Thesis Ref No_____

COMPARATIVE STUDY ON THE ANTIBODY RESPONSE OF INFECTIOUS BURSAL DISEASE IMMUNE-COMPLEX AND LIVE VACCINES AND SURVEY ON OVERALL DISEASE STATUS PROGRESS AND VACCINE MANAGEMENT IN COMMERCIAL POULTRY FARMS IN BISHOFTU TOWN, CENTRAL ETHIOPIA

MSc THESIS

BY:

NANOSHE TAYE JIMA

ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC HEALTH

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BISHOFTU, ETHIOPIA

COMPARATIVE STUDY ON THE ANTIBODY RESPONSE OF INFECTIOUS BURSAL DISEASE IMMUNE-COMPLEX AND LIVE VACCINES AND SURVEY ON OVERALL DISEASE STATUS PROGRESS AND VACCINE MANAGEMENT IN COMMERCIAL POULTRY FARMS IN BISHOFTU TOWN, CENTRAL ETHIOPIA

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

By Nanoshe Taye Jima

JULY 2024

BISHOFTU, ETHIOPIA

Signature Sheet

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public Health

As MSc research advisors, we here by certify that we have read and evaluated this Thesis prepared under our guidance by Nanoshe Taye. , The thesis is entitled: "**COMPARATIVE STUDY ON THE ANTIBODY RESPONSE OF INFECTIOUS BURSAL DISEASE IMMUNE-COMPLEX AND LIVE VACCINES AND SURVEY ON OVERALL DISEASE STATUS PROGRESS AND VACCINE MANAGEMENT IN COMMERCIAL POULTRY FARMS IN BISHOFTU TOWN, CENTRAL ETHIOPIA**"; we recommend that it be accepted as fulfilling the thesis requirement for the Degree of Master of Veterinary Science in Veterinary Microbiology.

Submitted by: Nanoshe Taye _____ _____

Name of student Signature Date

Approved for submittal to MSc research Thesis assessment committee:

Dr. Debebe Ashenafi _____ _____

Dr. Bedaso Mammo _____ _____

Prof. Dr. In. Jan Paeshuyse _____ _____

Approval sheet

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology Immunology and Veterinary Public Health

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by Nanoshe Taye Jima. The thesis is entitled: "Comparative study on the antibody response of infectious bursal disease immune-complex and live vaccines and survey on overall disease status progress and vaccine management in commercial poultry farms in Bishoftu Town, Central Ethiopia" and recommended that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Veterinary Microbiology.

	Signature	**Date**
Chairperson:		
Mr. Abebe Wirtu	_____	_____
External examiner:		
Dr. Getachew Abichu	_____	_____
Internal examiner		
Dr. Eyopb Hipha :	_____	_____
Advisor:		
Dr. Debebe Ashenafi Bekele	_____	_____
Co-Advisors:		
Dr. Bedaso Mammo	_____	_____
Prof. Dr. En. Jan Paeshuyse	_____	_____
Department Head		
Prof. Bekele Megersa	_____	_____

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STATEMENT OF THE AUTHOR

I hereby affirm that this thesis represents my original work, and I acknowledge all sources of material used in its creation. This thesis is being submitted as part of the requirements for a postgraduate (MSc) degree at Addis Ababa University College of Veterinary Medicine and Agriculture. It will be archived in the University/College Library for borrowing under library regulations. I solemnly declare that this thesis has not been submitted to any other academic institution to obtain any degree, diploma, or certificate. Brief quotations from this thesis are permissible without special authorization, provided proper acknowledgment of the sources is included.

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LIST OF ABBREVIATIONS

AGID	Agar Gel Immunodiffusion
BALT	Bronchus Associated Lymphoid Tissue
BF	Bursa of Fabricious
Bp	Base pair
CALT	Conjunctiva Associated Lymphoid Tissue
CAM	Chorio Allontioc Membrane
CD	Cluster of Differentiation
CMI	Cell Mediated Immunity
DsRNA	Double-stranded RNA
I-ELISA	Indirect Enzyme Linked Immunosorbent Assay
GALT	Gut Associated Lymphoid Tissue
HVR	Hypervariable Region
HVT	Turkey Herpes Virus
IBD	Infectious Bursal Disease
IBDV	Infectious Bursal Disease Virus
ICX	Immune Complex
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
MDA	Maternal Derived Antibody
MHC	Major Histocompatibility Complex
NF-Kb	Nuclear factor kappa B
NVI	National Veterinary Institute
ORF	Open Reading Frame
PAMP	Pathogen-Associated Molecular Patterns
PBMC	Peripheral Blood Mononuclear Cell
PM	Post Mortem
RT-PCR	Reverse Transcriptase Polymeraase Chain Reaction

