Thesis Ref No.__________

SEROLOGICAL INVESTIGATION OF INFECTIOUS BURSAL DISEASE IN POULTRY REARED UNDER BACKYARD PRODUCTION SYSTEM IN WOLAITA ZONE, SOUTHERN ETHIOPIA

MVSc. THESIS

BY

MIHRET AMAJO ARBA

ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, DEPARTMENT OF CLINICAL STUDIES

JUNE, 2021

BISHOFTU, ETHIOPIA

Thesis Ref No.__________

SEROLOGICAL INVESTIGATION OF INFECTIOUS BURSAL DISEASE IN POULTRY REARED UNDER BACKYARD PRODUCTION SYSTEM IN WOLAITA ZONE, SOUTHERN ETHIOPIA

By

Mihret Amajo Arba

A Thesis Submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of Requirement for the Degree of Master of Veterinary Science in Veterinary Epidemiology

June, 2021

Bishoftu, Ethiopia

Signature Sheet

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As MVSc research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance by Mihret Amajo, entitled: "**SEROLOGICAL INVESTIGATION OF INFECTIOUS BURSAL DISEASE IN POULTRY REARD UNDER BACKYARD PRODUCTION SYSTEM IN WOLAITA ZONE, SOUTHERN ETHIOPIA"**; I recommend that it can be accepted as fulfilling the thesis requirement for the Degree of Master of Veterinary Science in Veterinary Epidemiology.

Submitted by: Mihret Amajo __________________ ______________

Name of student Signiture Date

Approved for submittal to MSc research Thesis assessment committee:

Dr. Haileliul Negussie (DVM, Msc, PhD, Asso.Prof) __________________

Major Advisor

Dr. Haileleul Negussie (DVM, MSc, PhD, Associate Professor)

Department chairperson _______________ ________________

Signature Date

Approval Sheet

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As a member of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the Thesis prepared by: Mihret Amajo, entitled: "**SEROLOGICAL INVESTIGATION OF INFECTIOUS BURSAL DISEASE IN POULTRY REARD UNDER BACKYARD PRODUCTION SYSTEM IN WOLAITA ZONE, SOUTHERN ETHIOPIA"**; we recommended that it be accepted as fulfilling the thesis requirement for the degree of Master of Veterinary Science in Veterinary Epidemiology.

Dr. ________________ ________________

Chair man

Dr. ________________ ________________

External Examiner

Dr. _________________ ________________

Internal Examiner

Dr. Haileleul Negussie (DVM, MSc, PhD, Associate Professor)

Department chairperson _______________ ________________

Signature Date

STATEMENT OF THE AUTHOR

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

Brief quotations from this thesis are allowable without special permission provided that proper acknowledgment of sources is made, requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

Name: Mihret Amajo Signature: ______________

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June, 2021

ACKNOWLEDGEMENTS

First and foremost, I would like to honor and glorify the Almighty GOD for he has helped me in various ways in my life and Make my long-term dream come true and to be with me on my trip throughout my lifetime.

I would like to extend my deepest and sincere gratitude to my advisors Dr. Haileleul Negussie for his devotion, valuable advice, insight, and guidance from the beginning to the completion of the research work. This research could have not been completed without his enthusiasm and direction.

I owe a special thanks to National Animal Health Diagnostic and Investigation Center staff members and Wolaita sodo veterinary regional laboratory staff members for their munificent cooperation to carry out all the laboratory works.

My special thanks also go to my lovely husband, Titiwos Abera, for his contributions, encouragement, understanding, tolerance, and patience. I would like to extend my special gratitude, love, and respect to my family members; my mom Michu Godana, my dad Amajo Arba for their unconditional love, moral support, and inspiration throughout my study. I owe my deepest gratitude to all my friends and those who were aspiring and generous towards me.

Table of Contents **Pages**

STATEMENT OF THE AUTHOR ..... **I**

ACKNOWLEDGEMENTS ..... **II**

LIST OF TABLES **V**

LIST OF FIGURES **VI**

LIST OF APPENDICES ..... **VII**

LIST OF ABBREVIATIONS ..... **VIII**

ABSTRACT ..... **IX**

1. INTRODUCTION **1**

2. LITERATURE REVIEW **4**

2.1. History and Description of Infectious Bursal Disease Virus **4**

2.2. Epidemiology **6**

2.2.1. Host Range *6*

2.2.2. Distribution of IBD *6*

2.2.3. Transmission *8*

2.2.4. Risk factors of IBD *8*

2.2.5. Physicochemical characteristics of IBDV *9*

2.3. Pathogenesis **10**

2.4. Clinical Signs **12**

2.5. Diagnosis **12**

2.6. Control and Prevention of IBD **13**

2.6.1. Biosecurity measures *14*

2.6.2. Vaccination of IBD *14*

2.7. IBD Virus Vaccine Failure **16**

2.8. The Status of IBD in Ethiopia **17**

2.8.1. Vaccination of IBD in Ethiopia *18*

3. MATERIALS AND METHODS **20**

3.1. Description of the study area **20**

3.2. Study population **21**

3.3. Study Design **21**

3.4. Sample Size Determination **21**

3.5. Sampling Technique ... **22**
3.6. Questionnaire Survey ... **22**
3.7. Test Procedure and Interpretation ... **23**
3.8. Ethical consideration ... **24**
3.9. Data Management and Analysis ... **24**
4. RESULTS ... **25**
4.1. Questionnaire Survey ... **25**
4.1.1. Management of backyard poultry production system ... *25*
4.1.2. Health Care and Constraints ... *26*
4.2. The seroprevalence of IBD ... **28**
4.3. Major Risk Factors Analysis by Using Multiple Logistic Regression ... **30**
4.4. Prediction of the Occurrence of IBD in Association with Breed and No of the Flock ... **30**
5. DISCUSSION ... **32**
6. CONCLUSION AND RECOMMENDATIONS ... **37**
7. REFERENCES ... **38**
8. APPENDICES ... **48**

LIST OF TABLES **Pages**

Table 1. Types of poultry production, socioeconomic importance, and management system practiced 26

Table 2. Constraints of poultry production 27

Table 3. Previous outbreak and death history in association with treatment and isolation of sick chickens 27

Table 4.Results of multivariable logistic regression analysis on the occurrence of IBD as a function of various risk factors 28

LIST OF FIGURES **pages**

Figure 1. Morphology and structure of IBDV. 5

Figure 2. Worldwide geographical distribution of the acute forms of IBDV 7

Figure 3. Map of the study area 20

Figure 4. ggplot of predicted probability of the occurrence IBD in association with the number of flocks 31

Figure 5. ggplot of predicted probability of the occurrence IBD in association with the Breed. 31

LIST OF APPENDICES

Appendix 1.Serum Sample Collection Sheet For serosurvey 48

Appendix 2. Plat layout of I-ELISA Test 48

Appendix 3. I-ELISA testing procedure 49

Appendix 4. Questionnaire Sheet Format 50

Appendix 5. Ethical clearance 53

LIST OF ABBREVIATIONS

Ac-ELISA	Antigenic Capture Enzyme-Linked Immunosorbent Assay
AGID	Agar Gel Immunodiffusion Assay
at IBDV	Attenuated Infectious Bursal Disease Virus
avIBDV	Antigenic Variant Infectious Bursal Disease Virus
CEB	Chicken Embryo Bursa
CEK	Chicken Embryo Kidney
CFC	Chicken Fibroblast Cell
CI	Confidence Interval
cvIBDV	Classical Virulent Infectious Bursal Disease Virus
ELISA	Enzyme-Linked Immunosorbent Assay
HRP	Horse Radish Peroxides
IBD	Infectious Bursal Disease
IBDV	Infectious Bursal Disease Virus
I-ELISA	Indirect Enzyme-Linked Immunosorbent Assay
IFN	Interferon
IL	Interleukin
NAHDIC	National Animal Health Diagnosis and Investigation Center
OD	Optical Density
OIE	Office of International des Epizooties
OR	Odds Ratio
PDS	Participatory Disease Surveillance
vvIBDV	Very Virulent Infectious Bursal Disease Virus
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SNNPR	Southern Nation Nationalities and People Region

ABSTRACT

A cross-sectional study was conducted from December 2020 to May 2021 to investigate the epidemiology of the infectious bursal disease (IBD). Multistage sampling was implemented to determine the seroprevalence and assess the risk factors of IBD in unvaccinated backyard chickens and describe the distribution of disease concerning different risk factors in the Wolaita zone. A questionnaire survey was also carried out and revealed that various factors affect the occurrence of IBD. From 482 sera samples tested for IBDV antibodies using indirect enzyme-linked immunosorbent assay (I-ELISA), 236 samples were positive for IBDV antibodies with an overall prevalence of 48.96% (95% CI: 44.52-53.41). A statistically ($P < 0.05$) higher seroprevalence of IBD was found in exotic breed (n = 112; 57.14%) as compared to local breed (n = 124; 43.35%). Similarly, statistically higher seroprevalence of IBD was recorded in flock sizes ≥ 5 chickens (n = 201; 59.47%) as compared to < 5 chickens (n = 35; 24.3%). Based on the results of multivariable logistic regression the odds of occurrence of IBD was 0.67 times lower in local breeds than exotic breeds and ≥ 5 chickens had 4.33 times higher seropositive than < 5 chickens. There was a statistical association ($P<0.05$) between the treatment history and death in the flock. This study revealed that IBD was an important and wide speared problem in poultry production. Thus, appropriate biosecurity measures, regular serosurveilance, creating awareness to the owners, and frequent outbreak investigation for selecting appropriate vaccine strain and effective vaccine design and vaccination is important for the control of the disease.

Key Words: *Chicken, IBD, IBDV, Wolaita, Risk factors, seroprevalence, Backyard*, *IELISA,Ethiopia*

1. INTRODUCTION

Poultry production is a subset of livestock production that is an important agricultural activity in almost all developing communities in Africa and elsewhere. It is also one of the fastest-growing components of global agricultural demand because it has the unique opportunity to contribute to the sector while also improving the living standards of poor livestock keepers (Moges *et al*., 2010). The economy of emerging countries, in particular, is heavily reliant on chicken production in rural areas. Over 80% of Africa's poultry population is kept in backyard production systems (Alders and Pym, 2009). Because rural poultry makes up a larger proportion of a developing country's national flock population, it's worth paying attention to better management and breeding. If poultry is integrated with rural development programs, it can be a useful tool for quickly responding to poverty gaps. It has a short generation interval and a high rate of reproduction. Women, children, and the elderly are the most vulnerable members of society; therefore chickens are especially vital to them (Birol *et al*., 2010).

Ethiopian chicken production has a long history, plays a vital socio-economic role, and is characterized by low input and yield (Moges *et al*., 2010). Importing and distributing improved breeds to farmers in various locations of Ethiopia are being attempted to increase chicken productivity and contribution (Mulugeta and Tebkew, 2013). The imported chicken strains are moderate types that are less suited to the country's heat stress and illness concerns. Outbreaks of newly introduced diseases and/or epidemics of endemic diseases have been observed in conjunction with the intensification of poultry raising. Infectious Bursal Disease (IBD) is one of the diseases that is causing increasing concern in poultry (Zeleke *et al*., 2005).

IBD was initially reported in the United States in 1962 near the town of Gumboro, Delaware (Cosgrove, 1962), and was first documented in Ethiopia in 2002 at a privately held commercial poultry farm where 45-50% of the birds died (Zeleke *et al*., 2003). IBD is a notably communicable viral disorder of young chickens that causes high morbidity and mortality (Camilotti *et al*., 2016). It is one of the most economically important diseases that affect the growth of younger chickens which leads to considerable monetary losses in the poultry industry (Kim *et al*., 2000). The loss is attributed to high mortality, immunosuppression, and condemnation of the carcasses (Choudhary *et al*., 2017). The disease is characterized by the destruction of lymphocytes in the bursa of Fabricius (Aliyu *et al*., 2016)

IBD is caused by infectious bursal disease virus (IBDV), a double-stranded RNA virus belonging to the *Avibirnavirus* genus of the family *Birnaviridae* (Brandt *et al*., 2001). Based on the results of a virus neutralization assay, IBDV strains are divided into two groups: serotype 1 and serotype 2. Although serotype 1 viruses are pathogenic to chickens, they can be classified into attenuated (atIBDV), classical virulent (cvIBDV), antigenic variant (avIBDV), and very virulent (vvIBDV) subtypes based on mortality and bursal lesions (Sali, 2019) (Mahgoub, 2012). Serotype 2 viruses are a type of virus isolated from turkeys (McFerran *et al*., 1980). The IBDV genome is divided into segments A and B (Dey *et al*., 2019) (Sali, 2019). The isolate was referred to as an "infectious bursal agent" and was known as the true cause of IBD, and the term infectious bursal disease was proposed as the name of the disease that causes particular pathognomonic lesions of the cloacal bursa (Eterradossi and Saif, 2020).

