



WALLAGA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE

**SEROPREVALENCE OF INFECTIOUS BURSAL DISEASE AND ITS
ASSOCIATED RISK FACTORS IN BACKYARD CHICKENS
IN SELECTED DISTRICTS OF EAST WALLAGA ZONE, OROMIA, ETHIOPIA**

**DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND
PUBLIC HEALTH**

MSc. THESIS

BY:
HUNDERA WERKU

MAY, 2025

NEKEMTE, ETHIOPIA

**WALLAGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES**



**SEROPREVALENCE AND ITS ASSOCIATED RISK FACTORS OF
INFECTIOUS BURSAL DISEASE IN BACKYARD CHICKENS IN SELECTED
DISTRICTS OF EAST WALLAGA ZONE, WESTERN ETHIOPIA**

**A THESIS SUBMITTED TO THE SCHOOL OF VETERINARY MEDICINE
WALLAGA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY
MICROBIOLOGY**

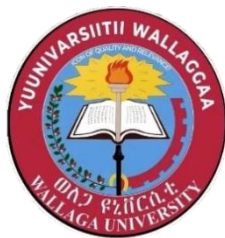
By:

Hundera Werku

Advisor: Lama Yimer (DVM, MVSc, Assistant Professor)

May, 2025

Nekemte, Ethiopia



WALLAGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
P.O. Box: 39 5, Nekemte, Ethiopia.

APPROVAL SHEET FOR SUBMITTING FINAL THESIS

As members of the examining board of the final MSc thesis open defenses, we certify that we have read and evaluated the thesis prepared by **Hundera Werku** entitled: **“Seroprevalence and Its Associated Risk Factors of Infectious Bursal Disease In Backyard Chickens In Selected Districts of East Wallaga Zone, Oromia, Ethiopia”** and recommend it to be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Microbiology

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STATEMENT OF THE AUTHOR

I declare that this thesis is my authentic and real work, that all sources and materials used for this has been properly acknowledged. This thesis has been dully submitted in partial fulfillment of the requirements for MSc degree at Wallaga University, School of Veterinary Medicine and is deposited at Wallaga university/college/school library to be made available to borrowers under rules of the library.

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Signature: _____

Date of submission: _____

Place: School of Veterinary Medicine, Nekemte, Ethiopia

DECLARATION

This is to certify that this thesis, entitled: "**Seroprevalence And Its Associated Risk Factors of Infectious Bursal Disease of Backyard Chickens in Selected District of East Wallaga Zone, Western Ethiopia**" was accepted in partial fulfillment of the requirements for the award of the degree of Master of Science by the school of graduate studies, Wallaga University, through the school of veterinary medicine; done by **Hundera Werku**, was a genuine work carried out by him under my guidance. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the research thesis requirements.

Student: Name: **Hundera Werku**

Signature: _____

Date: _____

Advisor: Dr. Lama Yimer(DVM, MSc, Assistant Professor)

Signature _____

Date _____

ACKNOWLEDGEMENTS

First of all, I am too pleased to thanks God, for giving me strength, power and granting me hope, love, patience, and blessing and also for his commitment and protection to me and my family.

My wonderful and deepest appreciation goes to my advisor Dr. Lama Yimer who advised me starting from the beginning proposal up to presentation of the final draft. No words to mention for his management, very nice approach, admirable assistance and open information. Next, I want to thanks Dr Demeke Zewde who facilitate and helping me during laboratory working and animal health institute (AHI) (sabeta) management for their willingness to host me their institutions for my laboratory work.

My honest appreciation is extended to Wallaga University School of Veterinary Medicine for allowing me to use any logistic and other access, especially internet service that is needed to complete master's thesis.

At the last but not the least, no phrase or words in any language can ever express my deep sense of love and gratitude to my wife and my Aunt Belayinesh Fite for her support both morally and financially.

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LIST OF ABBREVIATIONS

AC-ELISA	Antigen Capture Enzyme LinkedImmuno Sorbent Assay
BALT	Bronchial Associated Lymphoid tissues
CALT	Conjunctiva Associated lymphoid tissues
BF	Bursa of Fabricius
ELISA	Enzyme Linked Immuno Sorbent Assay
GALT	Gut Associated Lymphoid tissues
IBD	Infectious Bursal Diseases
IELISA	Indirect Enzyme Linked ImmunoSorbent Assay
IPNV	Infectious Pancreatic Necrosis Virus
MDAbs	Maternal Derived Antibodies
OD	Optical Density
OIE	Organization of International Epizootics
RT-PCR	Real Time Polymerase Chain Reaction
SP	Sample positive
SPF	Specific Pathogen Free
VNT	Viral Neutralization Test
VP	Viral Protein
VvIBDV	Very Virulent Infectious Bursal Disease Virus

ABSTRACT

Infectious Bursal Disease (IBD) is a highly contagious viral disease that poses a significant threat to poultry production globally, including in Ethiopia, where backyard chicken farming is a critical livelihood and food security source. Despite its economic impact, there is limited data on IBD seroprevalence and associated risk factors in the East Wallaga Zone of Western Ethiopia. A cross-sectional study was conducted from November 2024 to May 2025 in three districts (Wayu Tuka, Guto Gida, and Sibu Sire) with the aim of determining seroprevalence and associated risk factors of IBD in backyard chickens. A total of 340 serum samples were collected from backyard chickens and tested for IBDV antibodies using indirect ELISA (IELISA). Univariable and multivariable logistic regression analyses were employed to identify risk factors. The overall seroprevalence of IBDV was 88.53% (301/340). District-specific rates were 84.1% (Wayu Tuka), 88.9% (Guto Gida), and 92.7% (Sibu Sire). Significant associations were observed with age (adults: 92.4% vs. youngchickens: 71.0%; $P < 0.001$), breed (exotic: 94.7% vs. local: 83.5%; $P = 0.001$), and hygiene status (poor: 97.4% vs. good: 83.1%; $P = 0.017$). Multivariable analysis confirmed Sibu Sire district (OR = 5.70), exotic breeds (OR = 3.39), and poor hygiene (OR = 32.75) as high-risk factors. Generally, Infectious bursal disease is endemic in the study area, with high seroprevalence linked to management practices, breed, and hygiene. Therefore, targeted vaccination programs, improved biosecurity, and farmer education are urgently needed to mitigate economic losses. Moreover, the cross-sectional design precludes causal inferences, and the reliance on serology (without viral genotyping) limits insights into strain diversity. Hence, future studies that incorporate molecular techniques to characterize circulating strains and assess vaccine compatibility are needed.

Keywords: *backyard chickens, Ethiopia, IBD, risk factors, seroprevalence*

1. INTRODUCTION

Ethiopia has considerable livestock resource and poultry population is second ranked preceded by cattle. From the total population of chicken in Ethiopia, 99% are reared under the traditional backyard system of management, whereas 1% is under intensive management system. This flock provides with a yearly output of 72,300 metric tons of meat and 78,000 metric tons of eggs. Chickens are wide spread in the Ethiopia and are important to subsistence, economic and social livelihoods of a large human population. Chickens are especially important to women, children and aged individuals, who are the most vulnerable member of the society in terms of under-nutrition and poverty (Central Statistics Authority [CSA], 2009; Teklewold *et al.*, 2006).

Many biological and socio-economic factors are incriminating for the decrease of poultry population in Ethiopia, of which disease and poor animal health practices are the two most important responsible factors worth mentioning here. Among the different diseases causing losses in the poultry production in the country, infectious bursal disease is the top one. Infectious Bursal disease is a highly contagious immunosuppressive disease caused by a virus of the genus avibirnavirus of the family Birnaviridae and affects the immune system of young chicks throughout the world, occurring in more than 95% of member countries of OIE. Currently from those cited viral disease, IBD is the most important threat to poultry production in Ethiopia (Zeleke A, *et al.*, 2005).

