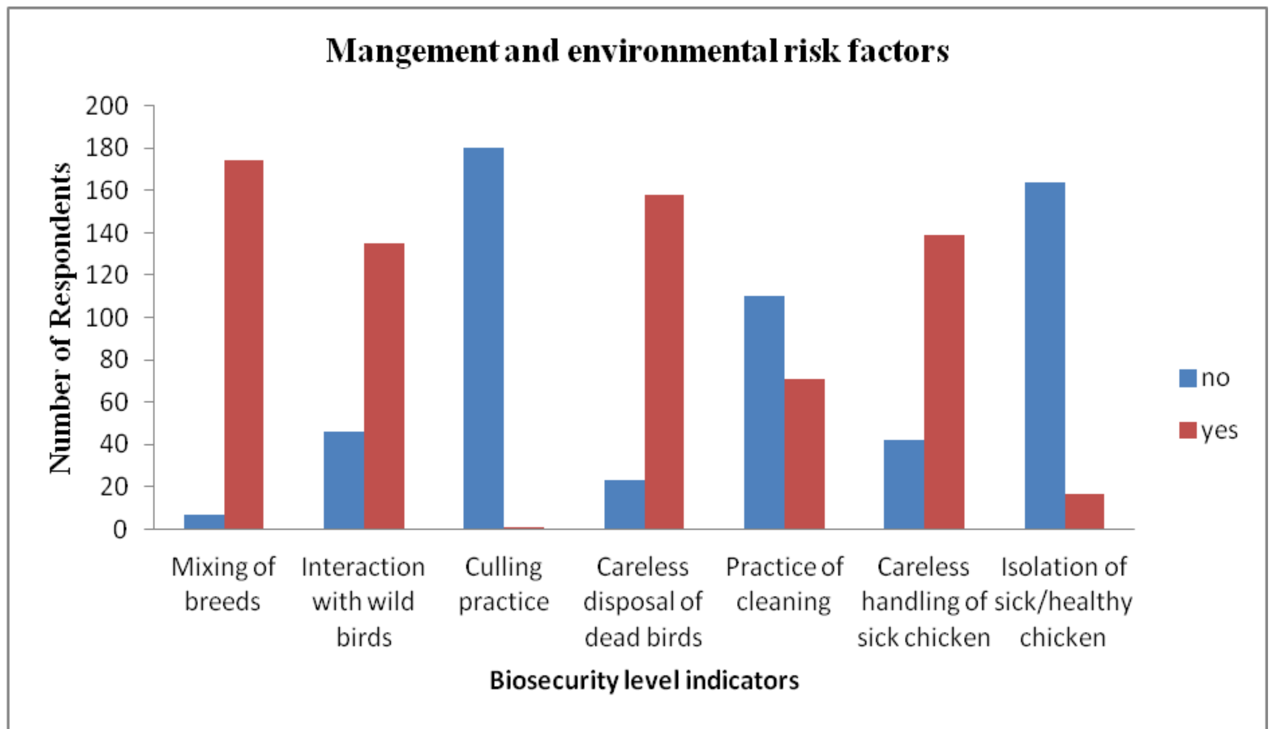

Figure 3: Factors that indicated risky backyard poultry production in the study areas

5. DISCUSSIONS

This cross-sectional study indicated that ILTV risk is prevalent in the study area. As there has never been history of vaccination, the sero-prevalence in the study area might be due to wild bird mediated infection of chickens with less virulent strains of ILTV (Bagust *et al*., 2000; Marangon and Busani, 2006). According to Birhan *et al*. (2022) the use of ILTV vaccine is not officially approved by the veterinary authority in Ethiopia. Furthermore, recent PCR confirmed ILTV strains in and around Bishoftu town and Liban Chuqala were attributed to wild ILTV strains (Galana *et al*., 2023).

The overall household/individual chicken level ILTV sero-prevalence detected in this study in backyard chickens using indirect ELISA was 27.9%, with variation from 22.5% in the highland to 32.9% in the midland agro-ecologic zone. In fact in some kebeles like Gurmuwoyidae of Soda zuria district the report is very high (38.9%), while in Hawora kebele of doyogena district the prevalence is low (5%). The variations in prevalence reported may be attributed to difference in the stage of disease infection when sampling at each kebele conducted. Besides, Ou and Giambrone (2012) described different strains of ILTV have different heat resistance, with ILTV maintaining infectivity for an extended period at lower temperature. Nevertheless the finding in this study indicates the presence of the risk in all agro-ecologies of the study area.

This finding (27.9%) is consistent with previous studies elsewhere like India and Swiss where there was a report of 26.77 % (Baksi *et al*., 2016) and 28.2% in fancy breed flock (Wunderwald and Hoop, 2002) respectively. Similar status was also noted in Island of Tobago, 30.7% (Jordan *et al*., 2018) and 30% in Belgium (Haesendonck *et al*., 2014). Perhaps this may entail the global importance of ILTV, even where the chicken production is intensified and managed relatively better.

In fact there are also reports where the prevalence is lower than our report, 17.33% in Bangladesh (Uddin *et al*., 2014), 13% in broiler flocks of Iran (Langeroudi *et al*., 2020) and 12% in Finland (Pohjola *et al*., 2017). Likewise there are also reports with higher sero-prevalence of 42.56% in Konya region of Turkey (Aras *et al*., 2018); 46.3% in California, USA (Derksen *et al*., 2018); 50% in Nigeria (Owoade *et al*., 2006); 56.25% in Algeria (Salhi *et al*., 2021) and 57.1% in Delmarva Peninsula (Johnson *et al*., 2004). Perhaps this may be attributed to the level of biosecurity and variation in study population.