IBDV infection ends up in lymphatic tissue depletion (Aliyu *et al*., 2016) and also the final destruction of the bursa which is that the transcendent highlight of its pathogenicity (Jenbreie *et al*., 2014). Most lesions of the disease are found within the bursa of Fabricius (Choudhary *et al*., 2017). After the acute phase of the infection subsides, the bursal follicles become repopulated with B cells, and immune competence is reestablished (Kim *et al*., 2000). The IBDV is communicable and

contact with infected birds and contaminated fomites may want to result in the spread of infection (Orakpoghenor *et al*., 2020). The virus is environmentally steady and resistant to many chemical and bodily agents (Eterradossi and Saif, 2020). Its spread out between flocks can be restricted via the implementation of strict biosecurity and a tremendous vaccination program (Lukert and Saif, 2003). The prevention of IBD outbreaks in the field has been achieved globally through vaccination (Jackwood and Sommer, 2002).

Frequent outbreaks and the high prevalence of the disease together with the involvement of various variants of IBDV are a big challenge to the juvenile poultry industry in Ethiopia (Mazengia, 2012). Seroprevalence of IBD in Ethiopia was found to be 83.1 % (Jenbreie *et al*., 2014). The most notable seroprevalence of IBD was found at Jimma and Bonga districts (96.4 %) (Debebe, 2016) whereas the least was recorded at Farta locale (21.70%) (Mazengia *et al*., 2010). Over the previous few years, 25 to 75% of the deaths in exotic and Local chickens have been associated with the IBD (Kegne and Chanie, 2014; Zeryehun and Fekadu, 2012) different studies showed that the vvIBDV genotype is circulating in Ethiopia (Tadesse and Jenbere, 2014). The IBD has increased at an alarming rate in many parts of Ethiopia and its occurrence throughout the country, which has increased commercial poultry production and even chickens in the backyard but is also a grave problem for the poultry industry.

Even though IBD is one of the most economical viral diseases of poultry in Ethiopia, there is confined nicely documented data in the current study area; hence much has been done to better understand disease epidemiology.

Therefore, this study was designed with the aims of:

- Estimating the seroprevalence of IBD in backyard chickens of Wolaita zone
- Assessing the potential risk factors for the disease prevalence

2. LITERATURE REVIEW

2.1. History and Description of Infectious Bursal Disease Virus

The IBD virus causes a highly contagious and acute disease in young chickens. Gumboro disease was named for the causal agent, which was first isolated in the United States in Gumboro, Delaware in 1957. On a broiler farm near Gumboro, Delaware, the classical serotype 1 IBDV triggered the first outbreak of IBD (Cosgrove, 1962). Because of gross changes in the kidney, the early opinion was that avian necrosis, or Gumboro disease, was caused by a variant nephrogenic strain of infectious bronchitis virus (IBV), as it turned out this misunderstanding occurred because the two infections were always present at the same time, and the causative agent was difficult to identify using diagnostic tools (Lasher and Davis, 1997). When it was discovered IBV immunized birds also showed changes in the bursa when infected with Gumboro disease, further research was done (statts, 2020). More IBD studies were effective in isolating an agent in embryonated eggs. The isolate was dubbed the "infectious bursal agent" and was discovered to be the cause of IBD, prompting the word" infectious bursal disease" to be proposed as the name for the disease that causes specific lesions at cloacal bursa (Hitchner,1970).

IBDV a member of the family *birnaviridae* of the genus *Avibirna* virus is the disease's etiological agent (Ashraf, 2005). *Avibirnavirus*, *Aquabirnavirus*, and *Entomobirnavirus* are the three genera that make up the *Birnaviridae* family. IBDV is the only virus in the *Avibirna* virus genus (Van den Berg *et al*., 2000). Based on the results of a virus neutralization assay, IBDV strains are divided into two groups: serotype 1 and serotype 2. Although serotype 1 viruses are pathogenic to chickens, they can be classified into attenuated (atIBDV), classical virulent (cvIBDV), antigenic variant (avIBDV), and very virulent (vvIBDV) subtypes based on mortality and bursal lesions (Sali, 2019) (Mahgoub, 2012). Serotype 2 viruses are a type of virus isolated from turkeys (McFerran *et al*., 1980).

IBDV is made up of two 3.2 kb (segment A) and 2.8 kb (segment B) double-stranded RNA (dsRNA) segments which are contained inside a 60 nm diameter single-shelled icosahedral capsid (Van Loon *et al.,* 2002). The virion's capsid is made up of a single layer of 32 capsomers that are arranged in 5: 3: 2 symmetries (Hirai and Shimakura, 1974). Segment A is polycistronic and encodes two major structural proteins (VP2 and VP3), one protease (VP4) that cleaves the viral polyprotein, and one nonstructural protein (VP5) that is expressed transiently at the virus's end of life cycle and is thought to be responsible for the virus's replication (Lombardo *et al*., 2000). Segment B is monocistronic and encodes the protein VP1, which is the viral RNA polymerase responsible for the release of viral particles (Van Loon *et al*., 2002). Letzel *et al*. (2007) found that protein VP2 contributes to the virus's antigenicity, tropism, and pathogenicity. The VP2 protein contains the epitopes that cause neutralizing and protective antibodies to be produced (Letzel *et al*., 2007).

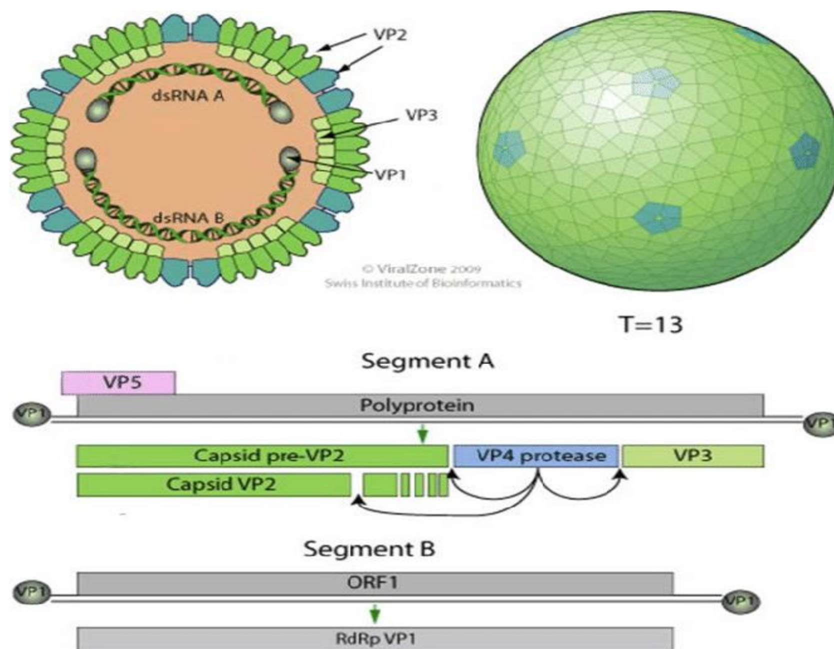

Figure 1. Morphology and structure of IBDV.

5-6 proteins are encoded by two segments (A, B). The free form of VP1 is located at the 5' genomic RNA end, where it is covalently bonded (VPg). The segments are approximately 2.8-3.6 kb in size. Source: (Viral Zone, 2009).

2.2. Epidemiology

2.2.1. Host Range

IBDV is only found in one kind of host. While turkeys, ducks, guinea fowl, and ostriches are infected with the virus through serology, the clinical disease is only seen in chickens (Van den Berg *et al*., 2000). The serotype IBDV is thought to be particularly specific to chickens that grow IBD after being infected with serotype 1 viruses. According to reports, serotype 2 of IBDV is more prevalent in many species of wild birds, with turkeys being the natural host (Deye *et al*., 2019). A virus that causes the IBD was recently isolated from a sparrow in China, implying that wild birds may be carriers (Wang *et al*., 2007). IBDV has not been shown to infect other animals or humans (Camilotti *et al*.,2016). Serotype I viruses affect all chicken breeds, but white leghorns have the most serious clinical signs and lesions, as well as the highest mortality rate (Eterradossi and Saif, 2008). The mortality rate associated with classic strain infections in fully susceptible flocks can range from 1 to 60%, with high morbidity of up to 100% (Muller *et al*., 2003).

2.2.2. Distribution of IBD

The first case of IBD, originally known as avian nephrosis, was identified in Gumboro, North Carolina, in 1957, and it quickly spread to other parts of the country between 1960 and 1964 (Cosgrove,1962). The most pathogenic strain of infectious IBDV is the vvIBD virus, which was first identified in Holland in 1986 (Van den Berg *et al*., 1991). The disease has reached most nations, including much of Asia, Central Europe and Russia, the Middle East, and South America, with only Australia, New Zealand, Canada, and the United States remaining free until 2008. There is a major difference in the vvIBDV strains of Africans, Asians, and Europeans, indicating that each continent evolved differently (Dey *et al*., 2019). Acute infection with classic mild or variant strains has a mortality rate of up to 5%. The variants inhibit the immune system more than the classic strains. The vvIBDV has the potential to destroy up to 50% of susceptible flocks (Shane, 2005).

IBD has now spread across the world and is affecting major poultry production areas (Zeryehun and Fekadu, 2012). In Ethiopia, an IBD outbreak has been recorded in 25-40 day-old broilers and layer chickens, with mortality rates ranging from 45 to 50%, overall mortality of 49.83 %, and seroprevalence of 93.30 % (*Zeleke et al*., 2005). The total mortality of chickens due to IBD was 72 % in young (1–70 days old) and 7% in adult (>70 days old) flocks, according to a case report study from Andasa poultry farm, with a 100% seroprevalence in non-vaccinated flocks (Woldemariam and Wossene, 2007).

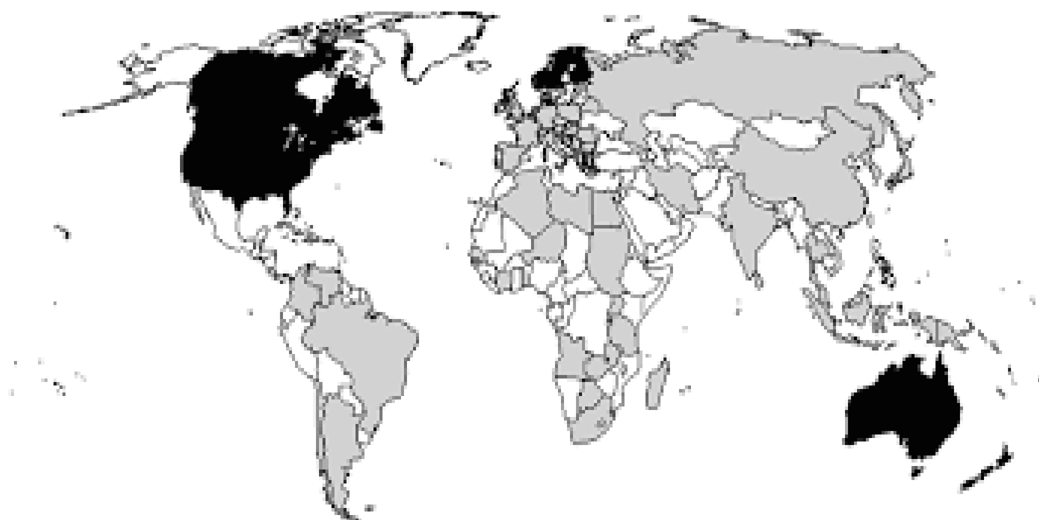

Figure 2. Worldwide geographical distribution of the acute forms of IBDV

Countries with acute forms have been recorded in gray. The absence of acute forms is depicted in black. IBD is not recorded in white-colored countries (Van den Berg *et al.*, 2000).

2.2.3. Transmission

The only way for IBDV to spread from diseased to disease-free chickens is through horizontal transmission. The fecal-oral route is the most common mode of transmission, but the respiratory and conjunctival routes have also been identified (Camilotti *et al*., 2016; Teshome *et al*., 2015). In multi-age groups, direct interaction of young birds with infected flocks causes a persistent "rolling" infection that is difficult to manage (Shane, 2005).