The virus has no envelope and transmission of the disease is exclusively horizontal (Eterradossi, *et al.*, 2020). According to kurukulasuriya (2017), chickens with infectious bursal disease (IBD) are especially susceptible to secondary infections and frequently pass away as a result of the illness. Particularly in recent years; it has resulted in large financial losses for the poultry industry worldwide. IBD's pathophysiology is characterized mostly by its impact on lymphoid organs, particularly the lymphoid cells in the bursa of Fabricius, which ultimately results in lymphoid depletion and bursa death. Chickens are especially susceptible to the virus between three and six weeks after hatching, when the bursa of Fabricius reaches its maximum size (Bolis *et al.*, 2003).

The reports from some parts of Ethiopia showed high prevalence of the disease. In the study conducted by Jenbreie on 2597 chickens a prevalence of 83.1% was recorded at Mekelle and Gondar districts using ELISA test. However, it is very difficult to indicate the general figure of the disease due to lack of, or uneven, reports from different parts of the country (Jenbreie S, *et al.*, 2013).

The study conducted so far in the country has limitation to show the exact picture of the disease. Although the disease is one of the main poultry health constraints in terms of viral diseases, responsible for marked economic losses in the backyard production system of a country, there is limited well documented information on the seroprevalence and associated risk factors of IBD. So far it was limitation published report has been found on the backyard chicken production system from Western Ethiopia, especially Guto gida, Sibul Sire and Wayu Tuka districts, even though production loss and chicken mortality reportedly occur in these districts.

1.1.Statement of Problem

Infectious Bursal Disease (IBD) is a highly contagious viral infection affecting young chickens, characterized by high morbidity and mortality rates. The disease is caused by the IBD virus (IBDV), which primarily targets the bursa of Fabricius, leading to immunosuppression and increased susceptibility to secondary infections. The prevalence of IBD varies significantly across different geographical regions and production systems, which complicates control strategies. Limited data on the current prevalence of IBD in specific areas. Additionally, environmental factors, management practices, and the genetic diversity of poultry populations may influence the spread and impact of the disease.

Infectious Bursal Disease (IBD) poses a significant threat to poultry production, particularly in developing countries. In East Wallaga Zone of Oromia, poultry farming is a vital source of livelihood and food security. However, the prevalence of IBD and its impact on poultry health and productivity remain poorly understood in this selected district.

Despite the known economic implications of IBD, there is limited data on the seroprevalence and associated risk factors of IBDV in East Wallaga. High mortality rates associated with IBD outbreaks can lead to substantial economic losses for farmers, jeopardizing food security and livelihoods. Despite its economic impact, data on seroprevalence and risk factors in backyard systems particularly in East Wallaga Zone are scarce. The absence of effective control strategies, coupled with poor biosecurity and low vaccination coverage, exacerbates the disease's spread. This study addresses critical knowledge gaps by assessing IBDV seroprevalence in backyard chickens, identifying district-specific and management-related risk factors and providing evidence for targeted interventions to reduce losses in backyard poultry. The findings will provide critical insights for stakeholders and policymakers to enhance disease management practices and improve poultry health in the selected district.

1.2.Objectives

1.2.1. General objective

- To assess the seroprevalence of Infectious Bursal Disease and associated risk factors in backyard chickens in selected district of East Wallaga Zone, Western Ethiopia.

1.2.2. Specific Objectives

- To determine the seroprevalence of Infectious Bursal Disease Virus in selected districts.
- To evaluate associations between serop Infectious Bursal Disease Virus positivity and risk factors.

2. OVERVIEW OF INFECTIOUS BURSAL DISEASE

2.1. Etiology

The genus Avibirnavirus, which is a member of the family Birnaviridae, is the source of the infectious bursal disease known as “Gumboro disease”. It is an icosahedral, non-enveloped, double stranded RNA virus with a bi-segmented genome. Viral proteins, VP2, VP3, VP4, and VP5, are encoded by the bigger segment, which is 3261 nucleotides long and has two open reading frames. The smaller segment B only encodes VP1, which is polymerase active. The two structural proteins that make up the viral capsid are VP2, and VP3. The VP2 protein contains the epitopes that cause the production of protective and neutralizing antibodies (Mahgoub *et al.*, 2012).

With a diameter of roughly 70 nm, the capsid has a T=13 icosahedral symmetry and is non-enveloped. It is made up of 260 trimers of VP2 that project radially from the capsid to form spikes. Virion retains the peptides that were produced by preVP2 C-terminal cleavages. Together with the genomic RNA, VP3 forms a ribonucleoprotein complex. The virion also contains trace levels of VP1 (Abdel-Alim and Saif, 2001). The family comprises three genera: *Entomobirnavirus*, which infects insects, *Avi Birnavirus*, whose type species is infectious bursal disease virus (IBDV), which infects birds, and *Aqua-birnavirus*, whose type species is infectious pancreatic necrosis virus (IPNV), which infects fish, mollusks, and crustaceans. There are two viral serotypes known as IBDV 1 and 2. Serotype 1 of the IBD virus is a significant pathogen for chickens. Since immunization with serotype 2 viruses did not provide protection against serotype 1, serotype 2 viruses are immunologically distinct from serotype 1 viruses (Delmas *et al.*, 2004).

2.1.1. Physical and chemical nature of the virus

The virus is not encapsulated and is resistant to both physical and chemical assaults due to its stability and hardness. It is stable at pH values between 2 and 11, but at 12, it loses its ability infectivity. Due to this resistance, even after extensive cleaning and disinfection, the virus can persist in chicken facilities for up to 4 weeks in the bone marrow of infected birds. It has been shown that the virus may infect feed, water, and waste for 52 days, as well as a henhouse for 122 days (Lukert and Saif 2003).

2.1.2. Antigenic variation of IBDV strains

Previously, variations in the virulence, antigenicity, tropism, and immunogenicity of circulating strains of the infectious bursal disease virus (IBDV) have been caused by alterations in the IBDV genome, which has also impacted the detection of antibodies (Zierenberg *et al.*, 2004). As a result, it's critical to carry out continuous surveillance in addition to prompt description, identification, and comparison of recently isolated IBDV with previously documented viruses. These recently reported antigenic variants have their basic basis in antigenic regions of the IBDV VP2 protein. The viral capsid protein, or VP2, is the primary immunogen that shields the host since it is the sole viral protein that produces neutralizing antibodies and guarantees serotype specificity (Van den Berg, 2000).

2.2. Epidemiology

2.2.1. Host Range

Serological response and isolation have shown that IBDV serotypes I and II can infect turkeys, ducks, and ostriches both spontaneously and experimentally; nonetheless, the infections are not harmful. Just hens are impacted by clinical disease. Many bird species have been found to either have antibodies against IBDV or be susceptible to infection, including gulls, falcons, crows, rooks, and wild pheasants (Campbell, 2001).

Serotype I viruses can infect any variety of chicken, although they have the highest mortality rate and the most severe lesions and clinical signs in white leghorns. In flocks that are fully susceptible, classic strain infections can have a high morbidity incidence of up to 100% and a fatality rate of 1-60%. On the other hand, vvIBDV strains cause 90–100%, 25–30%, and 50–60% of laying hen deaths in susceptible SPF leghorns (Muller *et al.*, 2003). According to reports, a high mortality rate is caused by susceptible hens infected with virulent strains of IBDV because they are able to mount a quick systemic cytokine-mediated immune response, which may lead to a shock-like state and final death (Asif *et al.*, 2007).