Besides, comparable overall sero-prevalence results with the current finding was also reported in Ethiopia including 19.4% in south and central (Tesfaye *et al*., 2019) and 27% in the central parts of the country (Habte *et al*., 2022) in both backyard and commercial productions. On the other hand, quite higher prevalence was also reported, 54.75% in backyard chicken in Bishoftu town (Roba *et al*., 2020) and 59.1% in both backyard and commercial farms in North Western Ethiopia (Birhan *et al*., 2022). This difference in the sero-prevalence of ILTV in Ethiopia may be attributed to difference in the concentration of commercial farms in the study areas.

Differences in ILTV sero-prevalence compared to this study could not be different from the context above. and it could be due to variations in the temporal and spatial distribution of the disease (Pitesky *et al*., 2014), in poultry density, poultry management system, chicken breeds, sample size, flock biosecurity level, ILTV vaccination practices and the specificity and sensitivity of the tests used.

Notably, the relatively lower prevalence in the present study was attributed to the small flock size of the backyard chickens and the low density of commercial or intensive poultry production in the study areas which is in agreement with the suggestion of Madsen *et al*. (2013) that described the lower risk of disease spread in small flock size of backyard chicken as well as Guy and Bagust (2003) that described the association of the concentration of farms in a given geographic area and the spread of the virus through wind.

Alongside screening chicken serum against anti-ILTV antibodies, potential risk factors that may be associated with sero-positivity of ILTV in backyard chicken were assessed. In this study, location (district/kebele), agro-ecologic zone, age, sex, breed and flock size were considered as potential risk factors, however the final mixed effect logistic regression analysis showed only age and flock size of chickens had a significant effect ($p < 0.05$).

The insignificant difference in the prevalence of ILTV ($p > 0.05$) confirmed among study zones in this study is in agreement with Salhi *et al*. (2021) and Tesfaye *et al*. (2019) but contradicts with Bhuiyan *et al*. (2019), Roba *et al.* (2020) and Birhan *et al*. (2022). The present finding may be attributed to the similarity in the rare distribution of commercial farms and chicken management system regardless of difference in agro-ecologic zone of the study areas.

Besides, in this study, sero-prevalence of ILTV significantly varied ($p < 0.05$) among the different age and flock size category of chickens. The sero-prevalence of ILTV was significantly higher ($p<$

0.05) in large flocks 48.6% and adult chicken 33.5%, as compared to small flocks 19.0% and young chickens 17.6% respectively.

Furthermore, in the final mixed model, the risk of exposure to ILTV was 4.3 times higher in large flocks than in small flocks. This may be attributed to the nature of backyard chickens constantly being mixed with chickens of unknown source and history in the neighboring household to such an extent that all the backyard chicken in the village can reasonably be considered a single flock (Gutierrez-Ruiz *et al*., 2000). In line with this, Garcia & Spatz (2020) and Gowthaman *et al*. (2020) mentioned that rearing different types and ages of poultry in the same area in combination with lax biosecurity and an increase in poultry production density as the factors that contributed to the increased ILTV incidence. Therefore, in this study it was assumed that the higher ILTV sero-prevalence in large flocks is associated with an increased risk of contact with different breeds and ages of chickens from different households. Likewise, in this study, adult chickens were 2.6 times more likely to be exposed to ILTV than young chickens which may be attributed to the likelihood of increased exposure of adult chickens due to their persistent existence in the operation.

In this study, a wide range of antibody titers was also observed with an overall mean of 792.61 ± 99.71 however, there was no significant difference in the mean antibody titers of backyard chickens between the different agro-ecologic zones (F= 1.65, *P=0.1937*). This may be attributed to the more or less similar environmental influence among the different agro-ecologic zones in the study areas, otherwise birds are more sensitive to heat stress than other domestic animals (Wang *et al*., 2018) and as Hirakawa *et al*. (2020) described heat stress is a major concern for the poultry industry as it negatively affects the immune functions. The maximum antibody titer was recorded in the lowland climatic zone, 12,013.6 followed by the highland, 11,500.94 and the midland, 7,725.55.

As per the interviewed backyard chicken owners response, the leading loss in poultry production in the study areas was due to high mortality in chicks (61.9%) followed by loss of weight or egg (31.5%). The main causes assumed for these losses were disease (50.3%) followed by predation (37.6%) and feed scarcity (12.1%). Among the syndromes reported, respiratory symptoms were most noted by the poultry producers (42%) followed by diarrhea (29.8%), dropping of the head or neck (18.8%) and circling or paralysis (9.4%) that corresponds to major disease symptoms which may be suggestive of respiratory, gastrointestinal and nervous diseases. In general, owing to season of frequent disease occurrence, the rainy seasons that is June to August (54.7%) followed by March to

May (21%) was reported to be the commonest and this is in agreement with the report of Hunduma (2010) in rift valley of oromia, Ethiopia.