The virus has a high contamination propensity and can be spread between infected and susceptible flocks through direct contact with excreting subjects or through indirect contact with any inanimate or animate contaminated vectors. For at least 14 days, the virus may be excreted in the feces. The feces excreted can contaminate water and feeds, as well as transmit the virus and spread the disease (Getachew and Fesseha, 2020). The virus can survive for more than 54 and 122 days in the shade due to its environmental persistence (Eterradossi and Saif, 2020). The droppings, water, and feed are still contagious after 52 days of contamination. Although the virus is not transmitted vertically, it can contaminate the surface of the eggshell, so the carrier birds should be removed from the recovered flocks. There is no indication that wild birds play a role in the disease's spread (Camilotti *et al*., 2016; Teshome *et al*., 2015). There is a study that indicates the mosquito can carry a nonpathogenic form of a virus that has been found in areas near chicken farms in southern Ontario, but there is no evidence that the mosquito is used as a vector in disease transmission (Eterradossi and Saif, 2020).

2.2.4. Risk factors of IBD

Various production methods have different risk factors for the incidence of infected bursal disease. Age, sex, breed, production type, chicken immune state, seasonal variation, agroecology, bio-security measures, social awareness, and a history of the infectious bursal disease are all factors to consider (Hailu *et al*., 2010; Zerihun and Fikadu, 2012; Natnael, 2015). Destitute sterile conditions, unseemly stocking

density, and coexisting diseases with E. coli, coccidiosis, and other bacterial contaminations favored the frequency of IBD. Chickens are profoundly vulnerable to the infection between 3 and 6 weeks after bring forth when the bursa of Fabricius comes to most extreme development and the onset of acute clinical signs (Farooq *et al*., 2003).

2.2.5. Physicochemical characteristics of IBDV

The virus is non-enveloped, tough, and defiant to physical and chemical agents, and, it continues in poultry premises indeed after careful cleaning and disinfection. The virus is viable for up to 4 weeks within the bone marrow of contaminated chickens. The infection has been appeared to stay irresistible for 122 days in a chicken house and 52 days in feed, water, and feces (Ashraf, 2005). The virus can survive for more than four months in the poultry farm due to its withstanding environmental factors such as heat, ultraviolet radiation, and disinfectants (Camilotti *et al*., 2016). As a result, the disease can remain contagious in the field, favoring its recurrence in consequent flocks. Since the virus is unusually resistant to inactivation by cooking, there is a risk of infection in backyard flocks from uncooked chicken meat products, even if the chickens appear to be in good health (Mandeville *et al*., 2000).

It is vulnerable to mutation, highly stable, and unaffected to several chemicals and disinfectants, such as phenolic derivative and quaternary ammonium compound, and may survive for up to four months in feces, bedding, polluted feed, and water under some circumstances. It also resists chloroform and ether therapy, however, the iodine complex has a deleterious impact on the virus; and is inactivated after 30 minutes at 70°C (Rani and Kumar, 2015). The viruses' infectivity was eliminated after alkaline pH (12) treatment and marginally reduced after acidic pH (2) treatment, suggesting that the viruses were less stable at alkaline than acid pH (Wang *et al*., 2007).

2.3. Pathogenesis

Pathogenesis is the mechanism by which a virus causes injury to the host, leading to death, disease, or immunosuppression as a result (Van den Berg et al., 2000). These injuries can be assessed at various levels, including the host, organ, and cell, and they are compounded in the acute stages of the disease. The bursa of Fabricius, which is a particular source of B lymphocytes in avian organisms, is the target organ of IBDV at its most developed stage. In chicks infected with a dangerous virus, a bursectomy will help them stay healthy (Deye et al., 2019). The bursa of Fabricius has been found to have elevated levels of viral antigens and infectivity titers, while the thymus and spleen have only traces of antigen and low virus titers (Muller et al., 2003).

IBDV is transported to the bursa of Fabricius by infected macrophages after infection, where it replicates intracytoplasmically in IgM+ B lymphocytes. The development of interferon (IFN) in the bursa of Fabricius is boosted by macrophage activation (Orakpoghenor et al., 2020). Pro-inflammatory cytokines including interleukin-6 (IL-6) and nitric oxide are released as a result.

These cytokines could worsen the bursal lesions (Eldaghayes *et al*., 2006). Furthermore, the IFN- produced during IBDV infection can cause apoptosis in infected and healthy B-cells in the surrounding area. The virus has been confirmed to spread to other lymphoid organs such as bone marrow, thymus, spleen, Peyer's patches, caecal tonsils, and Harderian glands after infection of susceptible chickens with vvIBDV (Eterradossi and Saif, 2020).

During the acute process of virus infection, the thymus shows significant atrophy and widespread apoptosis of thymocytes, but there is no indication that the virus replicates in thymic cells. Within a few days of virus infection, gross and microscopic lesions in the thymus are overcome, and the thymus returns to its natural state (Sharma *et al*., 2000). The caecal tonsils and bone marrow can assist the replication of IBDV at a later time. Inflammation of the bursa of Fabricius occurs 48 hours after infection (Ai) and a cytolytic improvement appears in all infected bursal IgM+ B cells

on days 3–4 Ai. According to a flow cytometric study, the IgM+ B-cell population is significantly smaller than the IgA and IgG+B-cell populations at days 7–21 Ai (Elankumaran *et al*., 2002). The bursal cortex's mesenchymally derived reticular cells, as well as the perarteriolar lymphoid sheaths, germinal base, and red pulp of the spleen, have all been confirmed to be relatively resistant to IBDV (Biro *et al*., 2011).

The lack of a B cell proliferative environment is associated with the occurrence of bursal follicular dendritic cell regression in IBD (Natnael, 2015). The infected birds are dehydrated with hemorrhages in the thigh and pectoral muscles which are common macroscopic lesions. IBDV replication causes extensive lymphoid cell destruction in bursal follicles, particularly in the bursa of Fabricius. The bursa becomes edematous, hyperemic, and creamy in color with prominent longitudinal striations early in the infection process. Later, the bursa's lymphoid follicles become necrotic, and the follicles in living birds will be devoid of lymphoid cells (Mazengia, 2012). The amount of mucus in the intestine increases. At 2 or 3 days after Ai, a yellowish transudate begins to cover the bursas of Fabricius. At 3-days Ai, the bursa of Fabricius starts to grow in size and weight. By 4 days Ai, it has doubled its normal weight, begun to shrink, and by 5 days Ai, it has returned to its normal weight. As the bursa of Fabricius returns to its normal scale, the transudate vanishes. At 8 days Ai, the bursa of Fabricius begins to atrophy (Mahgoub, 2012).

Immunosuppression will accompany recovery from illness or subclinical infection, with more severe effects if infection occurs early in life. Although IBDV's immunosuppression is primarily directed at B lymphocytes, it also affects cell-mediated immunity (Deye *et al*., 2019). Immunosuppression leads to decreased disease resistance and suboptimal response to vaccines provided during this period (Sharma *et al*., 2000).

2.4. Clinical Signs

The magnitude of clinical signs can be affected by the status of immunity, age, and genetic factor of infected birds with high virulence characteristics of the virus. Chickens infected between the ages of 3 and 6 weeks experience the most serious IBD symptoms. Within 2-3 days of exposure, the most vulnerable infected with the virus's most virulent strain develop sudden clinical symptoms, including extreme depression and ruffled feathers (Getachew and Fesseha, 2020). IBDV strains do not cause obvious clinical signs, but they do cause immunosuppression, which leads to subclinical infection and severe economic losses, as well as secondary infection, which contributes to mortality (Van den Berg *et al*., 2000). The virus's effect on T-cells and macrophages may be linked to immune suppression (Mazengia, 2012).

Ruffled feathers, depression, hurdling together, anorexia, prostration, and whitish diarrhea were among the clinical signs found in chicks in outbreaks, according to the reports of Aliyu *et al*. (2016). The infection may only last 5-7 days, and mortality spiked within 5 days of the beginning of IBDV. In a typical outbreak, 10-20% of the flock of 2- to 15-week-old chickens can exhibit rapid signs. Detecting any signs of illness is challenging. The presence of whitish or watery diarrhea, as well as soiled vent feathers, is one of the first signs. Afterward, anorexia, depression, shaking, extreme prostration, and death set in. With the swollen and bloodstained vent, the illness manifests itself as debilitation, vomiting, and depression (Sali, 2019).

2.5. Diagnosis

IBD-infected birds have microscopic and gross lesions that are suggestive of infection, but no pathognomonic clinical symptoms. Some of the detectable gross lesions of the infection include enlargement, change of color, hemorrhages, and atrophy of the bursa of Fabricius, as well as hemorrhages in the breast and leg muscles. Besides the hemorrhagic and cystic cavities seen in the bursa, microscopic lesions such as necrosis of lymphocytes and the presence of heterophils may be seen (Eterradossi and Saif, 2020).

IBDV infection is confirmed by pathological and histopathological lesions found in the bursa of Fabricius, as well as the detection of viral antigens using immune histochemistry. Inoculation of antibody-free embryonated chicken eggs with IBDV can be used to isolate the virus. The antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) or the agar gel precipitin assay can also be used to show viral antigens. AC-ELISA enables the recognition of vvIBDV with certain limitations (Muller *et al*., 2003).

RT-PCR is a technique for detecting viral RNA in homogenates of infected organs or embryos without taking into account the virus's viability (Afrin *et al*., 2020). Since IBDV load in the blood may substitute clinical signs and death as a measure of susceptibility or safety, the RT-PCR assay could provide a more humane alternative for assessing susceptibility to IBDV infection (Moody *et al*., 2000). In chicken embryo bursa (CEB) and chicken embryo kidney (CEK), classic strains of IBDV can be isolated (Ahimed *et al*., 2014). Antigenically, the very virulent strains are similar to the classic strains, but they can overcome high levels of maternally derived antibodies (Tomas *et al*., 2019).

2.6. Control and Prevention of IBD

Since the IBDV is contagious, infection was spread through contact with infected birds and polluted fomites (Orakpoghenor *et al*., 2020). The virus is unaffected by a variety of chemical and physical agents, and it is environmentally stable (Eterradossi and Saif, 2020). Its spread between flocks can be slowed by enforcing strict biosecurity and implementing a comprehensive vaccination program (Lukert and Saif, 2019).

2.6.1. Biosecurity measures

Several precautions, such as using “all-in/all-out” farming techniques, washing and disinfecting premises, and ensuring a time of rest between depopulation and restocking, are used to avoid IBDV. Both insects and rodents (such as rats and mice) must be removed before cleaning. All farm machinery is disassembled and relocated outside the farm buildings after the old bedding and dung have been removed and decomposed. After that, the clothes are washed in hot water (60°C) with detergent at a pressure of 80 to 150 bars (Van den Berg *et al*., 2000).

Controlling IBD, however, is difficult due to the integrated nature of commercial poultry activities, litter reuse, and the risk of contact with free-living wild birds (Eterradossi and Saif, 2020). While no therapeutic medication has been discovered to affect the course of the viral infection, birds may benefit from drugs that treat secondary agents (Arega, 2018).

2.6.2. Vaccination of IBD

IBDV is a stable virus that is resistant to a wide range of physical and chemical disinfectants. Despite adequate cleaning and disinfection procedures, IBDV infections continue to occur and spread (Van den Berg *et al*., 2000). The most common approach for controlling IBD is chicken vaccination (Nandhakumar, 2019). Vaccination success is determined by the vaccine strain chosen, vaccination schedule, and field isolate strains. Vaccination activities have helped to manage IBD outbreaks in the field (Fussell, 1998).

As a result, vaccination is unavoidable in high-infection environments and is needed to protect chickens from infection during their first weeks of life (Eterradossi and Saif, 2020). Live attenuated vaccines, immune-complex vaccines, live recombinant vectored vaccines expressing IBDV antigens, and inactivated oil-emulsion adjuvanted vaccines are the four main forms of vaccines available for the treatment of IBD (OIE, 2016).

To achieve active immunity, live vaccines made from classical and variant IBDV strains are passed through tissue cultures or embryonated chicken eggs are given, but interference from maternally derived antibodies (MDA) is a major issue in determining a successful live IBDV vaccination schedule (Garba *et al*., 2018). When chickens are vaccinated in the presence of high levels of maternally derived antibodies, the vaccine virus is neutralized and immunity is lost (Moraes *et al*., 2005).