SPF	Specific Pathogen Free
TNF	Tumor Necrosis Factor
VP2	Viral Protein 2
vvIBDV	very virulent Infectious Bursal Disease Virus

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ABSTRACT

The poultry production sector is vital for providing sustenance and enhancing food security in developing nations such as Ethiopia. However, this sector faces significant challenges, including infectious bursal disease (IBD). Evaluating the effectiveness of different vaccines for IBD is crucial for improving vaccination protocols and disease management. This study, conducted from November 2023 to May 2024, involved a controlled experimental study on 100 day-old chickens and a questionnaire survey of 93 poultry farms in Bishoftu town. The objectives were to gain insights into disease and vaccine management and to compare the immune response of chickens to various vaccines. The experimental study utilized a stratified randomized controlled trial with four groups of 25 chickens each. Group 1 received the CEVAC® TRANSMUNE vaccine, Group 2 received CEVAC® GUMBO L, Group 3 received IBD VIRUS LC – 75, and Group 4 served as a control. Vaccines were administered according to the manufacturer's recommendations. Blood samples (2 ml per chick) were collected on days 7, 14, 21, 28, 35, and 42 post-vaccination. Serum samples were analyzed for antibody response using a commercial indirect ELISA kit, and data were processed with STATA software. Survey results indicated that most respondents (64.52%) had 1 to 5 years of experience in the sector, and 50.54% were familiar with IBD and its impact. Key factors in vaccine selection included efficacy (45.16%), availability (19.35%), and cost (18.28%). Antibody titers produced by the vaccines varied: Trans-immune showed mean titers of 1298.814, 183.868, 38.864, 6280.42, 4424.76, and 6129.617 on days 7, 14, 21, 28, 35, and 42, respectively; Gumbo L showed titers of 21.46286, 2581.282, 3363.248, 3156.71, 3400.04, and 5973.526; and IBD LC-75 showed titers of 22.916, 642.136, 5270.4, 5653.071, 5793.221, and 6520.836 on the same days. All vaccines induced protective antibody titers, despite differences in the timing of responses. Continuous and updated surveillance on vaccine types and management practices is essential for effective disease control. Evaluating the immune response to vaccines in the presence of the challenge virus is also critical

Keywords: *Chickens, Efficacy, ELISA, IBD, Questionnaire survey, Vaccine*

1. INTRODUCTION

As a subsector of livestock production, poultry production has been observed to occupy an essential place within the agricultural activities in many developing countries, especially in Africa. This sector is growing very fast because it holds unique opportunities that help to improve local socio-economic environment (FAO, 2019). The primary reason for this phenomenon is the quick yield return of these animals, which requires minimal investment, a short generation interval, a small land requirement, and a fast reproduction cycle when compared to the majority of other livestock species (Korver, 2023). It serves as a method to provide sustenance and enhance food security, aiding in the reduction of poverty and malnutrition in developing nations such as Ethiopia (Tirfie, 2021). Ethiopia being home to about 57.01 million poultry (CSA, 2021), there is a low economic returns on poultry production and this scenario is linked to a number of issues, including, inadequate management, low genetic potential in the breeds that limit chicken output and different diseases like infectious bursal disease outbreaks (Terefe, 2018).

Infectious bursal disease (IBD), also referred to as Gumboro disease is an immunosuppressive condition that affects young chicks between the ages of 3 and 6 weeks and can result in either a clinical infection or a subclinical infection (Muller *et al*., 1979). In Gumboro, Delaware, the first incidence of IBD was documented in 1962 (Cosgrove, 1962). IBDV is a double-stranded non-enveloped RNA virus that belongs to the Birnaviridae family and the Avibirnavirus genus (Mahgoub, 2012).

As a preventive measure, the most widely used conventional vaccinations around the world are killed vaccines and attenuated live vaccines with classic or variant viral strains (Sze *et al*., 2016). With the advancement of technology, next-generation vaccines have been developed with the advantage of overcoming maternally derived antibodies (MDAbs) and are commercially available in the market such as the IBD vector vaccine using turkey herpes virus (HVT) as a vector for the IBDV viral protein 2 (VP2) gene (Bublot *et al*., 2007), and the Immune-complex vaccine that is a mixture of the intermediate plus strain with antibodies, which is taken up by macrophages till the MDAbs have been dropped (Prandini *et al*., 2016).

In 2002, 20 to 45-day-old broiler and layer chickens from commercial farms were the subject of the first IBD report in Ethiopia (Zeleke *et al*., 2005). Two IBDV genogroups were circulating in Ethiopia. A recent genogrouping investigation from Ethiopia revealed highly virulent strains in a new genogrouping three. The investigation supported the findings of recognized genogroups with Ethiopian isolates and the necessity of the evaluating the efficacy of the current vaccines (Bari, 2021).