2.2.2. Transmission

Vectors from vulnerable flocks to inanimate things (farm workers, livestock) facilitate the transmission (Sharma, 2000). Infected birds excrete the virus in their droppings for a minimum of 14 days. It is discharged in excrement, where it contaminates water, food, and litter, where it stays and usually spreads. The most common type of infection is oral; conjunctival and respiratory infections can also occur, but the virus is extremely infectious, meaning that the illness can be transmitted by direct touch with excreting patients or by indirect contact with anything contaminated. It's possible that the virus's remarkable resilience to numerous virucidal and disinfection treatments contributed to its rapid spread. Infected equipment could facilitate the spread of IBDV (Jackwood and Sommer-Wagner, 2010).

2.3. Pathogenesis

Once the virus enters the body through the mouth or the respiratory system, it can cling to host cell proteins such as the N-glycosylated polypeptide(s) that develop on the cell membrane of developing IgM⁺ B-cells. Because the virus only needs two to three days to incubate, pore-forming peptide (pep46) attached to the outer capsid of the infected particle may facilitate the virus's entry into the cytoplasm of infected cells. An *in vitro* study's results led to the proposal of an endocytic mechanism mediated by lipid drafts to allow attenuated IBDV to enter the cells in lymphocytes and macrophages of the gut-associated lymphoid tissues (GALT), IBDV starts the infection and replication process. The initial viraemia is identified by this stage of viral replication. The bursa of Fabricius (BF), the primary organ for widespread IBDV replication in the cytoplasm of intrabursal IgM⁺ B-cells, is reached by infected macrophages carrying the virus. The virus either destroys the circulating B-cells in secondary lymphoid tissues like GALT (gut-associated lymphoid tissue), CALT (conjunctiva), BALT (bronchial), and caecal tonsils, or it causes a second viraemia 16 hours after infection, which results in illness and death. When susceptible hens are infected with vvIBDV, the virus may spread to other lymphoid organs, including the spleen, Peyer's patches, cecal tonsils, and Harderian glands. IBDV infection causes noticeable inflammation in the BF as early as 48 hours after infection. All bursal IgM⁺ B-cells are infected and exhibit cytolytic alterations by day 3 or 4 pi.

The acute phase (7-10 days) of IBD can cause clinical symptoms and even mortality (Etteradossi and Saif, (2008).ore easily (Yip *et al.*, 2012).

2.4. Clinical Signs

Depending on the IBDV strain and presence of maternal immunity, infectious bursal disease can cause clinical or subclinical disease in young chicks. Results of infection depend on age and breed of chicken and virulence of the virus. Infections before 3 weeks old are usually subclinical. For both clinical and subclinical forms of the disease, all pathogenic IBDVs cause lesions in the cloacal bursa (bursa of Fabricius).

Early subclinical infections are the most important form of the disease because of economic losses. They cause severe, long-lasting immunosuppression due to destruction of immature lymphocytes in the cloacal bursa, thymus, and spleen. The humoral (B cell) immune response is most severely affected; the cell-mediated (T cell) immune response is affected to a lesser extent. Chickens immunosuppressed by early IBDV infections do not respond well to vaccination and are predisposed to infections with normally nonpathogenic viruses and bacteria. Common diseases are usually exacerbated by IBDV infections. Some strains of IBDV can cause subclinical infections in older birds (3–6 weeks old), leading to losses from poor feed efficiency and longer times to market. In these cases, immunosuppression is usually transient, and convalescent birds may recover most or all of their humoral immune function. However, secondary infections that occur during the transient immunosuppression can cause substantial economic losses (Hoerr, F.2010).

2.5. Pathology and Lesions

The bursa of Fabricius, the chicken's reservoir for B lymphocytes, is the main target of the virus, despite the fact that IBD affects other lymphoid organs. The virus will stimulate the mature and competent cells, causing them to proliferate, while the immature lymphocytes were die. The bursa is the primary site of macroscopic lesions, exhibiting all phases of inflammation subsequent to an acute infection (Muller, *et al.*, 2003).

The bursa is initially hypertrophic, edematous, and hemorrhagic; its color changes from white to cream, and a yellow transudate covers its serosa early in infection, according to autopsies done on birds who died during the acute phase (three to four days after infection). Serous transudate and a substantial mucous membrane infection, which give the bursal surface a yellowish color and are frequently accompanied by petechiae and hemorrhages, are characteristics of the most severe cases. The bursa returns to normal size by the fifth day and becomes atrophied to less than one-third of its original size by the eighth day. Furthermore, macroscopic lesions may also be seen in other lymphoid organs (the thymus, spleen, caecal tonsils, Harderian glands, and Payer's patches) during the acute phase of the disease brought on by hypervirulent strains (Eterradossi and Saif, 2008).

The postmortem examination of the affected animals revealed hypertrophy and white kidneys with deposits of urate crystals and cell debris, severely dehydrated carcasses, and frequently darker pectoral muscles with many petechiae hemorrhages. It is possible to see lumps in the pectoral and thigh muscles, which are most likely caused by a coagulation problem. The intestines may also contain mucus. Pale liver, bile stained, and possibly with gray foci on an enlarged spleen (Skeeles *et al.*, 1980).

2.6. Diagnosis

Initial diagnosis of infectious bursal disease is accomplished by the observation of gross lesions in the cloacal bursa. This is followed by microscopic analysis of the bursa for lymphocyte depletion in the follicles. Molecular diagnostic assays are most often used to identify IBDV in diagnostic samples. IBDV is most readily isolated from the cloacal bursa but may be isolated from other organs. RT-PCR assay is used to identify the viral genome in cloacal bursa tissue. Sequence alignments and phylogenetic analysis of the VP2 coding region has been used to further characterize the viruses into genogroups. Samples for molecular diagnostic testing are typically collected after maternal antibodies have waned. IBDV may be isolated in 8- to 11-day-old IBDV antibody-free chicken embryos with inocula from birds in the early stages of disease. The chorioallantoic membrane is more sensitive to inoculation than is the allantoic sac. Some strains of IBDV may also be isolated in cell cultures that include chicken embryo fibroblasts, cells from the cloacal bursa, and established avian and mammalian cell lines. Cell culture

adapted strains of IBDV produce a cytopathic effect and may be used for quantitative titration of the virus and virus-neutralization assays. Serologic testing can be used to detect antibodies against IBDV in convalescent chicks. Commercially available ELISA kits are most often used to quantitate IBDV antibodies. The presence of IBDV antibodies in chicks is not always an indication of infection because most young chicks have maternal antibodies (Jackwood D (2004), OIE (2012).

2.6.1. Histological Diagnosis

The identification of changes taking place in the bursa serves as the foundation for histological diagnosis. Hypervirulent IBDV strains have been shown to possess the capacity to induce histological lesions in non-bursal lymphoid organs, such as the spleen, bone marrow, or thymus (Inoue *et al.*, 1994, 1999). One benefit of using the histological diagnostic approach is that it may be used to diagnose both the acute and chronic, or subclinical, phases of the illness. Viral antigen detection: between the fourth and sixth day after inoculation, thin sections of the bursa of Fabricius were prepared to detect viral antigens specific to IBDV using immune peroxidase staining (Cho *et al.*, 1987) or direct and indirect immunofluorescence (Allan *et al.*, 1984).

2.6.2. Serological Diagnosis

In addition to being used to monitor vaccine responses, serological tests like AGID, ELISA, and VNT for antibody detection may provide extra information needed to diagnose infection in flocks that have not received vaccinations. The most widely used test for detecting and quantifying IBDV antibodies to assess vaccination response, natural field exposure, and deterioration of maternal antibody titer is the enzyme linked immune sorbent assay (ELISA). It is affordable, easy to use, rapid, and adaptable to computer software and automation. It can test many samples at once (Lukert and Saif, 2003).