The questionnaire data also showed that the majority of chickens were managed in a highly risky environment with poor biosecurity practices including common practices of mixing chickens with wild birds (74.6%) and chickens of different breeds (96.1%), lack of cleaning practice (60.8%), random disposal of dead birds (87.3%), lack of practice of isolation (90.6%), careless handling of sick chicken (87.9%) and lack of culling practices (98.9%). The practice of isolation of sick chickens from healthy ones was reported affirmatively in only 9.4% of the respondents but not in 90.6% of them. Besides, in the present study, when chickens got sick, 43.1 % of the respondents do nothing, 23.8% of them usually treat using traditional medicine and 16.1% sells out whereas only 17.1% of them consult veterinarians. Therefore, the poultry extension service providers need to focus on at least creating awareness to small holders on how to improve the biosecurity level and be benefited from the health and productivity of small poultry flocks.

In sum, based on my literature review, ILT is a recently reported poultry disease in backyard and commercial productions in Ethiopia. In the study area, backyard chickens are often reared within households and their health directly contributes to the livelihood of these families. However, the results of this study suggested that ILTV is widespread in backyard chicken in the study area that could have significant direct impacts on the livelihood and financial sources of the small holder as well as backyard chickens could serve as a reservoir of the infection and may jeopardize the commercialization of the poultry industry in the country. The results of this study also have important implications for the management and control of ILTV in backyard chickens. Therefore targeted vaccination programs, improved biosecurity measures and better management practices have been suggested to reduce the incidence and impact of ILTV on backyard chicken flocks.

Limitations of the Study

This study applied indirect ELISA to identify sero-positive chickens. It is well understood that indirect ELISA is more sensitive in detecting anti-ILTV antibodies in chicken; however the test has potential for cross-reactivity caused by the secondary antibody in the kit and does not allow differentiation of carrier chickens nor of vaccine and field strains (Garcia and Spatz, 2020). Besides, Munuswamy *et al*. (2019) described that ILTV-specific neutralizing antibodies can be detected in the serum within 5–7 days of tracheal infection and peaks at 21 days post infection that which later

diminishes for several months and persists at lower titer for a year or more. So it is likely that chickens at the later stage of infection to be missed out serologically by indirect ELISA. In addition, aside being a valuable screening tool for ILTV in chickens, ELISA should not be considered as the sole conclusive diagnostic method and other tests are often necessary to confirm the presence of the virus. To counteract this limitation of the test, the authors had planned to perform additional molecular investigation in collaboration with the National Veterinary Institute that promised support of the necessary materials for at least ten ILTV suggestive active case samples. Unfortunately, ILTV suggestive cases weren't observed in the study period as the outcome of infection depends on the virulence of the strain and co-infection with other respiratory pathogens (Gowthaman *et al.,* 2020) and hence this study was performed using only an indirect ELISA test. Therefore, considering these shortcomings in the serological test, use of an additional test could be considered in future studies.

6. CONCLUSION AND RECOMMENDATIONS

In this study 27.9% of the flocks had at least one ILTV sero-positive bird in the flock. Owing to the absence of supportive concrete evidence on ILTV vaccination in chicken in Ethiopia and the rare practice of regular vaccination activities in backyard chicken in the study area, the sero-conversion noted at reported scale in this study is likely to be due to field virus exposure. Therefore, the circulation of the virus in backyard chickens could be a significant threat to the growing commercialization of the poultry industry in Ethiopia.

Among the hypothesized risk factors older birds and birds of larger flock size were found to have strong association with the increasing number of sero-positivity.

Practices of mixing chickens with wild birds and chickens of different breeds coupled with lack of hygiene, lack of isolation and culling practices as well as random disposal of dead birds and careless handling of sick chicken were found to be highly risky in the transmission and maintenance of ILTV.

Thus, based on the above conclusions the following recommendations are forwarded

- There is a need to bring ILTV as one of the disease that need attention in backyard poultry production system of the country,
- Further research on isolation and molecular characterization of the virus particularly in the backyard chicken production system should be considered,
- Animal health extension service should be strengthened to improve the backyard chicken management and biosecurity practices.

7. REFERENCES

Abdo, W., Magouz, A., El-Khayat, F. and Kamal, T. (2017): Acute Outbreak of Co-infection of Fowl-pox and Infectious Laryngotracheitis Viruses in Chicken in Egypt. Pak. Vet. J. 37(3):321-325.

Aras, Z., Yavuz, O. and Golen, G.S. (2018): Occurrence of Infectious Laryngotracheitis Outbreaks in Commercial Layer Hens Detected by ELISA. J. Immunoas. Immunoch. 39(2).

Bagust, T.J., Jones, R.C. and Guy, J.S. (2000): Avian Infectious Laryngotracheitis. Rev. Sci. tech. Off. int. Epiz. 19(2):483-492.

Bakasi, S., Savaliya, B.F., Rao, N. and Panchal, M. (2016): Sero-prevalence of Infectious Laryngotracheitis of Poultry in India. Ind. J. Poul. Sci. 51(2):234-236.

Berhanu, M**.**, Sisay, D. and Rahmeto, A. (2019): First Report of Avian Infectious Laryngotracheitis Outbreak in Small Scale Chicken Flocks Around Hawassa City, Ethiopia. Bull. Anim. Hlth. Prod. Afr. 67(3):239-245.

Bhuiyan, Z.A., Ali, M.Z., Moula, M.M., Bary, M.A., Arefin, N., Giasuddin, M. and Khan, Z.M. (2019): Sero-prevalence of Major Avian Respiratory Diseases in Broiler and Sonali Chicken in Selected areas of Bangladesh. J. Adv. Vet. Anim. Res. 6(4):561-566.