Within a flock, titers can differ significantly, necessitating revaccination. It should also be remembered that vvIBDV can overcome the immunity offered by highly attenuated vaccine strains. On the other hand, fewer attenuated strains (also known as "hot vaccines") have been shown to cause lesions in the bursa follicles and, as a result, immunosuppression in vaccinated birds. In ovo vaccination (Muller *et al*., 2003) and post-hatch vaccination of broilers have been established using an "immune complex" vaccine, in which the vaccine virus is complexed in vitro with the appropriate amount of antibodies (Ivan *et al*., 2005). Although the exact working mechanism of the “immune complex” vaccine is unknown, it has been proposed that the virus is picked up by follicular dendritic cells (macrophages) and remains there until the decrease of maternal antibody (Jeurissen *et al*., 1998).

Layers are vaccinated with inactivated oil-emulsified vaccines to induce high titers of maternally derived antibodies that last throughout the laying period. Chickens are immunized with live vaccines shortly after hatching. The timing of vaccination is critical since antibodies originating from the mother could neutralize the vaccine. Within a flock, titers can differ significantly, necessitating revaccination (Muller *et al*., 2003). Inactivated IBD vaccines are often used to generate strong, long-lasting, and uniform levels of antibodies in breeding hens that have been primed by live vaccine or natural field virus exposure during rearing (Van den Berg *et al*., 2000).

Inactivated vaccines can be used in combination with live vaccines in young valuable birds with high MDA levels reared in areas with a high risk of exposure to virulent IBDV for successful vaccination (OIE, 2016). Chicks are protected from early immunosuppressive infections for one to three weeks by passive immunity, which can

be extended to four or five weeks by improving breeder immunity with oil adjuvant vaccines (Eterradossi and Saif, 2020). Vector vaccines are genetically modified vaccines in which a gene from one organism, the host, is introduced into the genome of another, the vector, to elicit a defensive immune response against both species. The only antigen that induces protective immunity to IBDV is VP2, which is expressed by vector viruses (Bublot *et al*., 2007).

2.7. IBD Virus Vaccine Failure

Early vaccination in flocks with unknown immune status, as well as the evolution of viruses circulating in the field, which leads to antigenic drift and a sharp rise in pathogenicity, is all factors that contribute to vaccine failure against infectious bursal disease. Inappropriate vaccine storage, vaccination techniques, the use of an expired vaccine, and the use of unrecommended doses are all factors that contribute to vaccine failure against IBD (Boudaoud *et al*., 2016).

Because of the antigenic gap between field strains and vaccine strains, conventional serotype 1 vaccines are unable to regulate antigenic variants (Bayyari *et al*., 1996). Until the mid-1980s, IBD was kept fully under control by vaccination and caused not more than 2% specific mortality in broiler flocks. Since then, vaccination failures have become ever more common and mortality rates have been rising all the time. A method for calculating antigenic distances between strains and determining how these distances contribute to cross-protection is the most important prerequisite for the development of vaccines against viruses with antigenic diversity. The evolution of human and equine influenza viruses in relation to vaccine strains was studied using antigenic cartography, a new computational tool for quantifying antigenic distances between strains (Mumford, 2007). This approach can be used to study other pathogens with antigenically variable antigens, such as IBDV (Boudaoud *et al*., 2016).

2.8. The Status of IBD in Ethiopia

Gumboro disease was first identified in Ethiopia in 2002 at a privately owned commercial poultry farm; the disease is thought to have been introduced simultaneously with the country's growing number of commercial state and private poultry farms (Zeleke *et al*., 2005). Where a 45-50% mortality rate was documented and diagnosed in commercial poultry (Jenbreie *et al*., 2014), followed by a government-owned poultry multiplication center (Woldemariam and Wossene, 2007) and a commercial broiler farm with serological tests (Kegne and Chanie, 2014). The disease is widespread, causing high mortality rates in chickens ranging from 49.89 % to 72 % (Zeleke *et al*., 2005).

The first study of IBD in Ethiopian backyard poultry was conducted in the Amhara region, which had received “improved” chicks from a commercial farm (Mazengia *et al*., 2010), and it was suggested that this was the source of the disease's introduction to village poultry. Since then, serological studies have revealed infection in native birds in several parts of the country, with the proportion of positive samples ranging from 75% to 96% (Chaka *et al*., 2012).

In Ethiopia, the juvenile poultry industry has been challenged by frequent outbreaks and the emergence of new strains of IBDV (Mazengia, 2012). The infectious bursal disease has been linked to 25 to 75 % of exotic and local chicken deaths/losses in recent years (Kegne and Chanie, 2014; Zeryehun and Fekadu, 2012). Several studies have shown that the vvIBDV genotype is circulating in Ethiopia (Kegne and Chanie, 2014); (Zeryehun and Fekadu, 2012; Tadesse *and* Jenbere, 2014).

Molecular characterization of the Ethiopian IBD virus isolates collected for the first time in 2005 from the samples from Kombolcha Poultry Multiplication Center, and in commercial and breeding poultry farms in Ethiopia between 2009 and 2011 (Jenbreie *et al*., 2014). As reported by Mekuriaw *et al*. (2017) the molecular analysis revealed the existence and circulation of vvIBDV in the Ethiopian chicken population.

The high prevalence of the disease in both chickens from the back yard and commercial chickens has been shown in serological studies carried out by several researchers at different periods (Mazengia, 2012); Kassa and Molla, 2012); Research indicates that the incidence of IBD is growing at an alarming pace in Ethiopia's poultry industry and is intensifying commercial poultry production even on the backyard of the chickens (Zeleke *et al*., 2005).

2.8.1. Vaccination of IBD in Ethiopia

The primary chicken embryo fibroblast and standard IBDV strains are now in use in Ethiopia to produce the IBDV vaccine. The production of local isolated vaccine IBDV provides absolute protection from the field challenge of IBDV (Aliy *et al*., 2020). CFC (Chicken fibroblast cell) based prepared vaccine is efficient for protecting the vvIBDV circulating in Ethiopia (Wakijira, 2018). Alternative vaccine strains of Ethiopian vvIBDVs may be more effective with vvIBDV attenuated cell culture (Jenberia *et al*., 2014).

Live attenuated and inactivated vaccines are commercially available IBDV vaccines, while recombinant and subunit vaccines have been licensed for some countries (Natnael, 2015). The ideal vaccination time depends on the level of the maternal antibody, the vaccine strain used, the vaccine breakthrough titer, and the field pressure of the IBDV (Mekuriaw *et al*., 2017). Inactivated vaccines to breeders of hens lead to long and high antibody levels in hatched chicks.

However, producers do not take inactivated vaccines in certain areas where a virulent IBDV has caused significant losses. But in hatched chicks of the unvaccinated breeder hens, the intensive live viral vaccination program is used. These chicks avoid the great risk of the disease's immunosuppressive form (Mazengia, 2012). Outbreaks occur in virulent conventional viruses, which are genetically linked to current attenuated traditional vaccine strains. Therefore, adequate vaccine use must be tackled before vaccine production involves attenuated vvIBDVs (Jenberia *et al*., 2014). The time to administer live intermediate IBD vaccine, which is significant, is

presently reported in Ethiopia, rather than IBD Vaccinations are given to chickens whose parents have vaccinated against IBD without determining the concentration and age of MDA have been determined. Therefore, it is essential to determine the optimum delivery time for the IBD vaccine, so that chickens are protected from IBDV field challenges. The optimum time frame is usually expected after the detection of IBDV MDA by ELISA during the first weeks of post-hatch (Fantay *et al*., 2015).

Various factors, including the lack of complete cross-protection among all strains, may cause vaccination programs to fail. As IBDV evolves further through genetic mutation, continuous monitoring of antigenic viruses is required. Infield virus, the ability of the IBDV to avoid immunity generated by current classic vaccines must be screened quickly for specific mutations that are known to cause antigenic drift. The local strains differ from the vaccine strains in recognized amino acids. Preparation of local vaccines in areas where outbreaks may not be controlled by commercially available vaccines is therefore proposed for the control of IBD (Shegu *et al*., 2020).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in the Wolaita Zone of the Southern Nations Nationalities and People (SNNP) region, Ethiopia. The study area is located in latitudes 6.4 to 7.1 degrees north and lengths 37.4 to 38.2 degrees east. The altitude is between 1.200 and 2.950 meters above sea level (masl). The area has three ecological zones: Kola (lowland <1500masl), Woina Dega (medium-altitude 1500-2300masl), and Dega (highland >2300masl). Bimodal rainfall is about 1000mm on average (lower in the lowlands and higher in the highlands). The mean monthly temperature in January is between 26°C and 11°C in August. The main and small rainy season usually runs between June and September and March and May, respectively (WZFEDD, 2005). Cattle, goats, sheep, equines, poultry (mainly local chickens, but also some improved varieties), and bees are among the livestock produced in the area with an estimated population of 685,886, 90,215, 87,525, 1951, 669,822, and 38,564 (hives) respectively. The zone has a total human population of 5,473,190. (Pound and Jonfa, 2006)

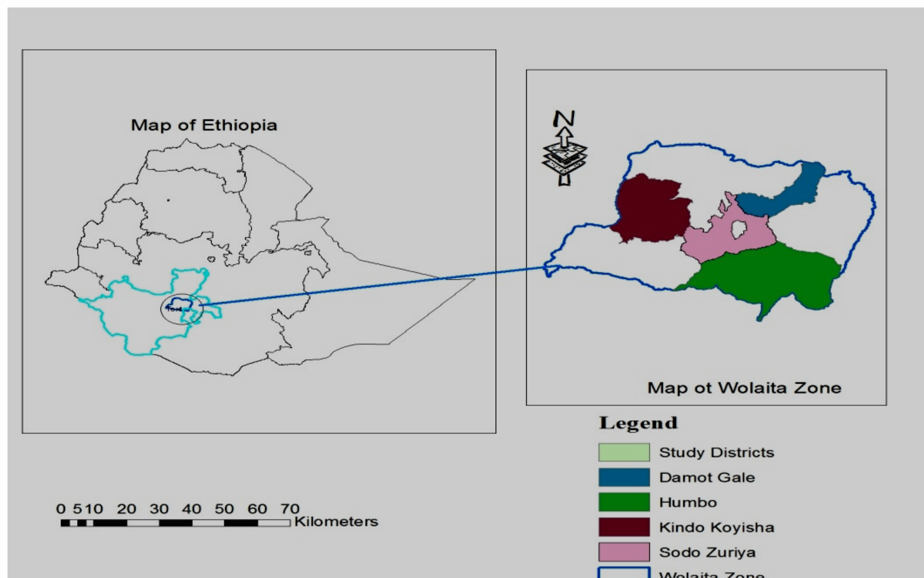

Figure 3. Map of the study area

3.2. Study population

The study animals were the backyard chickens found in different districts/kebeles in the Wolaita zone. The chickens were categorized into two age groups (< 3 weeks and ≥ 3 weeks) based on the development of the bursa of Fabricius, hence, producing a difference in the susceptibility of the age groups to IBD (De Hedt *et al*., 2005). A thorough survey was conducted for each household to evaluate the vaccination status for a selected chicken to be sampled. Accordingly, unvaccinated chickens against IBDV were included in this study. Chickens' flocks were categorized based on chicken numbers ≥ 5 and < 5 which was based on the average number of chickens per flock per household in the study area. Breeds of chickens that were exotic and indigenous were also considered as a risk factor in this study.

3.3. Study Design

A cross-sectional study was carried out from December 2020 to May 2021 to determine the seroprevalence of IBD in unvaccinated backyard chickens and to describe the distribution of disease concerning different risk factors in the Wolaita zone. The chicken level risk factors were included to assess their statistical association with the disease occurrence**.** Participatory epidemiology, specifically participatory disease surveillance (PDS) was conducted to assess the epidemiology of IBD in the study areas.

3.4. Sample Size Determination

Sampling was carried out using a multi-stage sampling technique. Four districts were selected based on agroecology from the Wolaita zone. Accordingly, Damot gale (midland), Humbo (lowland), Kindokoysha (lowland), and Sodozuria (highland) were included. From each district, five kebeles were randomly selected**.** A household flock is the unit of interest. Households that have a poultry flock were also randomly selected from the selected districts. The sample size was determined according to Thrusfield (2005). The expected prevalence of 82.9% was taken from a previous

study at Hawass by Jenberia *et al*. (2012) to determine the sample size with a 95% confidence interval (CI) and 5% desired absolute precision.

$$n = \frac{1.96^* (p)(1-p)}{d^2}$$

Where n= sample size; p= Expected prevalence; d= Desired level of precision (5%)

By considering the correction for the multi-stage sampling design effect (multiply of 2) (Thrusfield, 2005), the final sample size was= 436. Hence, a minimum of 436 chickens was required although 482 were sampled.