The antigen capture enzyme-linked immunosorbent assay (AC-ELISA) or the agar-gel precipitin assay can be used to identify viral antigens. The VN titers precisely correspond to the hens' defense against IBDV (Knoblich *et al.*, 2000). ELISA and monoclonal antibodies have been used

to distinguish between classic and variant strains. But these techniques could not be as sensitive and quick as molecular techniques (Jackwood, 2004).

2.6.3. Viral Isolation

The virus can be isolated in cell cultures, in embryonated eggs, or by vaccinating vulnerable birds. The best way is to vaccinate birds, as alternative methods can change the original characteristics of the field strains of IBDV (Rosenberger *et al.*, 1998).

2.6.4. Molecular Diagnosis

The majority of molecular identification efforts have been directed towards characterizing the vVP2 encoding region and the broader segment A of IBDV. A number of methods for characterizing RT-PCR product utilizing restriction endonucleases have been disclosed. These methods are called restriction fragment length polymorphism (RTPCR/RE) or RT-PCR-RFLP (Zierenberg *et al.*, 2001). The identification of enzymes that cut in restriction sites that are phenotypically important determines how valuable the information they offer. Restriction sites that are consistently linked to virulence still need to be characterized and verified, although several sites implicated in antigenicity have already been found.

2.7. Differential Diagnosis

The monitoring of symptoms and postmortem study of the pathognomonic lesions, namely the bursa of Fabricius, are crucial to the clinical diagnosis of acute forms of IBD. The disease progression reaches a mortality peak, after which there is recovery in five to seven days. The differential diagnosis for IBD includes conditions such as nephron pathogenic types of infectious bronchitis, avian coccidiosis, mycotoxicoses, stunting syndrome, and Newcastle disease in certain visceral forms. Bursal lesions are necessary for the diagnosis of IBD in all acute cases. In circumstances that are not clinically noticeable, bursa atrophy could be mistaken for other illnesses such infectious anemia or Marek's disease. Differentiating between these will be possible by a histological analysis of the bursa (Lukert and Saif, 2003).

2.8. Treatment

There is no known therapeutic intervention that can influence the progression of the viral infection; however, medications for symptom management can assist manage secondary agents and mitigate the consequences of immunosuppression in birds (Muller *et al.*, 2003).

2.9. Prevention and Control

There is no effective treatment for Infectious Bursal Disease. Since the virus is very stable in the environment, strict cleaning, disinfection, pest control and adequate downtime after depopulating a contaminated farm is key to reduce the risk of recurrent outbreaks. Control is based in passive protection of young chicks (MDA protecting chicks from early infection) and active immunization by vaccination.

2.9. 1. Management and Hygiene Procedures

The virus that a cause infectious bursal disease is extremely contagious and resistant to inactivation, which explains why it continues to exist in chicken farms even after disinfection and necessitates stringent management and hygiene procedures (Van den Berg, 2000).

2.9. 2. Vaccine and Vaccination

The principal approach to controlling many infectious diseases in poultry is to immunize hens with high-quality vaccinations. IBDV, on the other hand, is resistant to a wide range of disinfectants and is highly stable in the environment, but it is mostly managed by vaccination when administered according to the recommended schedule. Numerous investigations have highlighted the importance of rational immunization regimens and stringent biosecurity protocols in the management of IBD (Wagari, 2021).

The type of production, degree of biosecurity, local pattern of disease, status of maternally derived antibodies (MDAbs), availability of vaccines, prices, and possible losses are just a few of the many local elements that affect vaccinations and vaccination programs, which vary greatly. The importance of MDABs in defending chicks against IBDV has been demonstrated by

numerous earlier investigations. Progeny challenge studies, vaccination-challenge studies, and in vivo cross-protection experiments are commonly conducted to evaluate the effectiveness of IBDV vaccines and to identify the pathogenicity and antigenic characteristics of IBDV strains (Dormitorio *et al.*, 2007).

2.10. Economic Importance of Infectious Bursal Disease

Serious economic consequences result from IBD in poultry, which are impacted by the virus strain, flock breed, susceptibility, and current main and secondary infections in addition to environmental and managerial factors. Because of the high death rate associated with clinical IBDV, there are immediate costs. However, carcasses that are condemned because of skeletal muscle, thigh, and pectoral muscle hemorrhages can also result in significant financial losses (Wagari, 2021).

Due to the extreme immunosuppression of broilers and egg-laying hens, as well as their increased susceptibility to other infections and immunization failure, indirect losses from Gumboro disease occur. Delays in growth, decreased weight gain, decreased food conversion, prolonged fattening, lower production values, higher mortality, and decreased product quality are therefore the results (Sharma, 2000). The disease's economic significance has increased due to the prevalence of vvIBDVs. Vaccination was able to adequately manage the disease up until 1987, when the virus's strains became less virulent and killed fewer than 2% of cases. On the other hand, immunization failures and elevated mortality and morbidity have resulted from the incidence of vvIBDV. Acute clinical illness caused by IBDV has been recorded in 80% of OIE member countries (Van den Berg, 2000).

From the total population of chicken in Ethiopia, 99% are reared under the traditional backyard system of management, whereas 1% is under intensive management system. Chickens are wide spread in the Ethiopia and are important to subsistence, economic and social livelihoods of a large human population. Chickens are especially important to women, children and aged individuals, who are the most vulnerable member of the society in terms of under-nutrition and poverty. However, there has been a gradual decline in the Ethiopian poultry population.

According to the Central Statistical Authority (2004–2005), the Ethiopian poultry population was estimated at 85 and 31 million in 1954 and in 2005, respectively. Many biological and socio-economic factors are incriminating for the decrease of poultry population in Ethiopia, of which disease and poor animal health practices are the two most important responsible factors worth mentioning here. Among the different diseases causing losses in the poultry production in the country, infectious bursal disease is the top one (CSA, 2013; Teklewold *et al.*, 2006).

2.11. Status of Infectious Bursal Disease in Ethiopia

In Ethiopia, backyard chickens are mostly indigenous chicken breeds managed by individual households and maintained under scavenging regimens in the backyards with inadequate housing, feeding, and health care. In recent years, Ethiopia is experiencing rapid growth in the poultry sector. Improved breeds of chickens are distributed all over chicken-producing areas of Ethiopia from breeding and multiplication centers. In backyard chicken, poultry diseases including IBD are the major constraints of poultry production (Urgesa and Lensa., 2023).

Infectious Bursal Disease is a newly emerging disease of chicken in Ethiopia, as described by (Jembere *et al.*, 2005), the disease has been speculated to be introduced concurrent with the increased number of commercial state and private poultry farms flourishing in the country. Research and case reports coming from various regions of the country indicated that viral diseases are posing a growing threat to the young poultry industry flourishing in the country. As a result, viral illnesses like IBD are starting to seriously jeopardize poultry productivity. The Ethiopian juvenile poultry sector faced challenges because to the recurrent outbreaks of infectious bursal disease and the emergence of novel strains. Infectious bursal illness has been linked to 25–75% of exotic and cross-breed chicken fatalities and losses in the last several years (Wagari, 2021).