Birhan, M., Syoum, A., Ibrahim, S.M., Fentahun, T., Mohammed, A., Berhane, N., Bitew, M., Gelaye, E., Atanaw, M.B., Getachew, B., Desalegne, B., Abat, A.S., Berrie, K., Adamu, K. and Abayneh, T. (2022): Serological Evidence of Infectious Laryngotracheitis Infection and Associated Risk Factors in Chickens in Northwestern Ethiopia. Sci. Worl. J. https://doi.org/10.1155/2022/6096981.

Blacker, H.P., Kirkpatrick, N.C., Rubite, A., O'Rourke, D. and Noormohammadia, A.H. (2011): Epidemiology of Recent Outbreaks of Infectious Laryngotracheitis in Poultry in Australia. Aust. Vet. J. 89(3):89-94.

Chacon, J.L. and Ferreira, A.J.P. (2009): Differentiation of Field and Vaccine Strains of Infectious Laryngotracheitis Virus by DNA Sequencing. Elsev. Ltd. 27:6731-6738.

Chang, P.C., Chen, K.T., Shien, J.H. and Shieh, H.K. (2002): Expression of Infectious Laryngotracheitis Virus Glycoproteins in Escherichia coli and their Applications in Enzyme-Linked Immuno-SorbentAassay. Avi. Dis. 46(3):570–580.

Coppo, M.J.C., Noormohammadi, A.H., Browning. G.F. and Devlin, J.M. (2013): Challenges and Recent Advancements in Infectious Laryngotracheitis Virus Vaccines. Avi. Pathol. 42(3):195-205.

Craig, M.I., Rojas, M.F., Van Der Ploeg, C.A., Olivera, V., Vagnozzi, A.E. and Perez, A.M. (2017): Molecular Characterization and Cluster Analysis of Field Isolates of Avian Infectious Laryngotracheitis Virus from Argentina. Front Vet. Sci. 4:212.

Davison, A.J. (2010): Herpesvirus Systematics. Vet. Micro. 143:52-69.

Derksen, T., Lampron, R., Hauck, R., Pitesky, M. and Gallardo, R.A. (2018): Biosecurity Assessment and Sero-prevalence of Respiratory Diseases in Backyard Poultry Flocks Located close to and far from Commercial Premises. Avi. Dis. 62(1):1-5.

Dohoo, I., Martin, W. and Stryhn, H. (2009): Veterinary Epidemiologic Research. (2nd ed.). AVC. Inc. Charlotte town. P.E.I. pp. 239–249.

Dufour-Zavala, L. (2008): Epizootiology of Infectious Laryngotracheitis and Presentation of an Industry Control Program. Am. Ass. Avi. Pathol. 52(1):1-7.

El Houadfi, M., Kichou, F., Tazi, R.M., Mouahid, M. and Kissi, B. (2005): First report of Avian Infectious Laryngotracheitis in Morocco: Short communications. Vet. Rec. 156: 520-521.

El-Saied, M., El-Mahdy, M., Sakr, E., Bastami, M. and Shaalan, M. (2021): Anatomopathological, Ultrastructural, Immunohistochemical and Molecular Characterization of Infectious Laryngotracheitis Outbreaks in Poultry farms in Egypt (2018–2020). Braz. J. Vet. Pathol. 14(2):88-98.

Fuchs, W., Veits, J., Helferich, D., Granzow, H., Teifke, J.P. and Mettenleiter, T.C. (2007): Molecular Biology of Avian Infectious Laryngotracheitis Virus. Vet. Res. 38(2):261-279.

Galana, B.M., Dereje, A.W. and Belayneh, G. (2023): Isolation and Molecular Characterization of Infectious Laryngotracheitis Virus from Poultry in and around Bishoftu town and Liban Chuqala District. Res. squa. https://doi.org/10.21203/rs.3.rs2893189/v1.

Garcia, M., Spatz, S. & Guy, J.S. (2013): Infectious Laryngotracheitis. In: Swayne, D.E. (ed.) Diseases of Poultry (13th ed.). John Wiley & Sons, Inc. pp. 161–179.

García, M. and Spatz, S. (2020): Infectious Laryngotracheitis. In: Swayne, D.E. (ed.) Diseases of Poultry (14th ed.). John Wiley & Sons. Inc. pp. 189–203.

Garcia, M. and Zavala, G. (2019): Commercial Vaccines and Vaccination Strategies against Infectious Laryngotracheitis: What we have learned and knowledge gaps that remain. Avi. Dis. 63:325 -334.

Gowthaman, V., Kumar, S., Koul, M., Dave, U., Murthy, T.R., Munuswamy, P., Tiwarid, R., Karthike, K., Dhamac, K., Michalakf, I. and Joshi, S.K. (2020): Infectious Laryngotracheitis: Etiology, Epidemiology, Pathobiology and Advances in Diagnosis and Control - a Comprehensive Review. Taylor & Francis Group. 40(1):140-161.

Granzow, H., Klupp, B.G., Fuchs, W., Veits, J., Osterrieder, N. and Mettenleiter, T.C. (2001): Egress of Alphaherpesviruses: Comparative Ultrastructural Study. J. Virol. 75(6):3675-3684.

Gutierrez-Ruiz, E.J., Ramirez-Cruz, G.T., Camara Gamboa, E.I., Alexander, D.J. and Gough, R.E. (2000): A Serological Survey for Avian Infectious Bronchitis Virus and Newcastle Disease Virus Antibodies in Backyard (Free- range) Village Chickens in Mexico. Trop. Ani. Hlth. Prod. 32(6):381-390.