3.5. Sampling Technique

About 2 ml blood samples were collected using the disposable syringes from the wing vein of the chickens and stored for 2-4 hours or kept at room temperature until sera were separated. The separated sera were decanted into cryovial tubes, labeled, and transported to the National Animal Health diagnostic and investigation center (NAHDIC) laboratory using an ice-box. Then, the sera were stored at −20°C until analysis.

3.6. Questionnaire Survey

A semi-structured questionnaire was prepared to obtain relevant information on the backyard poultry management system in the study area. It was designed to both close and open-ended questions in the English language and was translated to local language for those respondents aiming for clarity as means of interview. Then, the questionnaire was distributed to the individuals to gather relevant data to the problem under study. Important data were recorded for household and flock level such as location, owner, source (brought in or homebred), history of IBD vaccination, and health status at the time of visit, sex, housing, other management methods. A total of 60 households were selected to participate in this study.

3.7. Test Procedure and Interpretation

The indirect ELISA diagnostic kit is used to check the presence of IBD-specific antibodies using a commercial IBDV ELISA test kit (Proflockplus IBD, Symbiotic Corporation, Frontera, and San Diego) following the recommended protocol of the manufacturer.

Micro wells were coated with purified IBDV antigens. Serum samples to be tested and controls were added to the wells. Anti-IBDV antibodies, if present, form an antigen-antibody complex. After washing, an anti-chicken horseradish peroxides (HRP) conjugate was added to the wells. It fixes to the antibodies, forming an antigen-antibody-conjugate-HRP complex. After elimination of the excess conjugate by washing, the substrate solution (TMB) was added. The resulting coloration depends on the number of specific antibodies present in the specimen to be tested in the presence of antibodies; a blue solution appears which becomes yellow after the addition of the stop solution. In the absence of antibodies, no coloration appears. The microplate is read at 450 nm. The result has been evaluated using the sample/positive value (S/P); The mean Optical Density (OD) value of the positive control serum is larger than 0.25 (OD PC > 0.25) in a valid IBD ELISA result, and the ratio of the mean values of the Positive and Negative Controls (OD PC and OD NC) is larger than 3. But, as this OD value was outside of these ranges, the IBD ELISA result was considered invalid.

$$S/P = \frac{\text{Sample OD} - \text{Negative control OD}}{\text{Positive control OD} - \text{Negative control OD}}$$

Samples with S/P > 0.3 and less than or equal to 0.3 were considered positive and negative respectively. Laboratory procedure was attached in (Appendice 3).

3.8. Ethical consideration

Ethical clearance for this study was obtained from Addis Ababa University College of veterinary medicine and agriculture minutes of animal research ethics and review committee (Ref number VM/ERC/22/06/13/2021)(Appendix 5).

3.9. Data Management and Analysis

The laboratory results and data from the questionnaire survey were recorded and managed in a Microsoft Excel spreadsheet before being analyzed with R version 3.6.2 and SPSS version 18.0 and both descriptive and analytical statistics were carried out. The occurrence of association between the dependent variable (seropositivity) and independent variables was analyzed by using logistic regression and an odds ratio was employed to assess the level effects of risk factors on IBD. A stepwise variable-selection strategy was used to make a final model using logistic regression. The prevalence of IBD was calculated by dividing the number of positive serum samples by the total number of chicken serum samples tested for IBD. For this analysis P-value, less than 0.05 was considered significant with a 95% confidence interval.

4. RESULTS

4.1. Questionnaire Survey

4.1.1. Management of backyard poultry production system

A questionnaire survey administered to 60 households indicated that poultry production is the main source of secondary income for the majority of farmers (81.66 %) and only 8.33% of respondents used it for home consumption**.** The remaining 10% were engaged in poultry production as the primary source of income. The majority (73.33%) of the respondents rear both layer and broiler chickens. The majority of farmers (78.33%) live with their chickens at home, whereas (21.66%) provide separate chicken houses. Almost all (100%) had the practice of cleaning poultry house but only 43.33% burn and burry the wastages and the remaining (66.7%) had the practice of disposing of the wastages by throwing away in open spaces. 100 % of the respondents allow their chickens to scavenge free area among them 50% provide supportive feeds to their chickens (Table 1).

Table 1. Types of poultry production, socioeconomic importance, and management system practiced

	Frequency	Percent (%)
Types of poultry productions		
Layer	7	11.66%
Broiler	9	15%
Both	44	73.33%
Socioeconomic Importance		
Home consumption	5	8.33%
The primary source of income	6	10%
A secondary source of income	49	81.66%
Management practice		
Provide supportive feed	30	50%
Dwell at home	47	78.33%
Lives in a separate house	13	21.66%
Cleaning poultry house	60	100%
Burn and bury wastage	26	43.33%%
Throw away in open space	34	56.66%

4.1.2. Health Care and Constraints

No chicken owner ever had their bird vaccinated. 50% of the respondents have information about IBD. 10% of the respondents said their chickens had contact with the wild birds and 96.66% of the respondent's compliant diseases, predators, housing, and feeding problems as major challenges for poultry production (Table 2).

Table 2. Constraints of poultry production

Constraints	frequency	Percent (%)
Lack of vaccination	60	100
Disease and predators	40	66
Housing and Feeding problem	60	100
Contact with Wild birds	6	10
Lack of IBD knowledge	30	50

About 58.3% of people surveyed have no practice of treating their chickens, and 84% of the people who do not treat their chickens have a history of their chickens died. Only 21.66% of the respondents isolate sick chickens. 63.3% prove that there was an IBD outbreak previously in the study areas among them 77% of respondents had a lack of practice of isolating sick chickens. There was a statistical association ($P<0.05$) between the treatment history and isolation of sick chickens with death in the flock. The previous outbreaks had also a statistical association ($P<0.05$) with the isolation of sick chickens in the flock (Table 3).

Table 3. Previous outbreak and death history in association with treatment and isolation of sick chickens

Variables	**Death in the flock**		**P-value**	**The previous outbreak of IBD**		**P-value**
	Yes	No		Yes	No	
Treated	6(17.1%)	29(82.9%)	0.001	19(54.3%)	16(45.7%)	0.147
Not treated	21(84%)	4(16%)		19(76%)	6(24%)	
Isolate sick chickens	1(7.69%)	12(92.31%)	0.006	3(23%)	10(77%)	0.002
Not isolate sickchickens	26(55.3%)	21(44.7%)		35(74.5%)	12(25.5%)	

4.2. The seroprevalence of IBD

From 483 sera samples tested IBDV antibodies using indirect ELISA, 236 samples were positive for IBDV antibodies with an overall prevalence of 48.96% (95% CI: 44.52-53.41). Although there was no statistical association ($P>0.05$), the highest seroprevalence was found from Humbo district (n = 63; 55.75 %) followed by Sodozuria district (n = 67; 51.54%), Damot gale district (n = 49; 46.23%), and Kindokoysha district (n= 57; 42.85%).

The investigation of the effects of sex on disease prevalence in this study reveals female and male chickens had nearly comparable percentages of seroprevalence (48.75 % vs. 49.37 %). There was no statistically significant difference between the sexes groups ($P>0.05$). IBD seroprevalence was 51.96 % in the chicken < 3 weeks of age group and 51.84 % in the ≥ 3 weeks of age group, with no statistically significant association ($P>0.05$). A statistically ($P< 0.05$) higher seroprevalence of IBD was found in exotic breed (n = 112; 57.14%) as compared to local breed (n = 124; 43.35%). Similarly, higher seroprevalence of IBD was recorded in flock sizes ≥ 5 chickens (n = 201; 59.47%) as compared to < 5 chickens (n = 35; 24.3%). This variation was statistically significant ($P < 0.05$) as shown in Table 4.

Table 4.Results of multivariable logistic regression analysis on the occurrence of IBD as a function of various risk factors

Variable	Total no. of samples tested	Seropositives	Seroprevalence (%)	OR	95% of CI	P-value
District						
Damotgale	106	49	46.22	-	-	-
Humbo	113	63	55.75	0.38	-0.14-0.91	0.049
Kindokoysha	133	57	42.85	-0.13	-0.65-0.377	0.981
SodoZuria	130	67	51.53	0.21	-0.30-0.72	0.177
Breed						
Local	286	124	43.35	-0.396	-0.78--0.01	0.003
Exotic	196	112	57.14	-	-	-
Sex						
Male	160	79	49.37	0.024	-0.35-0.402	0.9703
Female	322	157	48.75	-	-	-
Age						
< 3 weeks	102	53	51.96	0.15	-0.28-0.59	0.5682
≥3weeks	380	197	51.84	-	-	-
Flock size						
≥ 5 chicken	338	201	59.47	1.466	1.03 - 1.91	0.001
< 5 chicken	144	35	24.3	-	-	-

4.3. Major Risk Factors Analysis by Using Multiple Logistic Regression

To determine the independence of effects of each risk factor in connection to disease prevalence, a multiple logistic regression analysis was used. Because of the insignificant effect, explanatory variables with nonsignificant associations with the occurrence of IBD were removed from the model. The two risk factors, breed and flock size, had a significant relationship with IBD occurrence and were taken as major risk factors of IBD. The odds of the occurrence of IBD in the local breed were 0.67 times lower than that of the occurrence in exotic breeds. The odds of the occurrence of the IBD in ≥ 5 chickens were 4.33 times higher than < 5 chickens (Table 5).

Table 5. Stepwise multivariate logistic regression analysis of risk factors

Risk factors	Coefficients	P-value	OR	95%CI
Local to exotic	0.67	0.044	-0.396	-0.784 -0.0104
≥ 5 chicken to < 5 chicken	4.33	0.001	1.466	1.033 - 1.918

4.4. Prediction of the Occurrence of IBD in Association with Breed and No of the Flock

From the categories of the significant explanatory variable number of flock size ≥ 5 chickens is predicted to have a high probability of IBD occurrence than the categories with < 5 chickens. As shown in the plot, the predicted probability of IBD in flocks with ≥ 5 chickens is between 0.55 and 0.65 (figure 4), which was higher than the predicted probability in flocks with < 5 chickens (between 0.2 and 0.3). The predicted probability of IBD occurrence is also higher in exotic breeds than in local breeds, ranging from 0.3 to 0.65 and 0.25 to 0.55 respectively (figure 5).

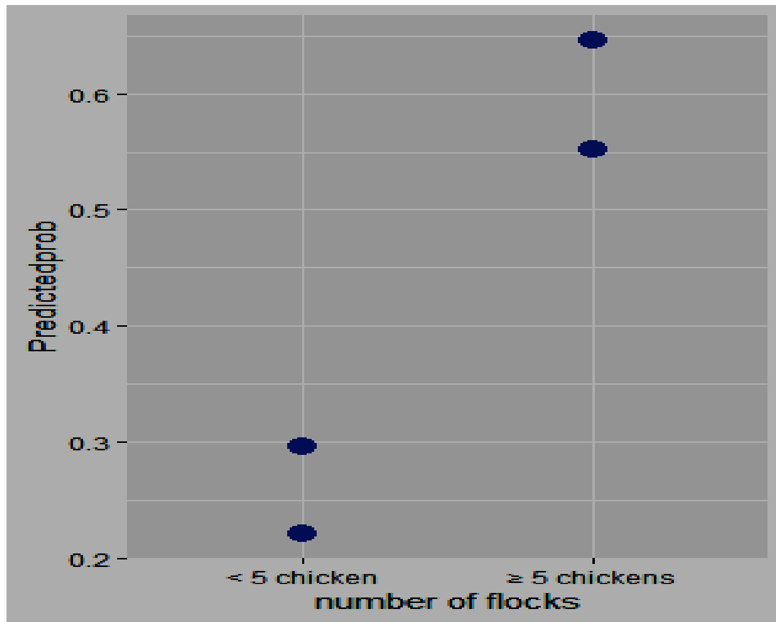

Figure 4. ggplot of predicted probability of the occurrence IBD in association with the number of flocks

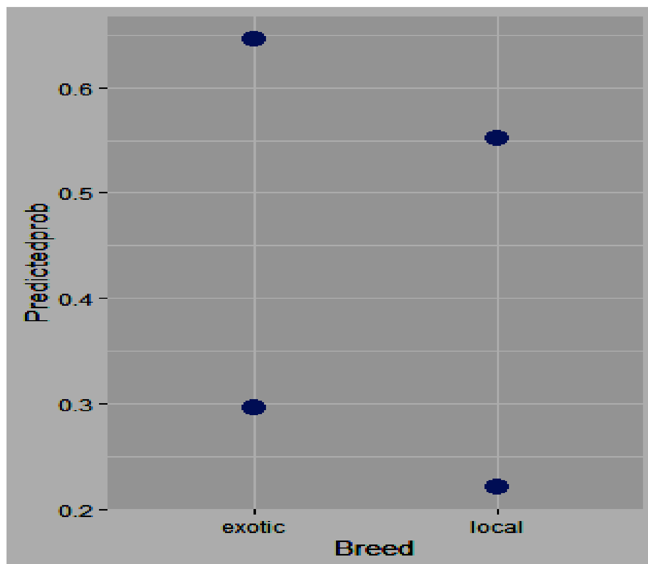

Figure 5. ggplot of predicted probability of the occurrence IBD in association with the Breed.