The samples were processed in both cases at the National Veterinary Institute in Ethiopia using chicken fibroblast cell culture for the purpose of isolating the viruses. The isolates that proved to be positive were then sent to the OIE-IBD Reference Laboratory in France for additional antigenic and genomic characterization, where they were found to be highly virulent IBD viruses as well as virulent classical viruses. Furthermore, the prevalence of IBDV mortality in chickens is a major financial loss for chicken traders, especially those who purchase young chickens and raise them for several weeks after purchase. The disease is evident in small-scale commercial flocks and backyard poultry farms across the nation (Jembere *et al.*, 2014).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in selected district of East Wallaga Zone of Oromia Regional State, western Ethiopia. Wayu Tuka was situated in the western region of Ethiopia, 324 kilometers from Finfine, the nation's capital. There are two town kebele and ten rural kebele in the district. The Wayu Tuka district lies between latitudes 80° North and longitudes 340° East. The district has a total land area of 45,339,300 km². The region receives rainfall in two distinct seasons: spring (April–May) and summer (June–November), which is the main rainy season. The study area had a temperature of 26°C and average annual rainfall of 1400–2400 ml. According to the livestock development department of agricultural office of the district, the total livestock population of in Wayu Tuka district is about 268,660 out of which cattle was about 99,558, sheep 32,805, goat 20,851, poultry 100,272, horse 7,780, donkey 6,308 and mule 1,086 (WTAO, 2023).

Sibu Sire district was situated around 273 kilometers west of the Ethiopian capital, Addis Ababa, on the Addis Ababa Nekemte main road. Sire is the town at the middle of the district, located at 9°04'59.9" N and 36°49'59.9" E. The district's elevation ranges from 1,200 to 2,400 meters above sea level (m.a.s.l.), with an average of 1,830 meters. 7.53% of the region's agro ecology is highland, 74.2% is midland, and 18.7% is lowland. With brief wet seasons from March to May and the main rainy season from June to September, it exhibits a bimodal rainfall pattern (84 % of rain is predicted). Rainfall in the region ranges from 1,000 to 2,400 mm, with an average of 1,700 mm per year. The districts lowest and highest recorded temperatures were 10.9°C and 33.9°C, respectively. The district has the following livestock populations: 190,166 cattle, 35,952 sheep, 36,512 goats, 193,155 poultry, 12,249 horses, 16,402 donkeys, and 1,196 mules (SSAO, 2023).

Guto Gida District was located around 331 Km West of Addis Ababa. It is located between 1350 and 2400 meters above sea level (Masl) in latitude and longitude 9°5 'N 36°33'E /9.083°N 36.550°E. Its climate was classified as highland (0.26%), midland (46.74%), and lowland (53%).

The average annual rainfall was between 1800 and 2200 mm, while the average temperature ranged between 12 to 27 °C.

Rainfall in the region occurs in two distinct seasons: June to September is the long rainy season, whereas March, April, and May are the short wet season. The Guto Gida people follow a mixed farming style, which involves raising both crops and livestock. In the district, there are 86,724 cattle, 8,589 horses, 14,171 sheep, 11,821 goats, and 57,695 chickens (CSA, GGAO, 2023).

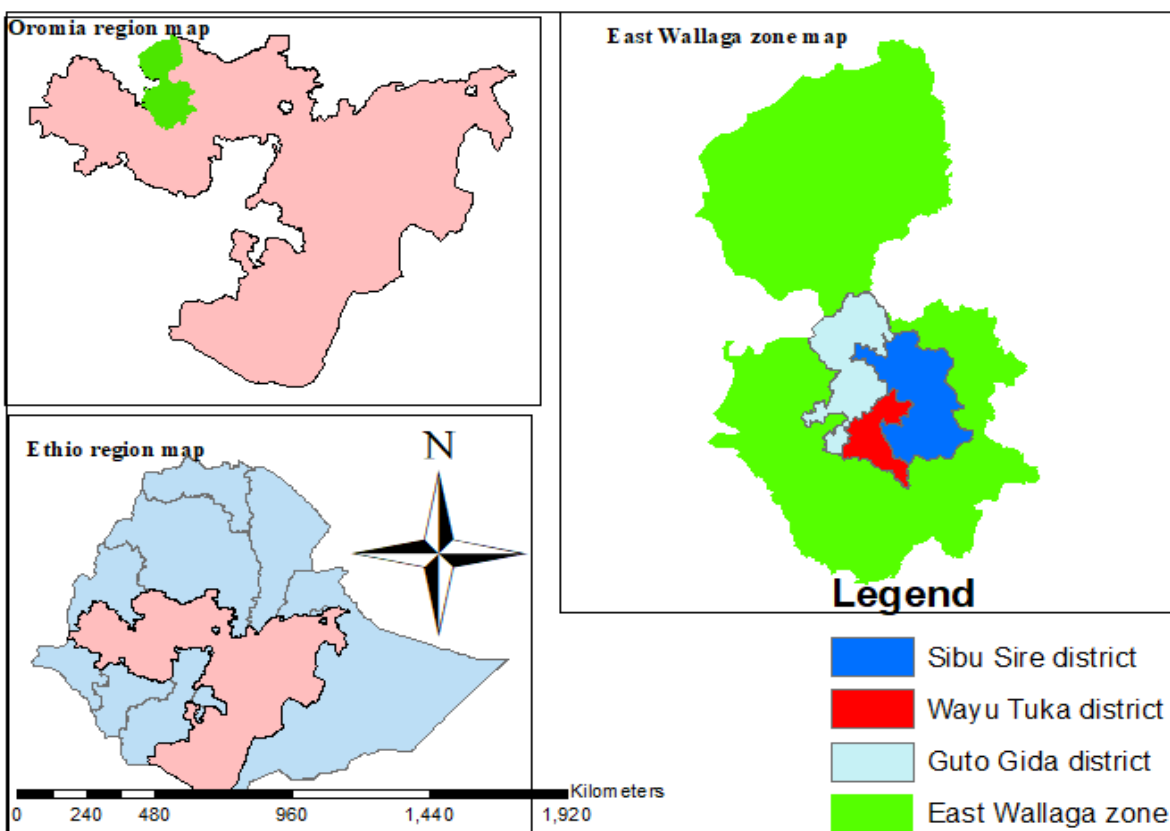


Figure 1: Map showing the study area

Source: - Created using Arc GIS software

3.2. Study Population

The study population consisted of backyard chickens aged over three month from three districts in East Wallaga Zone, Western Ethiopia: Wayu Tuka, Guto Gida, and Sibu Sire district. Chickens were categorized by: Age (young (3 month up to one years) and Adult (>1 years), determined subjectively via physical traits (crown size, spur length, xiphoid cartilage flexibility) (Magwisha *et al.*,2002) and information from the poultry farmers. Chickens were classified as their origin of selected districts. Based on sex, chickens were also grouped as male and female and breed (Local and exotic), flock size, hygiene condition (Good, medium, or poor (based on management practices) (chali *et al.*, 2024).

3.3. Study Design

A cross-sectional study was conducted from November 2024 to May 2025 to determine IBDV seroprevalence and identify risk factors. Blood samples were collected from randomly selected, apparently healthy chickens. The number of samples were determined and allocated proportionately to each district, PA, and household. Three Pas /kebele/ were chosen from each district and chicken was chosen at random using a basic simple random sample technique.

3.4. Sample Size Determination and Sampling Methods

3.4.1. Sample size determination

The required sample size for the study was determined based on the formula of (Thrusfield, 2018): Therefore the sample size was determined using the 95% confidence interval at 66.93% expected prevalence (Debela *et al.*, 2022) and 0.05 desired absolute precision.

$$n = \frac{(1.96)^2 * P_{exp} (1-P_{exp})}{d^2}$$
$$n = \frac{(1.96)^2 * 0.6693 (1-0.6693)}{0.05^2} = 340$$
$$n = \text{Required sample size}$$

Where: P_{exp} = Expected prevalence
 d = Desired level of precision at 95% confidence interval

Accordingly, a total of 340 poultry was included in this study for sample collection.