Guy, J.S. and Bagust, T.J. (2003): Laryngotracheitis. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R. and Swayne, D.E. (eds.) Diseases of Poultry (11th ed.). Iowa State Press. pp. 121-134.

Guy, J.S. and Garcia, M. (2008): Laryngotracheitis. In: Saif, Y.M., Fadly, A.M., Glisson, J.R., Mc-Dougald, L.R., Nolan, L.K. and Swayne, D.E. (eds.) Diseases of Poultry (12th ed.). Iowa State Press. pp. 137-146.

Habte, T., Gerber, P.F., Ibrahim, F., Groves, P.J. and Walkden-Brown, S.W. (2022): Sero-prevalence of Major Respiratory Diseases of Chickens in Central Ethiopia in Different Chicken Production Systems. Poult. Sci. Ass. Inc. 101:102065-102074.

Haesendonck, R., Verlinden, M., Devos, G., Michiels, T., Butaye, P., Haesebrouck, F., Pasmans, F. and Martel, A. (2014): High Sero-prevalence of Respiratory Pathogens in Hobby Poultry. Avi. Dis. 58(4):623-627.

Hidalgo, H. (2003): Infectious Laryngotracheitis: A Review. Braz. J. Pou. Sci. 5(3):157-168.

Hirakawa, R., Nurjanah, S., Furukawa, K., Murai, A., Kikusato, M., Nochi, T. and Toyomizu, M. (2020): Heat Stress Causes Immune Abnormalities via Massive Damage to Effect Proliferation and Differentiation of Lymphocytes in Broiler Chickens. Front. Vet. Sci. 7:46.

Hunduma, D., Regassa, C., Fufa, D., Endale, B. and Samson, L. (2010): Major Constraints and Health Management of Village Poultry Production in Rift Valley of Oromia, Ethiopia. American-Eurasian J. Agri. Environ. Sci. 9(5):529-533.

Johnson, Y.J., Colby, M.M., Tablante, N.L., Hegngi, F.N., Salem, M., Gedamu, N. and Pope, C. (2004): Application of Commercial and Backyard Poultry Geographic Information System Databases for the Identification of Risk factors for Clinical Infectious Laryngotracheitis in a Cluster of Cases on the Delmarva Peninsula. Int. J. Poult. Sci. 3(3):201-205.

Jones, R. (2010): Viral Respiratory Diseases (ILT, aMPV infections, IB): are they ever under control? Brit. Poult. Sci. 51(1):1-11.

Jordan, B.A., Gongora, V., Hartley, D. and Oura, C. (2018): A Review of Eight High-Priority, Economically Important Viral Pathogens of Poultry within the Caribbean Region. Vet. Sci. 5(14):1-12.

Kaboudi, K., Nciri, J., Amara, A., Laarbi, I., Moalla, N., Bouzouaia, M. and Ghram, A. (2016): Histopathological and Molecular Diagnosis of Infectious Laryngotracheitis in Tunisia: First report. Int. J. Liv. Res. 6(10):34-45.

Kammon, K., Shabba, J., Asheg, A. and Abouzeed, Y. (2020): Isolation, Serological and Molecular Detection of Infectious Laryngotracheitis Virus (ILTV) in Chickens in Libya. Appro. Poult. Dairy & Vet. Sci. 7(4):654-664.

King, A., Adams, M., Carstens, E. and Lefkowitz, E. (2012): Family Herpesviridae: Ninth Report of the International Committee on Taxonomy of Viruses. Elsev. Acad. Press. pp. 193-210.

Kirkpatrick, N.C., Mahmoudian, A., Colson, C.A., Devlin, J.M. and Noormohammadi, A.H. (2006): Relationship between Mortality, Clinical Signs and Tracheal Pathology in Infectious Laryngotracheitis. Avi. Pathol. 35(6):449-453.

Langeroudi, G.A., Hosseini, H., Fallah, M.H., Aghaeean, L., Esmaeelzadeh Dizaji, R., Ziafati, Z., Modiri, A., Almasi, Y., Gholamian, B., Ashouri, A. and Moghadam, Z.N. (2020): Serological Survey of Infectious Laryngotracheitis in Broiler Flocks, Iran, 2018. I. J. Virol. 14(1):1-5.

Madsen, J.M., Zimmermann, N.G., Timmons, T. and Tablante, N.L. (2013): Prevalence and Differentiation of Diseases in Maryland Backyard Flocks. Avi. Dis. 57(3):587-594.

Magouz, A., Medhat, S.H., Abou-Asa, S. and Desouky, A. (2018): Detection of Infectious Laryngotracheitis Virus (Gallid herpesvirus-1) from Clinically Infected Chickens in Egypt by Different Diagnostic Methods. I. J. V. R. 19(3):194-201.

Marangon, S. and Busani, I. (2006): The Use of Vaccination in Poultry Production. Rev. Sci. tech. Off. int. Epiz. 26(1):265-274.

McGeoch, D.J., Dolan, A. and Ralph, A.C. (2000): Toward a Comprehensive Phylogeny for Mammalian and Avian Herpesviruses. J. Virol. 74(22):10401–10406.

Menendez, K.R., Garcia, M., Spatz, S. and Tablante, N.L. (2014): Molecular Epidemiology of Infectious Laryngotracheitis: A Review. Avi. Pathol. 43(2):108–117.