5. DISCUSSION

A major source of high-quality animal protein is the production of poultry. Nevertheless, the high incidence of infectious diseases in poultry particularly in smallholdings poses a great risk to their productivity and survival. Despite vaccination and efforts to improve control, IBD has continued to affect poultry production (Adebiyi *et al*., 2018). The current investigation, which was conducted on backyard chickens in four districts of the Wolaita zone, demonstrated the existence of IBDV specific antibody in the absence of vaccination, indicating that domestic chickens were exposed to the virus in the field. Although no evident clinical signs of the disease were found during sample collection in chickens grown in the area, it is speculated that this could be due to field exposure of chickens to the virus, which resulted in them becoming subclinically infected at an early age. In this study, an attempt was made to estimate the seroprevalence of IBD in apparently healthy chickens and identify risk factors associated with the occurrence of the disease.

In this study, 236 chickens tested positive for IBD among 482 examined through the I- ELISA test, resulting in an overall seroprevalence of 48.96 %. IBDV is extensively spread and is a concern in all types of production systems and breeds of chickens in the research areas, as evidenced by the greatest seroprevalence of IBD in this study. Similar studies conducted at different times and places reported different prevalences.

The current finding was relatively comparable to the earlier reported prevalence of 45.5%, 48%, and 49.3% documented by Zegeye *et al.* (2015), Sero-prevalence of IBD in backyard chickens around Mekelle, El-Yuguda *et al.* (2009), Newcastle disease and IBD among village chickens in Borno State, Nigeria, and Ndanyi (2004), A study to determine causes of mortality and the effect of IBD vaccination in village chickens in Taita Taveta district of Kenya respectively. A recent study by Lemma *et al*. (2018) and Natinael (2015) in Ethiopia and Abraham-Oyiguh *et al*. (2015) in southeast Nigeria reported a closely comparable prevalence of 51.7%, 51.6%, and 51.6%, respectively.

The observed prevalence in the current study could be attributed to scavenging characteristics of chickens by covering long areas, failure to vaccinating chickens, improper management practices, disposal of dead chickens as well as different wastages by throwing in open spaces, replacing flocks from the local market, contact with wild birds, and contact of chickens with other neighborhoods could be possible reasons for the high prevalence value. Rising together the various ages of village chickens might make the infection persistent in a given flock (El-Yuguda *et al*., 2009).

On the contrary, seroprevalence documented in this survey showed a higher value when compared to the previous reports of 38.39% by Tesfaye (2008) from the east shewa zone, 29.04% (Bahirdar), and 21.70% (Farta) by mazengia *et al*. (2010), and 34.7% by Sharma *et al*. (2014) from West Indies, and On the other hand, a higher prevalence than the present study was reported with the prevalence of 90.3 % Jenbreie *et al*. (2012) in Ethiopia, 83% reported by Tadesse and Jenbere (2014) in backyard chickens at selected districts of eastern Ethiopia, Tesfaheywet and Getnet (2012) reported an overall seroprevalence of 82.2% in chickens managed under backyard production system in Central Oromia and seroprevalence of 96.4% was reported by Debebe (2016) from Jimma and Bonga district of southwest Ethiopia. In addition, a higher prevalence of IBD reports from the current study was 63% from Yobe state Nigeria Sule *et al*. (2013), 82.2 by Zeryehun and Fekadu (2012), and Kassa and Mola (2012) seroprevalence of 75% from West Gojam and 72% from North Gonder.

The reasons for the results change were attributable to the sample collections after outbreaks of IBD. This is in accord with the results of the Mekele samples collected after the outbreak season with the greatest seroprevalence of (90.3%) in 2012 (Jenbreie *et al*., 2012), and also the difference in prevalence maybe because some of the researchers use the AGID test which is less sensitive than the ELISA kit utilized in the current investigation. It is agreed with the International Office for Epizootics Manual (OIE, 2004), which identified ELISA as the most optimal, sensitive, and

specific tools for the diagnosis of viral antibodies in serological terms. The lower IBD prevalence observed at the present study area was because most households had a low tendency of rearing the improved chicken breeds, and instead, they depended exclusively on the indigenous local breeds.

The present study revealed seropositivity for IBD in all study districts of the study areas with an overall seroprevalence of 48.96% in chickens, indicating the spread of the IBD virus throughout the districts of the study areas. The study showed the level of prevalence of IBD has no significant variations between the four study districts but Kindo koysha district (42.85%) is considered as low prevalence and the highest prevalence recorded in Humbo district (55.75%). Damot gale districts have a prevalence of (46.23%) and sodo zuria (51.54%). No statistical significance was seen in agroecology based on the prevalence result from the four districts with different agroecology and this result agrees with Desalegn (2015), Girma *et al*. (2017), and Chaka *et al*. (2012). IBDV is particularly resistant to various environmental conditions and can survive for a long time in the environment (Kassa and Molla 2012). This indicates no particular environmental setting can prevent or affect the disease's incidence.

seroprevalence was found to be closure in male chickens (49.37%) and female chickens (48.75%) the results were not statistically significant($p<0.05$). Sex-wise prevalence of IBD occurrence agrees with the report of Lemma *et al*. (2019), Tadesse and Jenber (2014), and Girma *et al*. (2017). This could be attributed to male and female chickens being exposed to a common source of infection, such as contaminated fecal feed when scavenging seed or transmitting IBDV from the newly-purchased chickens (Sule *et al*., 2013). The horizontal transmission among the age groups of chickens which were raised together is suggestive of a nonsignificant association of IBD occurrence and age groups. Almost equal prevalence between< 3 weeks (51.96%) and ≥3weeks (51.84%) age groups. This finding is supported by previous reports of Zeryehun and Fekadu (2012), Lemma *et al*. (2019), and *Sule et al*. (2013). The disease is present in all main areas of chicken production around the

world and it can be shown in all age groups (Van Den Berg *et al*., 2000).

In this study, breed and number of flocks were found to be the potential risk factors and there was a statistically significant difference observed (P<0.05). In indigenous breeds, the lower seroprevalence (43.35%) relative to exotic breeds (57.14%) has been discovered. This result was consistent with earlier findings of Hailu *et al*. (2010), Jembere *et al*. (2012), and Zeryehun and Fekadu (2012). A similar observation was reported by Tadesse and Jenbere (2014) who explained higher seroprevalence of the infection was recorded in exotic breeds than local breeds. This difference could be because indigenous breeds are more resistant than exotic ones to most diseases In this study, the relation between flock size and IBD occurrence was identical with Desalegn's (2015) findings that the lower prevalence was observed in flock size less than or equal to fours. The seroprevalence of IBD in flock size ≥ 5 chicken was (59.47%) which was greater than flock size <5 (24.3%).

Risk factors for IBD were assessed through a questionnaire survey and it revealed that the disease was endemic in the study areas. In the area studied, more important restrictions on poultry production were predation, diseases, and feed shortages. The majority of farmers (owners), instead of providing supplementary food to their chickens, let them scavenge. This is due to the farmers' low knowledge that poultry is seen as a side business this is in agreement with the report of Desta and Wakeyo (2012). There were frequent outbreaks of IBD in the area and resulting in the deaths of chickens. Most of the farmers don't pay attention to their birds' health. Most of the farmers (78.33%) dwell at home with the chickens while (21.66%) provide separate chicken houses. Such stressful situations expose various diseases for birds and can contribute to disease transfer from birds to people or vice versa. This can also make birds exposed to predator injury, mechanical damage, cannibalism, uncomfortable rest. Even if they know the advantage of poultry production over the other farming systems, not give due consideration as they lack hope in the sector as there are constraints above their control that affect the poultry production system for a long time in the area. There was a statistical association (P<0.05) between the treatment

history and isolation of sick chickens with death in the flock. The previous outbreak had also a statistical association ($P<0.05$) with the isolation of sick chickens in the flock. This indicates the disease occurrence in the flock was related to the farmer’s reluctance to manage their chicken's health. The vaccine of IBD is not practiced in backyards poultry production systems in the area.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed the seroprevalence and the associated potential risk factors of IBD in backyard chickens of the Wolaita zone. The overall seroprevalence of IBD in the present study was 48.96%; this result suggests that IBDV is widely disseminated in the study areas. Large flock size and exotic breeds of the chickens have high exposure to the IBD than the other bird-related risk factors included in this study. The higher frequency of IBD found is that farmers are unaware of disease management measures and no vaccination schedule undertaken in the area under research; vaccinations and strict biosecurity are the techniques of prevention of the disease.

Therefore the following recommendations are forwarded according to the aforesaid conclusion:

- Farmers should be trained about the disease, backyard flock disease prevention mechanisms, and report the IBD disease occurrence to the stakeholders in the area.
- The administration of the planned chicken vaccination program and the appropriate management measures should be given attention to the backyard production system to reduce IBD and to enable an appropriate program of control in the field of study.
- A further investigation is required to investigate the IBD viral strains circulating in the study are.

7. REFERENCES

ADEBIYI, A. I., AF, F., OA, O., and OE, A. (2018): Infectious bursal disease in the live-bird market and smallholding birds in two states of southwest Nigeria. *Malaysian Journal of Veterinary Research*, ***9*(1):** 2-57.

Abraham-Oyiguh, J., Adewumi, M. O., Onoja, A. B., Suleiman, I., Sulaiman, L. K., Ahmed, S. J., and Jagboro, S. T. (2015): Seroprevalence of infectious bursal disease virus in local chickens in Udu Local Government Area of Delta State, southeast Nigeria. *Journal of Immunoassay and Immunochemistry*, ***36*(4)**: 398-404.

Afrin, F., Chowdhury, M. M. R., Cho, C. Y., Kim, S., Roh, H. J., Kim, D. K. and Saha, S. (2020): Efficacy of two diagnostic tests for the detection of infectious bursal disease viruses in chicken from different types of bursal samples. *African Journal of Microbiology Research*, ***14*(1)**: 1-7.

Ahmad, A. N., Hussain, I., Akhtar, M. and Bibi, F. (2014): Comparative study of commercially available infectious bursal disease vaccine with egg attenuated live vaccine. *Pakistan J. Zool*, ***46*(4)**: 959-966.

Alders, R. G., and Pym, R. A. E. (2009): Village poultry: still important to millions, eight thousand years after domestication. *World's Poultry Science Journal*, **65(2)**: 181-190.

Aliy, A., Shiferaw, S., Shegu, D., Senbeta, B., Guyassa, C., and Bulbula, A. (2020): Diagnostic Techniques of Infectious Bursal Disease Virus: A Review.*Life Science Journal*, **17(6)**. 10.7537/marslsj170620.01

Aliyu, H. B., Saidu, L., Jamilu, A., Andamin, A. D. and Akpavie, S. O. (2016): Outbreaks of virulent infectious bursal disease in flocks of battery cage brooding system of commercial chickens. *Journal of Veterinary Medicine*, 10.1155/2016/8182160

Arega, A. M. (2018): Review on infectious Bursal disease: Threat for the Ethiopian poultry industry. *International Journal of Advanced Life Sciences*, **11**: 52-65.

Ashraf, S. (2005): *Studies on infectious bursal disease virus* (Doctoral Dissertation,

TheOhioStateUniversity),http://rave.ohiolink.edu/etdc/view?acc_num=osu1124124381.