3.4.2. Sampling methods

To ensure representative data for assessing IBDV seroprevalence and associated factors in backyard poultry systems, the sampling made were Purposive sampling selection based on poultry density, agro ecology and accessibility Three PAs per district (nine totals) were randomly selected based on the willingness of the farm owners to allow sample collection and due to logistic and financial reasons. Simple random sampling techniques was followed to select the chickens from house within Pas and Chickens Samples were systematically allocated to districts (Wayu Tuka: 113; Guto Gida: 117; Sibu Sire: 110).

3.5. Study Techniques

3.5.1. Sample collection

For serological detection, sterile 3 mL disposable syringes were used to collect blood samples from the brachial vein of the chickens (Siegmann, 2013). A 3-ml syringe was used to collect 1.5 to 2 ml of blood after the humeral region of the wing vein has been cleaned with 70% alcohol. The syringe was then placed almost horizontally until the blood clots at 37 °C for 24 hours or before the serum is separated. To help the serum seep out after clotting, the syringe was put back in a vertical inverted posture. The separated serum was transferred to cryovials, labeled. Serum was stored at 4-8°C until shipment takes place, for maximum of 7 days.

Then, it was transported by using ice box bag to Sebeta Animal Health Institute, Ethiopia and stored at -20 °C until the Indirect Enzyme-Linked Immunosorbent Assay (IELISA) was performed to detect antibodies against the IBD virus (Bedasa *et al.*, 2022).

3.5.2. Serological test and procedure

An antibody against the IBD was measured from sera that were collected. As directed by the kit manufacturer (IDvet, France), the mean titer among the flock samples was determined. In a study assessing different serological methods for diagnosing IBD, Indirect ELISA demonstrated the highest sensitivity among the tested assays. Specifically, it recorded a sensitivity of 81.1% for detecting IBDV antibodies in local chickens (Daodu *et al.*, 2018).

Antibodies against the IBD virus were found in chickens using the indirect ELISA technique. Five hundred fold (1:500) dilutions were used, to put it briefly. All pre dilution plate wells except the controls received approximately 245µl of dilution buffer 14. Then, 5µl of sample was added to each well, excluding the controls. Wells A1 and B1 received 100µl of negative control, while wells C1 and D1 received 100µl of positive control. Then 90µl of dilution buffer 14 and 10µl of the pre diluted samples was dispersed into appropriate 92 well plates coated with IBDV viral antigen, and the plate was covered and incubated at room temperature for 30 minutes.

At the end of the incubation period, 300µl of the wash solution was used to wash the plate three times. Next, 100µl of horseradish peroxidase conjugate was added to each well. After 30 minutes of room temperature incubation, the plate was rinsed three times for the second round and 100µl of the substrate solution was added to each test well. The test wells were then left to incubate for 15 minutes at room temperature in a dark location. A micro plate reader was used to measure the absorbance at 450 nm after 100µl of stop solution was introduced to each well to stop the reaction. If the positive control's mean optical density (OD) was higher than 0.25 ($OD_{pc} > 0.25$), and the ratio of the positive and negative controls' mean ODs (OD_{PC} and OD_{NC}) was larger than 3, then the indirect ELISA test was considered valid.

Therefore, serum is regarded as having sufficient protective antibody against IBD if the estimated S/P ratio is greater than 0.3, while less protective antibody is seen as having a value less than 0.3. The IBD antibody level in each field chicken serum tested and the IBD ELISA kit-positive and non-reactive sera are compared in the IBD ELISA titer values that were obtained. Consequently, it is crucial to first confirm that the IBD ELISA positive and normal

positive control serum values obtained are legitimate, as explained in the section above on "As say Control Values." Each test serum's Sp (sample to positive ratio) was necessary in order to evaluate the test results. Next, using the following formula as instructed by the manufacturer, the sample to positive ratio was determined:

SP = sample absorbance – average normal control absorbance

Corrected positive control absorbance An IBD ELISA titer is calculated by the following suggested equation by the manufacturer:

$$\text{Log}_{10} \text{ Titer} = (1.172 \times \text{Log}_{10} \text{ Sp}) + 3.614 \text{ then,}$$

$$\text{Titer} = \text{Antilog of Log}_{10} \text{ Titer}$$

If Sp (sample to positive control) value was ≥ 0.3 the IBD antibody status was considered to be positive but < 0.3 was taken as negative (Chala *et al.*, 2023).

3.6. Data Management and Analysis

Data collected from the field and the serological test were coded and stored in MS Excel spreadsheet and transferred to SPSS version 20 software package. The number of animals that tested positive for i-ELISA was divided by the total number of animals to get the overall prevalence. Breed, age, sex, flock size, and management concerns are examples of dependent variables. The relationship between possible risk factors and the disease was assessed using the chi-square test. For components that were strongly correlated, the degree of correlation between potential risk factors and IBD serological status was measured using odds ratio (OR) estimates. The final multivariable logistic regression model was used to analyze potential risk factors found in the univariable analysis. A p-value < 0.05 was used as the significance criterion for inclusion or exclusion in the final model. Statistical significance was defined as a 95% CI with a significance threshold of $P < 0.05$ for all analyses.

4. RESULTS

4.1. Prevalence of Infectious Bursal Disease Antibodies

A total of 340 serum samples from backyard chickens across three districts (Wayu Tuka, Guto Gida, and Sibü Sire) were tested for IBDV antibodies using indirect ELISA. The overall seroprevalence was **88.53% (301/340)**, indicating widespread exposure to IBDV in the study area. Chickens from Sibü Sire had the highest seroprevalence (**92.7%**), followed by Guto Gida (**88.9%**) and Wayu Tuka (**84.1%**). However, the Chi-square test revealed no significant association between district and seropositivity ($\chi^2 = 4.136, P = 0.126$). Similarly, sex was not significantly associated with seropositivity, with males showing 90.0% and females 88.1% seroprevalence ($\chi^2 = 0.223, P = 0.637$). Flock size also showed no significant association ($\chi^2 = 1.98, P = 0.370$), with seroprevalence rates of 85.8% , 90.8%, and 87.5%. In contrast, age was significantly associated with seroprevalence ($\chi^2 = 23.02, P < 0.001$), with adult chickens exhibiting higher seropositivity (92.4%) compared to young chickens (71.0%). Breed type also showed a significant association ($\chi^2 = 10.431, P = 0.001$), with exotic breeds having higher seroprevalence (94.7%) than local breeds (83.5%). Additionally, hygiene status significantly influenced seropositivity ($\chi^2 = 8.175, P = 0.017$), with poor-hygiene flocks showing the highest seroprevalence (97.4%), followed by medium (86.8%) and good (83.1%) hygiene conditions (Table 1).

Table 1: Association of seroprevalence of IBD Virus and different risk factors

Risk Factors	Category	Sample tested	Sample positive (Seroprevalence %)	χ^2	P-value
Districts	Wayu Tuka	113	95(84.1%)	4.136	0.126
	Guto Gida	117	104(88.9%)		
	Sibü Sire	110	102(92.7%)		
Sex	Male	80	72(90.0%)	0.223	0.637
	Female	260	229(88.1%)		
Flock size	Small	148	127(85.8%)		

	Medium	184	167(90.8%)	1.98	0.370
	Large	8	7(87.5%)		
Age	Adult	278	257(92.4%)	23.02	0.000
	Young	62	44(71.0%)		
Breed type	Local	188	157(83.5%)	10.43	0.001
	Exotic	152	144(94.7%)	1	
Hygiene status	Good	59	49(83.1%)	8.175	0.017
	Medium	205	178(86.8%)		
	Poor	76	74(97.4%)		

4.2. Univariable Logistic Regression Analysis

The univariable logistic regression analysis highlights the significant roles of district (specifically Sibü Sire), age (older birds being less likely to be seropositive), breed type (exotic breeds being more likely to be seropositive), and hygiene status (poor hygiene increasing the risk of sero-positivity) in IBDV sero-positivity. Thus districts, breed type, age, and hygiene status were taken to multivariable logistic regression for further analysis. However, sex and flock sizes were not associated with IBDV sero-positivity (Table 2).