Molini, U., Aikukutu, G., Khaiseb, S., Kahler, B., Westhuizen, V.J., Cattoli, G. and Dundon, W.G. (2019): Investigation of Infectious Laryngotracheitis Outbreaks in Namibia in 2018: Short communications. Trop. Anim. Hlth. Prod. https://doi.org/10.1007/s11250-019-01918-x.

Munuswamy, P., Mariappan, A.K., Dhama, K. and Reddy, M.R. (2019): Avian Infectious Laryngotracheitis. In: Malik, Y.S., Singh, R.K. and Yadav, M.P. (eds.) Recent Advances in Animal Virology. Springer Singapore. pp. 72-90.

Oldoni, I., Rodríguez-Avila, A., Riblet, S.M., Zavala, G. and García, M. (2009): Pathogenicity and Growth Characteristics of Selected Infectious Laryngotracheitis Virus Strains from the United States. Avi. Pathol. 38(1):47-53.

Ou, S.C. and Giambrone, J.J. (2012): Infectious Laryngotracheitis Virus in Chickens. Worl. J. Virol. 1(5):142-149.

Owoade, A.A., Ducatez, M.F. and Muller, C.P. (2006): Sero-prevalence of Avian Influenza Virus, Infectious Bronchitis Virus, Reovirus, Avian Pneumovirus, Infectious Laryngotracheitis Virus and Avian Leukosis Virus in Nigerian Poultry. Avi. Dis. 50(2):222-227.

Parra, S., Nuñez, L. and Ferreira, A. (2016): Epidemiology of Avian Infectious Laryngotracheitis with Special Focus to South America: an update. Braz. J. Poul. Sci. 17(1):117-120.

Pitesky, M., Chin, R.P., Carnaccini, S., Sentíes-Cué, C.G., Charlton, B., Woolcock, P.R. and Shivaprasad, H.L. (2014): Spatial and Temporal Epidemiology of Infectious Laryngotracheitis in Central California: 2000–2012. Avi. Dis. 58(4):558-565.

Pohjola, L., Tammiranta, N., Ek-Kommonen, C., Soveri, T., Hänninen, M.L., Fredriksson Ahomaa, M. and Huovilainen, A. (2016): A Survey for Selected Avian Viral Pathogens in Backyard Chicken Farms In Finland. J. Avi. Pathol. http://dx.doi.org/10.1080/03079457.2016.1232804.

Ramdani, H., Zeghdoudi, M., Merdaci, L., Tahri, M., Madi, S. and Aoun, L. (2021): Retrospective Epidemiological Study of Infectious Laryngotracheitis in Broilers from 2011 to 2019. Rev. Rom. Med. Vet. 31(1):64-68.

Roba, Y., Tadesse, D., Assefa, Z. and Tesfaye, A. (2020): Sero-prevalence of Infectious Laryngotracheitis Disease in Backyard Chickens in Villages of Ada'a district, Oromia Region, Ethiopia: first report. Trop. Ani. Hlth. Prod. 52:3109–3112.

Salhi, O., Messaï, C., Ouchene, N., Boussaadi, I., Kentouche, H., Kaidi, R. and Khelef, D. (2021): Indicators and Risk Factors of Infectious Laryngotracheitis in Layer Hen Flocks in Algeria. Vet. worl. 14(1):182-189.

Shehata, A.A., Halami, M.Y., Sultan, H.H., Abd El-Razik, A.G. and Vahlenkamp, T.W. (2013): Chicken Embryo Origin-like Strains are Responsible for Infectious Laryngotracheitis Virus Outbreaks in Egyptian Cross-bred Broiler Chickens. Virus Genes. 46:423-430.

Shittu, I., Sulaiman, L.K., Gado, D.A., Egbuji, A.N., Ndahi, M.D., Pam, E. and Joannis, T.M. (2016): Sero-epizootiological Investigation of Infectious Laryngotracheitis Infection in Commercial Poultry of Plateau state, North Central Nigeria. J. Immunoas. Immunoch. http://dx.doi.org/10.1080/15321819.2016.1151439.

Tamilmaran, P., Kumar, R., Lakkawar, A.W., Uma, S. and Nair, M.G. (2020): Occurrence and Pathology of Infectious Laryngotracheitis(ILT) in Commercial Layer Chicken. J. Ento. Zoo. Stud. 8(2):1575-1579.

Tesfaye, A., Mesfin, S., Teshale, S., Tadewos, K., Abebe, G., Tafese, K., Chala, D., Chala, G., Hailu, H., Sintayehu, G. and Fanose, T. (2019): Infectious Laryngotracheitis in Commercial and Backyard Chicken Production Systems in Central and South Ethiopia (First report). J. Appl. Poult. Res. 28:1324–1329

Thrusfield, M. (2018): Veterinary Epidemiology (4th ed.). John Wiley & Sons Ltd. pp. 270 - 284.

Uddin, M.I., Sen, A.B., Islam, M.S., Das, S., Sultana, N., Ripa, R.N., Kashem, A. and Kamaruddin, K.M. (2014): Sero-epidemiology of Infectious Laryngotracheitis (ILT) in the Commercial Layer Farms of Chittagong district, Bangladesh. Advan. Anim. Vet. Sci. 2(6):316-320.

Wang, Y., Saelao, P., Chanthavixay, K., Gallardo, R., Bunn, D., Lamont, S.J., Dekkers, J.M., Kelly, T. and Zhou, H. (2018): Physiological responses to heat stress in two genetically distinct chicken inbred lines. Poult. Sci. 97:770–780.