Bayyari, G. R., Story, J. D., Beasley, J. N., and Skeeles, J. K. (1996): Antigenic characterization of an Arkansas isolate of infectious bursal disease virus. *Avian diseases*, 588-599. https://doi.org/10.1017/S0043933916000775

Biro, E., Kocsis, K., Nagy, N., Molnar, D., Kabell, S., Palya, V., and Olah, I. (2011): Origin of the chicken splenic reticular cells influences the effect of the infectious bursal disease virus on the extracellular matrix. *Avian Pathology*, **40(2)**: 199-206.

Birol, E., Asare-Marfo, D., Ayele, G., Mensah-Bonsu, A., Ndirangu, L. K., Okpukpara, B., and Yakhshilikov, Y. (2010): *Investigating the role of poultry in livelihoods and the impact of HPAI on livelihoods outcomes in Africa: evidence from Ethiopia, Ghana, Kenya, and Nigeria*: 10.22004/ag.econ.97084

Boudaoud, A., Mamache, B., Tombari, W. and Ghram, A. (2016): Virus mutations and their impact on vaccination against infectious bursal disease (Gumboro disease). *Rev Sci Tech*, **35(3)**: 875-897.

Brandt, M., Yao, K., Liu, M., Heckert, R. A. and Vakharia, V. N. (2001): Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. *Journal of virology*, **75(24)**: 11974-11982.

Bublot, M., Pritchard, N., Le Gros, F. X. and Goutebroze, S. (2007): Use of a vectored vaccine against the infectious bursal disease of chickens in the face of high-titred maternally derived antibody. *Journal of comparative pathology*, **137**: 581-584.

Camilotti, E., Moraes, L. B. D., Furian, T. Q., Borges, K. A., Moraes, H. L. D. S. and Salle, C. T. P. (2016): Infectious bursal disease: pathogenicity and immunogenicity of vaccines. *Brazilian Journal of Poultry Science*, **18(2)**: 303-308.

Chaka, H., Goutard, F., Bisschop, S. P., and Thompson, P. N. (2012): Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia. *Poultry Science*, **91(4)**, 862-869.

Choudhary, U. K., Tiwary, B. K., Prasad, A. and Ganguly, S. (2012): Study on the incidence of infectious bursal disease in and around Ranchi. *Indian Journal of Animal Research*, **46(2)**: 156-159.

Cosgrove, A. S. (1962): A new disease of chickens: avian nephrosis. *Avian diseases*, **6(3)**: 385-389.

Desalegn, J. M. (2015): Epidemiology of Village Chicken Diseases: A Longitudinal Study on The Magnitude and Determinants of Morbidity and Mortality-The Case of Newcastle And Infectious Bursal Disease. Addis Ababa University College of Veterinary Medicine and Agriculture, Department of Clinical Study, Debre-Zeit, Ethiopia.

Desta, T. T., and Wakeyo, O. (2012): Uses and flock management practices of scavenging chickens in Wolaita Zone of southern Ethiopia. *Tropical animal health and production*, **44(3)**: 537-544.

Dey, S., Pathak, D. C., Ramamurthy, N., Maity, H. K. and Chellappa, M. M. (2019): Infectious bursal disease virus in chickens: prevalence, impact, and management strategies. *Veterinary Medicine: Research and Reports*, **10**: 85.

Elankumaran, S., Heckert, R. A. and Moura, L. (2002): Pathogenesis and tissue distribution of a variant strain of infectious bursal disease virus in commercial broiler chickens. *Avian diseases*, **46(1)**: 169-176

Eldaghayes, I., Rothwell, L., Williams, A., Withers, D., Balu, S., Davison, F. and Kaiser, P. (2006): Infectious bursal disease virus: strains that differ in virulence differentially modulate the innate immune response to infection in the chicken bursa. *Viral immunology*, **19(1)**: 83-91.

El-Yuguda, A. D., Baba, S. S., Ibrahim, U. I., and Brisibe, F. (2009): Newcastle disease and infectious bursal disease among village chickens in Borno State, Nigeria. *Family Poultry*, **18(1)**: 16-23.

Eterradossi, N. and Saif, Y. M. (2020): Infectious bursal disease. Diseases of poultry, Pp 257-283.

Fantay, H., Balcha, E., Tesfay, A. and Afera, B. (2015): Determining optimum time for administration of live intermediate vaccine of infectious bursal disease to chickens at Mekelle farm. *J. Vet. Sci. Technol*, **6**: 223-227.

Farooq, M., Durrani, F. R., Imran, N., Durrani, Z., and Chand, N. (2003): Prevalence and economic losses due to infectious bursal disease in broilers in Mirpur and Kotli districts of Kashmir. *Int. J. Poult. Sci*, **2**: 267-270.

Fussell, L. W. (1998): Poultry industry strategies for control of immunosuppressive diseases. *Poultry Science*, **77(8)**: 1193-1196.

Garba, S., Mera, U. M., Musa, U., Onifade, K. I., Raji, A. A., Jibrin, M. S. and Yakubu, Y. (2018): Immunomodulatory Effects of Neem (Azadirachta indica) Leaf Aqueous Extracts in Cockerels Vaccinated and Experimentally Infected with Infectious Bursal Disease Virus. *International Journal of Sciences: Basic and Applied Research*, **37(2)**: 72 -89

Getachew, Y., Fesseha, H., 2020: Infectious Bursal Disease in Poultry Production - A Review 22, 185–196. *Global Veterinaria*, **22**: 185 – 196.

Girma, M., Kebede, B., and Megarsa, B. (2017): Seroprevalence of Infectious Bursal Disease in Backyard Chickens of Six Districts of North Shewa Zones of Oromia and Amhara Regions, Ethiopia. *SOJ Veterinary Sciences,* **3(1)**: 10.15226/2381-2907/3/2/00129

Hailu, D., Melese, B., Moti, Y., and Mekedes, G. (2010): Seroprevalence of infectious bursal disease in backyard chickens of Oromia regional state, Ethiopia. *Veterinary Research (Pakistan)*, **3(4)**: 89-93.

Hirai, K, and Shimakura, S. (1974): Structure of infectious bursal disease virus. *Journal of virology*, **14(4)**: 957-964.

Hitchner, S. B. (1970): Infectivity of infectious bursal disease virus for embryonated eggs. *Poultry Science*, **49(2)**: 511-516.

Jackwood, D. J., and Sommer, S. E. (2002): Virulent vaccine strains of infectious bursal disease virus not distinguishable from wild-type viruses with the use of a molecular marker. *Avian diseases*, **46(4)**: 1030-1032.

Jenbreie, S., Ayelet, G., Gelaye, E., Kebede, F., Lynch, S. E., and Negussie, H. (2012): Infectious bursal disease: seroprevalence and associated risk factors in major poultry rearing areas of Ethiopia. *Tropical animal health and production*, **45(1)**: 75-79.

Jenbreie, S., Lynch, S. E., Kebede, F., Christley, R. M., Gelaye, E., Negussie, H., and

Ayelet, G. (2014): Genetic characterization of infectious bursal disease virus isolates in Ethiopia. *Acta Tropica*, **130**: 39-43.

Jeurissen, S. H., Janse, E. M., Lehrbach, P. R., Haddad, E. E., Avakian, A., and Whitfill, C. E. (1998): The working mechanism of an immune complex vaccine that protects chickens against infectious bursal disease. *Immunology*, **95(3)**: 494.

Nandhakumar, D., Rajasekhar, R., Logeshwaran, G., Ravishankar, C., Sebastian, S. R., Anoopraj, R., and John, K. (2019): Identification and genetic analysis of infectious bursal disease viruses from field outbreaks in Kerala, India. *Tropical animal health and production*, **52**: 989–997.

Kassa, S. A. and Molla, W. (2012): Seroprevalence of infectious bursal disease in backyard chickens of North West Ethiopia. *SJCS*, **1(1)**: 20-25.

Kegne, T. and Chanie, M. (2014): Review on the incidence and pathology of the infectious bursal disease. *British Journal of Poultry Sciences*, **3(3)**: 68-77.

Kim, I. J., You, S. K., Kim, H., Yeh, H. Y. and Sharma, J. M. (2000): Characteristics of bursal T lymphocytes induced by infectious bursal disease virus. *Journal of virology*, **74**(**19**): 8884-8892.

Lasher, H.N. and Davis, V.S. (1997): History of Infectious Bursal Disease in the USA the first two decades. *Avian disease*, **Pp** 11-19.

Lemma, F., Zeryehun, T., and Kebede, A. (2019): Seroprevalence of Infectious Bursal Disease in Non-vaccinated Village Chicken in Jigjiga and Harar Districts, Eastern Ethiopia. *Journal of Veterinary Science & Technology,* **10(1)**:572-577

Letzel, T., Coulibaly, F., Rey, F. A., Delmas, B., Jagt, E., Van Loon, A. A. and Mundt, E. (2007): Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease virus. *Journal of virology*, **81**(**23**): 12827-12835.

Lombardo, E., Maraver, A., Espinosa, I., Fernández-Arias, A. and Rodriguez, J. F. (2000): VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. *Virology*, **277(2)**: 345-357.

Lukert, P. D., and Saif, Y. M. (2003): Infectious bursal disease. Diseases of poultry,

11th ed, *Iowa State University Press, Ames, IA*, **Pp** 161–179.

Mahgoub, H. A. (2012): An overview of the infectious bursal disease. *Archives of virology*, **157**(**11**): 2047-2057.

Mandeville, W. F., Cook, F. K., and Jackwood, D. J. (2000): Heat lability of five strains of infectious bursal disease virus. *Poultry Science*, **79**(**6**): 838-842.

Mazengia, H., Bekele, S. T., and Negash, T. (2010): Incidence of infectious bursal disease in village chickens in two districts of Amhara Region, Northwest Ethiopia. *Livestock Research for Rural Development*, **21(12)**: 214.

Mazengia, H. (2012): Review on major viral diseases of chickens reported in Ethiopia. *Journal of Infectious Diseases and Immunity*, **4(1):** 1-9.

McFerran, J. B., McNulty, M. S., McKillop, E. R., Connor, T. J., McCracken, R. M., Collins, D. S., and Allan, G. M. (1980): Isolation and serological studies with infectious bursal disease viruses from fowl, turkeys, and ducks: demonstration of a second serotype. *Avian Pathology*, **9(3**): 395-404.

Mekuriaw, A., Bitew, M., Gelaye, E., Mamo, B. and Ayelet, G. (2017): Infectious bursal disease: outbreak investigation, molecular characterization, and vaccine immunogenicity trial in Ethiopia. *Tropical Animal Health and Production*, **49**(**6**): 1295-1302.

Moges, F., Mellesse, A., and Dessie, T. (2010): Assessment of village chicken production system and evaluation of the productive and reproductive performance of local chicken ecotype in Bure district, North West Ethiopia. *African Journal of Agricultural Research*, **5(13)**: 1739-1748.

Moody, A., Sellers, S. and Bumstead, N. (2000): Measuring infectious bursal disease virus RNA in blood by multiplex real-time quantitative RT-PCR. *Journal of virological methods*, **85(1)**: 55-64.

Moraes, H. L. D. S., Salle, C. T. P., Nascimento, V. P., Salle, F. O., Rocha, A. C. G. P., Souza, G. F. and Artencio, J. O. (2005): Infectious bursal disease: evaluation of maternal immunity and protection by vaccination of one-day-old chicks against challenge with a very virulent virus isolate. *Brazilian Journal of Poultry Science*, **7(1)**: 51-57.

Muller, H., Islam, M. R. and Raue, R. (2003): Research on infectious bursal disease

the past, the present and the future. *Veterinary microbiology*, **97(1-2)**: 153-165.

Mulugeta, A., and Tebkew, A. (2013): Evaluation of indigenous chicken productivity by using a questioner survey, in selected Chagni town, Awi-administrative zone, Amhara Region, Ethiopia. *World Journal of Agricultural Sciences*, **1(1)**: 026-035.

Mumford, J. A. (2007): Vaccines and viral antigenic diversity. *Revue scientifique et technique (International Office of Epizootics)*, **26(1)**: 69-90.

Natnael, T. (2015): Pathological and Seroprevalence Studies on Infectious Bursal Disease in Chickens in and around Bahir Dar, North West, Ethiopia. An MSc Thesis presented to Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Pathology and Parasitology. Debrezeit, Ethiopia.