Table 2: Results of univariable logistic regression analysis of seropositivity to IBD Virus

Variables	Categories	Sample tested	Sample positive (%)	95% CI	P-Value
	Wayu Tuka	113	95(84.1%)	**	
Districts	Guto Gida	117	104(88.9%)	1.51 (0.705-3.260)	0.287
	Sibü Sire	110	102(92.7%)	2.41(1.004-5.815)	0.049
	Male	80	72(90.0%)	**	
Sex	Female	260	229(88.1%)	0.821(0.361-1.86)	0.637
Flock size	Small	148	127(85.8%)	**	
	Medium	184	167(90.8%)	1.62 (0.823-3.206)	0.162

	Large	8	7(87.5%)	1.157 (0.135-9.89)	0.894
Age	Adult	278	257(92.4%)	**	
	Young	62	44(71.0%)	0.2 (0.099-0.405)	0.00
Breed type	Local	188	157(83.5%)	**	
	Exotic	152	144(94.7%)	3.554(1.582-7.98)	0.002
Hygiene status	Good	59	49(83.1%)	**	
	Medium	205	178(86.8%)	1.34(0.610-2.969)	0.462
	Poor	76	74(97.4%)	7.55 (1.586-35.95)	0.011

4.3. Multivariable Logistic Regression Analysis

With Wayu Tuka as the reference district, the analysis reveals that Sibuloba has a statistically significant association with IBDV seropositivity (OR = 5.70, 95% CI: 1.951-16.678, P = 0.001). This indicates that, even after controlling for other factors, poultry in Sibuloba are significantly more likely to be seropositive for IBDV compared to those in Wayu Tuka. Guto Gida, however, does not show a statistically significant association (OR = 1.74, 95% CI: 0.731-4.133, P = 0.211).

Age remains a highly significant predictor of IBDV seropositivity. Compared to adult chickens (reference group), younger chickens have a significantly lower likelihood of being seropositive (OR = 0.073, 95%, P < 0.001). This strong negative association suggests that younger chickens are far less likely to have antibodies against IBDV, even after accounting for other variables in the model. Breed type continues to be a significant predictor. Compared to local breeds (reference group), exotic breeds are 3 times more likely to be seropositive for IBDV (OR = 3.385, 95%, P = 0.011). Hygiene status also emerges as a significant predictor in the multivariable model. Compared to good hygiene status, both medium (OR = 2.754, 95%, P = 0.035) and poor (OR = 32.75, 95%, P < 0.001) hygiene statuses are significantly associated with increased IBDV seropositivity (Table 3).

Table 3: Results of the multivariable logistic regression model for the predictors of seropositivity to IBD Virus

Variables	Categories	Sample tested	Sample positive	OR(95% CI)	P- value
Districts	Wayu Tuka	113	95(84.1%)	**	
	Guto Gida	117	104(88.9%)	1.74 (0.731-4.133)	0.211
	Sibu Sire	110	102(92.7%)	5.70(1.951-16.678)	0.001
Age	Adult	278	257(92.4%)	**	
	Young	62	44(71.0%)	0.073 (0.029-0.184)	0.00
Breed type	Local	188	157(83.5%)	**	
	Exotic	152	144(94.7%)	3.385(1.324-8.654)	0.011
Hygiene status	Good	59	49(83.1%)	**	
	Medium	205	178(86.8%)	2.754(0.1.075-7.057)	0.035
	Poor	76	74(97.4%)	32.75 (5.560-193.016)	0.00

5. DISCUSSIONS

Poultry production is a significant source of high quality animal protein. Nonetheless, the high prevalence of infectious diseases in poultry poses a significant threat to productivity and survival. (Adebiyi O.*et al.*,2018). In the absence of immunization, the current investigation found that backyard chickens in three areas of western Oromia had IBDV specific antibodies, suggesting that the chickens had been exposed to the virus in the wild. In the current investigation, an indirect ELISA with an IBDV specific antibody was used to examine 340 chickens from different management regimes of backyard chickens.

The present study revealed a high overall seroprevalence (88.53%) of Infectious Bursal Disease Virus (IBDV) antibodies in backyard chickens across East Wallaga Zone, indicating widespread exposure to the virus. This finding aligns with prior studies in Ethiopia, such as Tesfayew *et al.* (2012) and Kassa & Mollila (2012), who reported seroprevalences of 82.2% and 73.5%, respectively. The slightly higher prevalence in this study may reflect ecological variations in management practices, agroecological conditions, or the sensitivity of the indirect ELISA used. The ubiquitous presence of IBDV underscores its endemicity in backyard poultry systems, likely driven by limited management and high viral environmental stability coverage (Van den Berg, 2000; Eterradossi & Saif, 2008).

The finding that chickens from Sibu Sire district was significantly associated with the disease virus seropositivity aligns with the understanding that geographical location and local management practices can influence disease prevalence. Variations in seroprevalence across different regions can often be attributed to factors such as poultry density, biosecurity measures, and vaccination coverage, which are critical in controlling infectious diseases in poultry populations (Dawood *et al.*, 2018; El-Sayed *et al.*, 2020). High poultry density can facilitate the rapid spread of pathogens, including IBDV, as close contact among birds increases the likelihood of transmission (Khan *et al.*, 2019). In regions with high-density poultry farming, the risk of outbreaks can be exacerbated by inadequate management practices, which may include insufficient sanitation, lack of proper isolation of new chickens, and poor management of equipment and personnel (Hassan *et al.*, 2021). Conversely, areas with low vaccination coverage

may experience higher rates of infection, leading to increased seropositivity among the poultry population (Suleiman *et al.*, 2020).

The negative association between age and IBDV seropositivity is indeed an intriguing finding. In this study, adult chickens exhibited a high seroprevalence of 92.4%, while young chickens showed a significantly lower seroprevalence of 71.0%. This discrepancy may be attributed to as chicken's age; their immune response may diminish, leading to a reduced ability to maintain protective antibody levels against IBDV. This phenomenon has been documented in various studies, indicating that younger chickens may not respond as robustly to infections or vaccinations compared to younger chickens (Khan *et al.*, 2019). Management practices often change as chicken's age. Younger chickens may be kept in environments with lower exposure to the virus due to improved biosecurity measures or changes in flock management, which could lead to lower seroprevalence (Hassan *et al.*, 2021). This finding contrasts with some studies that report increasing seroprevalence with age, as birds accumulate exposure over time. Ganapathy *et al.* (2016)

The increased seropositivity in exotic breeds compared to local breeds is consistent with observations in other countries. A study in Nigeria by Abdu *et al.* (2011) found that exotic breeds were more susceptible to IBDV infection than local breeds. This could be attributed to several factors: Exotic breeds may have different genetic backgrounds that influence their immune responses. Research has shown that local breeds often possess better adaptive immune mechanisms, which can provide them with enhanced resistance to viral infections like IBDV. In contrast, exotic breeds may lack these advantageous traits, making them more vulnerable to infections (Abdu *et al.*, 2011). Exotic breeds may also have different responses to vaccination compared to local breeds. Studies have indicated that local breeds often show a more robust immune response to vaccines, which can lead to better protection against diseases like IBDV (Mansour *et al.*, 2019). These factors collectively contribute to the observed differences in seropositivity between exotic and local breeds.