Wernery, U. (2016): Infectious Diseases. In: Samour, J. (ed.) Avian Medicine (3rd ed.). Mosby, Missouri, USA. pp. 434-521.

Wolfrum, N. (2020): Infectious Laryngotracheitis: an Update on Current Approaches for Prevention of an Old Disease. J. Anim. Sci. 98(1): 27-35.

Wunderwald, C. and Hoop, R.K. (2002): Serological Monitoring of 40 Swiss Fancy Breed Poultry Flocks. Avi. Pathol. 31(2):157-162.

8. APPENDIX

Appendix 1: Semi-structured questionnaire

Questionnaire survey to assess the management and environmental risk factors associated with ILT

Reminder to enumerators

- Make brief introduction to household chicken owner before interviewing, greet them the local way if possible, exchange names and make explanations on the purpose and objectives of your interview
- Make a relaxed approach and present the questions clearly and patiently so that the respondent understands and give a valuable answer
- Avoid leading questions and putting your personal opinion on the questionnaire answer format
- Avoid using technical/scientific terms while presenting your interviews

Enumerator's Name___________________________Date_______________Agro-ecology: LL/ML/HL

Part I. Demographic characteristics of the household owner in the study area

Region: ______________________________ Zone: ___________________ Woreda:___________

Kebele : ______________________________Village: ______________________________________

Name of the respondent:____

Age of the respondent: ________________________ Sex of the respondent: __________________

Level of education of the respondent:

- Illiterate (not write and read)
- Primary school (1-6 grades)
- Secondary school (7-12 grades)
- Tertiary school (college/university)

Part II. General Information related to chicken production and health status in the study area

1. How many, which breeds and types of chicken do you have currently?

Chicken type	Number of chickens by breed		
	Local	Exotic/hybrid	Total
Layers			
Pullets			
Cocks			
Total			

2. Where do your chickens reside the day time?

- o Scavenging
- o Partially housed

3. Do you encounter any health problem in your chicken?

- o Yes
- o No

4. If the response to question number **3** is yes, which of the following syndromes are usually seen?

Disease Syndromes	**Yes**	**No**
Diarrhea		
Difficulty of respiration, coughing, nasal or ocular discharge		
Circling and paralysis		
Dropping of the wing and head		

5. Which one is the major loss encountered in your poultry production**?**

- o Death of chicks
- o Weight or egg loss
- o others

Part III. Management and environmental risk factors associated with chicken production in the area

6. Where is your chicken housed at night?

Availability of night shelter for chicken	Yes	No
a. In the main house with people		
b. In a separate house for chicken only		
c. In a separate house but with other animals		

7. Which one is the major source of feed for your chickens?

- o Scavenging only
- o Scavenging supplemented with household scarps or crop byproducts
- o Scavenging supplemented with industrial byproducts

8. Is there mixing up of different breeds, breeding stocks and ages of your chicken with each other or with neighboring chicken?

- Yes
- No

9. Is there any interaction between your chicken and wild birds?

- Yes
- No

10. Is it possible for you to avoid mixing of diseased chicken from healthy ones with the necessary physical barriers?

- Yes
- No

11. What do you do when your chicken is sick of unknown disease?

Activities	Yes	No
a. Doing nothing		
b. Brought to the market		
c. Treat with household medicines		
d. Seek veterinary consultation		

12. Do you have practice of culling of chickens sick of known/unknown severe disease?

- Yes
- No

13. If a chicken is suddenly died among your flock, how do you disposed off?

Activities	Yes	No
a. By simply throwing in to an open air		
b. By burning		
c. By burial		

14. Do you have a practice of cleaning chicken waste regularly?

- Yes
- No

15. Which one of the following is the main challenge to your chicken production in your area?

- Predators
- Disease
- Others

16. Season of frequent occurrence of disease in your chicken?

- September to November
- December to February
- March to May
- June to August

17. What do you think is the major predisposing factor for the occurrence of disease in your area?

____

____

Appendix 2: Chicken data collection format during sample collection and serum analysis

Date: ______________________Zone _________________ District_______________

village________________kebele____________Agro-ecologic Zone: :________________

HH No	Owner's name	Flock size	Sampled chicken				ELISA result	Antibody titer
			Code	Breed	Sex	Age		

Appendix 3: Household chicken flock sampling frame sheet

Date: ________________________Zone ___________________ District_________________

village__________________kebele_____________Agro-ecologic Zone: :__________________

HH No	Owner's Name	Name of Kebele	Name of Village	Total flock size	Flock size by breed & sex			
					local		Exotic	
					M	F	M	F

APPendix 4: Indirect ELISA procedure

Description and principle

- Micro-wells are coated with purified ILT antigen
- Samples to be tested and controls are added to the wells
- Anti-ILT antibodies, if present, form an antigen-antibody complex
- After washing, an anti-chicken horseradish peroxidase (HRP) conjugate is added to the wells. It fixes the antibodies, forming an antigen-antibody-conjugate-HRP complex.
- After elimination of excess conjugate by washing, the substrate solution(TMB) is added
- The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested:
  - ✓ In the presence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution
  - ✓ In the absence of antibodies, no coloration appears
- The micro-plate is read at 450 nm

Kit Components

Reagents supplied with the kit

- Micro-plates coated with purified ILT antigen
- Positive control
- Negative control
- Concentrated conjugate (10X)
- Dilution buffer 14
- Dilution buffer 9
- Dilution buffer 3
- Wash concentrate (20X)
- Substrate solution
- Stop solution (0.5M)