Ndanyi, M.R. (2004). A study to determine causes of mortality and the effect of infectious bursal disease (IBD) vaccination in village chickens in Taita Taveta district of Kenya. MSc. Thesis, Department of Veterinary Microbiology and Network of Smallholder poultry Development, the Royal Veterinary and Agriculture University, Frederiksberg, Denmark.

Office of international des epizootics. (2004): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. *Office international des epizootics*.

Office international des epizootics (2016): Infectious bursal disease (Gumboro disease); Version adopted by the World Assembly of Delegates of the OIE.

Orakpoghenor, O., Oladele, S. B., and Abdu, P. A. (2020): Infectious bursal disease: Transmission, pathogenesis, pathology, and control an overview. *World's Poultry Science Journal*, **76(2)**: 292-303.

Pound, B., and Jonfa, E. (2006): Cattle in Southern Ethiopia: Participatory studies in Wolaita and Konso woredas. *Report from the FARM-Africa*.

Rani, S., and Kumar, S. (2015): Evaluation of infectious bursal disease virus stability at different conditions of temperature and pH. *Biologicals*, **43(6)**: 515-518.

Sali, K. (2019): Overview of Methods Used in the Diagnosis of Infectious Bursal Disease. *Veterinary Medicine Open Journal*, **4(1)**: 9-17.

Sharma, J. M., Kim, I. J., Rautenschlein, S. and Yeh, H. Y. (2000): Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Developmental and Comparative Immunology*, **24(2)**: 223-235.

Sharma, R. N., Tiwari, K. P., Chikweto, A., Thomas, D., DeAllie, C., Stratton, G., and Bhaiyat, M. I. (2014): Seroprevalence of five important viral diseases in commercial chickens in Grenada, West Indies. *International Journal of Poultry Science*, **13(5)**: 299.

Shegu, D., Sori, T., Tesfaye, A., Belay, A., Mohammed, H., Degefa, T., .and Gelaye, E. (2020): Sequence-based comparison of field and vaccine strains of infectious bursal disease virus in Ethiopia reveals an amino acid mismatch in the immunodominant VP2 protein. *Archives of virology*, **165(6)**: 1367-1375.

Singh, K. C. P., and Dhawedkar, R. G. (1992): Prevalence of subclinical infectious bursal disease and its significance in India. *Tropical animal health and production*, **24(4)**: 204-206.

Statts, L. E. (2020): *Characterization of antigenic variants of infectious bursal disease virus* (Doctoral dissertation, University of Delaware). https://udspace.udel.edu/handle/19716/28321

Sule, A. G., Umoh, J. U., Abdu, P. A., Ajogi, J., Jibrin, U. M., and Gidado, A. S. (2013): A serological survey for infectious bursal disease virus antibodies among village chickens in Yobe State Nigeria. *International Journal of Agricultural Sciences*, **3(7)**: 596.

Tadesse, B., and Jenbere, S. (2014): Sero-prevalence of infectious bursal disease in backyard chickens at selected woredas of Eastern Ethiopia. *Journal of Biology, Agriculture and Healthcare www. iiste. org. ISSN*, **4(17)**: 2224-3208.

Tesfaye, R. (2008): Seroprevalence of infectious bursal disease in nonvaccinated backyard local chickens of East Showa zone, Akaki, Debrezeit, and Adama. *DVM Thesis, FVM, AAU, Ethiopia*.

Thrusfield, M. (2005): Veterinary Epidemiology 3rd ed. Black well Science Ltd, London, pp.178 - 236.

Teshome, M., Fentahunand, T. and Admassu, B. (2015): Infectious bursal disease (Gumboro disease) in Chickens. *British Journal of Poultry Sciences*, **4(1)**: 22-

28.

Tomas, G., Marandino, A., Courtillon, C., Amelot, M., Keita, A., Pikula, A. and Domańska-Blicharz, K. (2019): Antigenicity, pathogenicity and immunosuppressive effect caused by a South American isolate of infectious bursal disease virus belonging to the "distinct" genetic lineage. *Avian Pathology*, **48(3)**: 245-254

Van den Berg, T. P., Gonze, M., and Meulemans, G. (1991): Acute infectious bursal disease in poultry: isolation and characterization of a highly virulent strain. *Avian Pathology*, **20(1)**: 133-143.

Van den Berg, T. P., Eterradossi, N., Toquin, D. and Meulemans, G. (2000): Infectious bursal disease (Gumboro disease). *Revue scientifique et technique (International Office of Epizootics)*, **19(2)**: 509-543.

Van Loon, A. A. W. M., De Haas, N., Zeyda, I. and Mundt, E. (2002): Alteration of amino acids in VP2 of very virulent infectious bursal disease virus results in tissue culture adaptation and attenuation in chickens. *Journal of General Virology*, **83(1)**: 121-129.

Wakjira, K. (2018): Vero cell adaptation, immunogenicity and efficacy test of vaccine strain (LC-75) infectious bursal disease virus for Vero cell-based vaccine development in Ethiopia, MSc Thesis, Addis Abeba University, Faculty of veterinary medicine, Debrezeit, Ethiopia.

Wang, Y. S., Wang, Z. C., Tang, Y. D., Shi, Z. L., He, K. W., Li, Y. and Lu, C. P. (2007): Comparison of four infectious bursal disease viruses isolated from different birds species. *Archives of virology*, **152(10)**: 1787-1797.

Woldemariam, S. and Wossene, A. (2007): Infectious bursal disease (Gumboro Disease): case report at Andasa poultry farm, Amhara region. *Ethiopian Veterinary Journal*, **11(1)**: 151-155.

WZFEDD. (2005): Zonal basic socioeconomic and demographic information, Wolaita Zone Finance and Economic Development Department (WZFEDD).

Zegeye, S., Tsegaye, Y., Abreha, H., and Awol, N. (2015): Sero-prevalence of infectious bursal disease in backyard chickens around Mekelle, Northern Ethiopia. *African Journal of Biotechnology*, **14(5)**: 434-437.

Zeleke, A., Gelaye, E., Sori, T., Ayelet, G., Sirak, A., and Zekarias, B. (2005): Investigation on infectious bursal disease outbreak in Debre Zeit, Ethiopia. *International Journal of poultry science*, **4(7)**: 504-506.

Zeryehun, T. and Fekadu, G. (2012): Seroprevalence of infectious bursal disease in chickens managed under backyard production system in Central Oromia, Ethiopia. *African Journal of Microbiology Research*, **6(38)**: 6736-6741.

8. APPENDICES

Appendix 1. Serum Sample Collection Sheet For serosurvey

Sample code	Districts	Age	Breed	Previous outbreak	Vaccination status	Sex	Production system	No of flocks
1								
2								
3								

Appendix 2. Plat layout of I-ELISA Test

OD	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	5										
B	NC	6										
C	PC	7										
D	PC	8										
E	1	9										
F	2	10										
G	3	11										
H	4	12										

Appendix 3. **I-**ELISA testing procedure

Allow all reagents to come to room temperature before use.

1. The negative and positive controls are supplied ready to use. Do not add dilution buffer to the control wells A1, B1, C1, and D1. Controls are to be tested un-diluted. Add:
    - 5 µl of each sample to be tested
    - 245 µl of Dilution Buffer 14 to all wells except to control wells
2. In the ELISA microplate add:
    - 100 µl of the negative control to well A1 and B1
    - 100 µl of the positive control to well C1 and D1
    - 90 µl of Dilution Buffer 14 to as many as wells as there are samples to be tested(not to control wells)
    - 10 µl of pre-diluted samples as prepared above
3. Cover the plate and incubate 30 Minutes ±3 min at 21 °C
4. Prepare the conjugate 1x by diluting the concentrate conjugate 10x to 1:10 on Dilution Buffer 3.
5. Empty the wells. Wash each well 3 times with approximately 300 µl of the washing solution 1x. Avoid drying of the wells between washes.
6. Add 100 µl of the conjugate 1x to each well.
7. Cover the plate and incubate 1x to each well
8. Empty the wells. Wash each well 3 times with approximately 300 µl of the washing solution 1x. Avoid drying of the wells between washes.
9. Add 100 µl of substrate solution to each well.
10. Cover the plate and incubate 15 min ±2 min at 21 °C (±5°C) in the dark.
11. Add 100 µl of stop solution to each well to stop the reaction.
12. Read and record the OD.at 450 nm.

Appendix 4. Questionnaire Sheet Format

Informants consent for the participation in the study

I-- (name of informant) hereby give my full consent and consciousness to participate in this study and declare that to the best of my knowledge the information that I have provided is true, accurate, and complete.

Date-------------------------------- (Signature/Thump impression of informant) ------------------

Part I Informants' details

1. 1. Name--

2. Sex A) Male ☐ B) Female ☐

3. Age A) 18-25 ☐ B) 26-33 ☐ C) 34-41☐ D) 42-49 ☐ E) 50-57☐ F) 58 &above ☐

4. Educational status A) Illiterate ☐ B) primary ☐ C) secondary ☐ D) diploma and above ☐

5. Marital status A) Single ☐ B) Married ☐ C) Divorced/separated ☐

6. Location/Residents--

7. Position related to poultry production. A) Farm attendant ☐ B) Farm owner ☐C) field veterinarians ☐ D) Para veterinary staffs ☐

8. Experience in poultry production? A high experience☐ B) Medium experience☐ C) low experience ☐ D) No experience ☐

Part II Data on the epidemiological investigation of IBD virus

1. Type of birds A) Broiler ☐ B) Layer ☐ C) Both ☐

2. Flock size (no of birds in the home)? A) <5 ☐ B) $\geq$5 ☐

3. Purpose of poultry production A) Primary income source ☐B) Secondary income source ☐ C) Home consumption ☐

4. Replacement of flock A) local market ☐ B) commercial farms ☐C) Hatching at home ☐ D) Local and commercial ▫ E) local and hatching ☐ F) All☐

5. Housing systems A) Separate Chicken house ☐ B) Dwell at home ☐ C)

cage system

6. Cleaning poultry house A) Yes ☐ B) No ☐

7. Frequency of cleaning A) Daily ☐ B) Weekly ☐ C) Monthly ☐

8. Disposal of waste A) Burnt ☐ B) burial ☐ C) Thrown away ☐

9. Feeding system A) Scavenging ☐ B) Providing supplementary feeds ☐ C) Both ☐

10. Supplementary feed A) Yes ☐ B) No ☐

11. Water source for the chickens A) Tap ☐ B) bore whole ☐ C) Hand-dug well☐ D) river ☐

12. Death of chickens in your flock A) Yes ☐ B) No ☐

13. Reason of death A) Predators ☐ B) Disease ☐ C) Accident ☐ D) All ☐

14. Common clinical signs observed A) Diarrhea ☐B) Coughing ☐ C) Twisted neck ☐ C) ruffled feather☐ D) Soiled Vent feather ☐ E) Swollen and blood stained Vent ☐ F) Others__________

15. Treatment of sick chickens A) Yes ☐ B) No ☐

16. Isolation of sick chickens A) Yes ☐ B) No ☐

17. Disposal of dead chickens A) Giving to carnivores' ☐ B) Throwing environment ☐ C) Burn and burry ☐ D) Others___________

18. Information about IBDV A) Yes ☐ B) No ☐

19. Age groups of affected birds? A) < 3 weeks ☐ B) between 3 and 6 weeks ☐ C) > 6 weeks ☐ D) other specify____________

19. Previous outbreak of IBD in the area A) Yes ☐ B) No ☐

20. Season of IBD occurrence A) out man ☐ B) spring ☐ C) winter ☐D) summer ☐

21. Age groups of affected birds A) ≤ 3 ☐ B) 3 – 6 ☐ C) ≥ 6 ☐ D) All ☐

22. Mention clinical signs of IBD A) white water diarrhea ☐ B) ruffled feather ☐ C) Swollen and blood stained vent ☐ D) All ☐ E) A & B F) Other__________

23. Do you know the source of infection A) Yes ☐ B) No ☐

24. Do you know the source of infection A) Yes ☐ B) No ☐

25. Contact with wild birds A) Yes ☐ B) No ☐

26. Contact with neighbor chickens A) Yes ☐ B) No ☐

27. Vaccination history A) Yes☐ B) No ☐

28. Major challenges of poultry production A) Disease and mortality ☐ B) predators ☐ C) Housing and feeding problem ☐ D) A & B ☐E) All ☐ F) others____________

29 Any other control measure undertaken in the home? _____________

Appendix 5. Ethical clearance