This finding aligns with numerous studies worldwide that have demonstrated the importance of hygiene and sanitation in controlling IBDV and other poultry diseases. For instance, Saif *et al.*

(2019) emphasized that effective biosecurity measures, including proper hygiene practices, are essential for minimizing the risk of infectious diseases in poultry populations. The extremely high odds ratio (OR = 32.75) for poor hygiene highlights the profound impact of inadequate biosecurity on IBDV transmission in the study area. This suggests that farms with poor hygiene practices are significantly more likely to experience outbreaks of IBDV, reinforcing the need for stringent biosecurity protocols. Furthermore, the finding that medium hygiene was also significant indicates that even moderate improvements in hygiene can substantially reduce the risk of IBDV infection. This is consistent with observations from other studies, which have shown that enhancing biosecurity measures, such as improving sanitation and reducing pathogen exposure, can lead to lower disease prevalence in poultry (Hassan *et al.*, 2021; El-Sayed *et al.*, 2020).

Generally, the high seroprevalence suggests subclinical IBDV circulation is pervasive, posing risks for immunosuppression and secondary infections. While vaccination remains the cornerstone of control (Müller *et al.*, 2012), its absence in backyard systems exacerbates vulnerability. The study supports the integration of targeted vaccination programs (e.g., hatchery-based MDA boosting) and community-led biosecurity measures, as advocated by Mansour *et al.* (2019). Additionally, the breed and age disparities call for further research into seroconversion and vaccine efficacy in diverse genetic backgrounds.

6. COCLUSIONS AND RECOMMENDATIONS

Infectious bursal disease (IBD) has a major impact on poultry health, besides causing significant economic losses. The present study revealed the widespread seroprevalence of Infectious bursal disease Virus in backyard chickens across the surveyed districts of East Wallaga Zone, indicating endemic circulation. Among key risk factors associated with seropositivity, (Sibu Sire), age (younger chicken's), breed type (exotic breeds) and hygiene status (medium and poor) were identified as significant independent predictors of IBDV seropositivity. Chickens from Sibu Sire district had the highest seroprevalence linked to local management practices. Younger chicken's showed lower seropositivity suggesting waning immunity or reduced exposure. Exotic breeds were more likely to be seropositive than local breeds. The decreased seroprevalence in young chickens warrants further investigation into age-related immunity. The increased seropositivity in exotic breeds underscores the importance of breed-specific management strategies. Finally, the strong association between poor hygiene and IBDV seropositivity emphasizes the critical role of biosecurity in disease control. Poor hygiene increased odds of seropositivity, underscoring biosecurity's. Generally, the findings demonstrate IBDV's endemic circulation in the area, posing risks to poultry productivity and rural livelihoods and if not intervened uncontrolled IBD threatens food security and livelihoods in poultry-reliant communities.

Therefore based on the study findings, the following recommendations are forwarded:

- ✓Biosecurity Measures: Promote hygiene improvements (e.g., regular coop cleaning, disinfection) to reduce viral persistence.
- ✓Farmer Education: Train farmers on IBD symptoms, prevention, and the importance of breed-specific management.
- ✓Vaccination Programs: Implement routine vaccination tailored to local IBDV strains, prioritizing high-risk districts like Sibu Sire.
- ✓Surveillance: Establish longitudinal studies to monitor IBDV trends and vaccine efficacy.
- ✓Policy Action: Integrate Infectious Bursal Disease control into national poultry health strategies, with support for backyard producers.

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8. APPENDICES

Appendix A: Indirect Enzyme Linked Immuno-sorbent Assay (IELISA) principles and procedures.

General information:- this indirect ELISA kit is designed to detect antibodies directed against the infectious bursal disease (IBD) Virus also known as Gumboro disease. It is a quantitative test for the detection of IBDV specific antibodies in chicken serum.

Kit components

1. Reagents
2. Microplates coated with purified IBDV antigen.
3. Positive control.
4. Negative control.
5. Concentrated conjugate (10x).
6. Dilution buffer 3
7. Dilution buffer 14
8. Wash concentrate(20x)
9. Substrate solution
10. Stop solution (0.5M)

Materials required but not provided

1. Mono or multichannel pipettes
2. Disposable tips
3. 96-well pre-dilution microplate
4. Distilled or deionized water
5. Manual or automatic wash system
6. 96-well microplate reader

Precautions and warnings

- Handle all biological material as potentially infectious.
- Wear protective gloves /protective clothing / eye or face protection when handling samples and reagents to the product material safety data sheet for additional information
- Do not pipette by mouth
- Do not expose the substrate solution to bright light nor to oxidizing agents.
- All waste should be properly decontaminated prior to disposal.

Preparation of reagents

- ❖ Sample preparation
- ❖ Wash solution preparation

4. Controls

- ✓ PC = positive control and NC = Negative control are supplied ready to use. Do not add dilution buffer to the control wells. A1, B1, C1, and D1 controls are to be tested undiluted.

Testing procedure:-

Samples, however, are tested at a final dilution of 1:500 in dilution buffer 14 (1:50 pre-dilution followed by 1:10 dilution in the microplate).

1. In a pre-dilution plate, set aside wells A1, B1, C1 and D1 for the control and add.
 - 5 micro litter of each sample to be tested.
 - 245 micro litter of dilution buffer 14 to all well except to control wells A1, B1, C1 and D1

Note: it is recommended to respect the indicated order of deposit to be able to visually control addition of sample to each well.

2. In the ELISA microplate add:
 - 100 microlitter of the negative control to wells A1 and B1.
 - 100 microlitter of the positive control to wells C1 and D1.
 - 90 microlitter of dilution buffer 14 to as many wells as there are samples to be tested (NOT to control wells A1, B1, C1 and D1).

- 10 microlitter of the pre diluted samples as prepared above.
3. Add 100 microlitter of the conjugate 1x to each well.
 4. Cover the plate and incubate 30 min $\pm$ 3min at 21c⁰ ($\pm$ 5c⁰)
 5. Empty the wells. Wash each well 3 times with at least 300 microlitter of the wash solution 1x. Avoid drying of the wells between washes.
 6. Add 100 microlitter of the substrate solution to each well.
 7. Cover the plate and incubate 15min $\pm$ 2min at 21c⁰ ($\pm$ 5c⁰) in the dark.
 8. Add 100 microlitter of the stop solution to each well in order to stop the reaction. The stop solution should be added in the same order as in step N^o9
 9. Read and record the O.D at 450nm

Validation

The test is validated if:

The mean OD value of the positive control (ODpc) is greater than 0.250

$$OD_{pc} > 0.250$$

The ratio of the mean values of the positive and negative controls (ODpc and ODnc) is greater than 3.

$$OD_{pc}/OD_{nc} > 3$$

Interpretation

For each sample calculate the S/P ratio and antibody titer as follows.

$$S/P \text{ ratio} = \frac{OD_{\text{sample}} - OD_{nc}}{OD_{pc} - OD_{nc}}$$

Results are interpreted

S/P value	ELISA antibody titer	IBD immune status
$S/P \leq 0.3$	$\text{titers} \leq 875$	Negative
$S/P > 0.3$	$\text{titer} \geq 875$	Positive

Note: IDEXX has instrument and software systems available which calculate results and provide data summaries.

Appendix B: Images taken during the study period in selected districts of the East Wallaga zone, western Ethiopia 2024/2025 G.C.



Serology laboratory working in animal health institute, sabeta microbiology lab.