Materials required but not provided with the kit

- Mono or multi-channel pipettes capable of delivering Volumes of 5 µl, 10 µl, 100 µl and 300 µl
- Disposables tips.
- 96-well micro-plate reader

- Distilled or deionized water
- Manual or automatic wash system
- 96-well pre-dilution micro-plate

Solution Preparation

- Prepare the wash Solution (1X) by diluting the Wash Concentrate (20x) to 1/20 in distilled or deionized water
- Prepare the Conjugate 1X by diluting the concentrated conjugate 10X to 1:10 in Dilution Buffer 3

Testing Procedure

ID Screen® ILT indirect ELISA test was carried out in order to detect the specific antibodies for GaHV-1. Each serum was tested according to the manufacturer's instruction at a final dilution of 1:500 in dilution Buffer, such that the 1:50 in a pre-plate (pre-dilution) and the 1:10 in the coated ELISA micro-plate (dilution).

- In a pre-dilution plate set aside wells A1, B1, C1 and D1 for the controls, and add
  - ✓ 5 µl of each sample to be tested.
  - ✓ 245 µl of Dilution Buffer 14 to all wells except to control wells (A1, B1, C1 and D1)
- In the ELISA micro plate, add
  - ✓ 100 µl of the Negative Control to wells A1 and B1,
  - ✓ 100 µl of the Positive Control to wells C1 and D1,
  - ✓ 90 µl of Dilution Buffer 9 to all wells as there are samples to be tested excluding the control wells (A1, B1, C1 and D1).
  - ✓ 10 µl of the pre-diluted samples as prepared above
- Cover the plate and incubate for 60 min ± 5 min at 21°C ± (5°C)
- Empty the wells.
- Wash each well 3 times with approximately 300 µl of the wash Solution 1X and avoid drying of the wells between washes.
- Add 100 µl of the Conjugate 1X to each well
- Cover the plate and incubate for 60 min ± 5 min at 21°C ± 5°C).
- Empty the wells.

- Wash each well 3 times with approximately 300 µl of the Wash Solution 1X. Avoid drying of the wells between washes
- Add 100 µl of the Substrate Solution to each well.
- Cover the plate and incubate for 15 min ± 2 min at 21°C (± 5°C) in the dark.
- Add 100 µl of the Stop Solution to each well in order to stop the reaction.
- Read and record the OD at 450 nm.

The presence or absence of antibodies to ILTV was determined by comparing the value of sample to positive control OD (S/P) ratio. Following manufacturer's recommendations, the positive threshold for ILTV was a sample-to-positive (SP) ratio of 0.3 or greater. The relative level of antibody to ILTV in the sample is determined by calculating the sample to positive (S/P) ratio according to the methods provided by the manufacturer of the test kit and it was calculated using the following formula:

$$\frac{S}{P} = \frac{ODS - ODNC}{\mathrm{ODPC} - ODNC}$$

If S/P value was > 0.3, then the ILT antibody status was considered to be positive but S/P ≤ 0.3 was taken as negative

Validity of the test result was verified based on the manufacturers' recommendation that, the test is valid if the mean OD value of the positive control (ODPC) is greater than 0.25 or if the ratio of the mean value of the positive control and the negative control (ODPC and ODNC) is greater than 3. The mean ODPC was 0.63+0.59/2=0.61 and that of the mean ODNC was 0.05+0.05/2=0.05. Therefore, since the ODPC value (0.61) was greater than 0.25 and the ratio of the ODPC to the ODNC were 12.2 which is also greater than 3 then we can conclude that test result is valid.

9. BIOGRAPHY

The author, Demeke Hailu, was born in December 1975 in the city of Gondar, Ethiopia. He completed his primary and secondary education at Abiyot FIrae Elementary and Fasiledes Comprehensive Schools in Gondar City respectively. In 1993, he passed the ESLCE (Ethiopian School Leaving Certificate Examination) and obtained a high school certificate that enabled him to continue his education in higher institutions.

He joined the Faculty of Veterinary Medicine at Addis Ababa University in the academic year 1993/94 and graduated with a degree of Doctor of Veterinary Medicine in July 2000. After graduation, he was employed by the Amhara National Regional Government as district animal health team leader mainly with responsibilities of coordinating the overall animal health activities as well as a veterinary practitioner from September 2000 to June 2006.

He then left his government post and joined a short term project under the University of Edinburgh from July 2006 to May 2007 entitled “Rabies control project in domestic dogs, wild life and public health in Ethiopia” which focused primarily on vaccination of domestic dogs residing near the habitats of red fox in the Semien Mountain of north Gondar, Abune Yosef mountain of the north wollo and in the Bale mountains.

After termination of the project contract and a brief break, destiny called him to the Southern Region of Ethiopia and re-employed in June 2007 in the Department agriculture in the then Sidama zone and served as animal health team leader until May 2016. He then promoted to the regional bureau of Livestock and Fisheries as senior animal health expert and is serving until today in the Bureau of Agriculture.

In the meantime, he joined the Hawassa University, college of law extension program from 2011/12-2016/17 and awarded a Bachelor of Arts Degree (LL.B). Last but not least on 2022, he rejoined the Hawassa University, Faculty of Veterinary Medicine, this time to pursue his Master of Science in Veterinary Epidemiology at the School of Postgraduate Studies.