The current food initiative called "Bounty to Basket (Ye Lemat Tirufat)" implemented by the Ethiopian government aims to increase productivity in food production, improve nutritional situation, and develop Ethiopia's agricultural and poultry industry (Dessie *et al*., 2023). Thus, the constantly occurring diseases in poultry comprise the sustainability of Ethiopia's poultry production (Asfaw *et al*., 2021). Highlighted with considerable economic implications associated with IBD, there is a need to develop and evaluate improved IBD vaccines in efforts to check the impact of these pathogens that poses significant threat to expanding poultry industry (Mazengia, 2012). Routine surveys enables tracking of changes in perception over time thus aids in identifying cultural shifts that may likely affect efforts towards disease control (Collett *et al*. , 2020).

Ethiopian commercial poultry farms have enhanced their biosecurity and make routine vaccinations for their chickens (Shegu *et al*., 2020). Hatchery vaccination is gradually assuming popularity in light of the rising prominence of poultry farming and the need to increase competency of vaccine delivery through more efficient routes such as subcutaneous injection at one-day-of-age or in ovo vaccination (Isihak *et al*., 2021). It is crucial to track the antibody response after vaccination as maternal antibodies give immunity for a certain duration (Cazaban *et al*., 2018). IBD vaccines are currently employed extensively in Ethiopian attempts to control the disease. Nonetheless, a number of post-vaccination outbreaks have been reported. Despite the identification of highly virulent strains (Bari, 2021), there is limited research on the effectiveness of existing vaccines against these strains. One locally produced and several imported vaccines are used in Ethiopia. And comparing different types of vaccines available for Gumboro disease is crucial. Because, knowledge on the effectiveness of various types of vaccines is essential for improving vaccination protocols and disease management in poultry farming (Ravikumar *et al*., 2022). Although choosing the right vaccine to immunize against this viral infection is of utmost importance, there is limited published works, which directly addresses the comparison of different

Gumboro disease vaccines available in Ethiopia. And, there is no study done in Ethiopia that has compared the immune response of live vaccines with immune complex. Therefore, this study was conducted with the following general and specific objectives:-

General objective

- Assessing public awareness and perceptions of Gumboro disease and its vaccine, and comparing the immune responses of chickens to different Gumboro vaccines

Specific objectives

- ✓ Surveying Gumboro disease management (vaccines and related)
- ✓ Comparing immune response of chickens towards three commonly used Gumboro vaccines in poultry farms
- ✓ Evaluating efficacy of live and immune complex Gumboro vaccine

2. LITERATURE REVIEW

2.1. Etiology

Infectious bursal disease (IBD), which is induced by the Infectious bursal disease virus (IBDV), is characterized as an acute and highly transmittable infectious disease. The etiological factor primarily targets the bursa of Fabricius, where B lymphocyte maturation and differentiation occur, leading to B lymphocyte necrosis and disintegration (Ingaro *et al*., 2013), thereby causing profound immunosuppression and heightening the vulnerability to other pathogens in chickens aged 3–6 weeks (He *et al*., 2019).

Infectious bursal disease virus is a double-stranded RNA virus belonging to the genus Avibirnavirus of the family Birnaviridae. The genome of the IBDV is bi-segmented and divided into segments A and B (Hon *et al*., 2008). IBDV multiplies in growing B cells, in the bursa of Fabricious (Reddy *et al*., 2023). The definitive division of VP2 found in IBDV plays a crucial role in the replication mechanism of the virus by harboring significant immune dominant epitopes and triggering the generation of neutralizing antibody (Vakharia *et al*., 1994).

Serotypes 1 and 2 have been identified as two serotypes based on virus neutralization studies. Only serotype 1, as being harmful to chickens, despite the fact that both serotypes can naturally infect chickens, turkeys, ducks, guinea fowls, and ostriches (Dey *et al*., 2019). Infectious Bursal Disease virus have historically been divided into four phenotypes based on their pathogenicity and antigenicity traits: classic, variant, very virulent, and attenuated (van den Berg *et al*., 2004). Despite the existence of high levels of maternal antibodies to the classic strains of IBDV, variant and vvIBDV strains have been isolated from disease outbreaks (Jenberie *et al*., 2014).

2.2 IBDV genome structure and characteristic

IBDV particles have a non-enveloped, icosahedral capsid with a diameter of about 60-75nm (Böttcher *et al*., 1997). The larger segment A (approximately 3400 base pairs (bp) contains two open reading frames (ORF). The larger ORF of segment A is monocistronic and encodes a polyprotein that is auto-processed after several steps into mature VP2 (48 kDa), VP3 (33–35 kDa),

and VP4 (24 kDa) (M¨uller & Becht, 1982; Azad *et al*., 1985, Azad *et al*., 1987; Hudson *et al*., 1986; Kibenge *et al*., 1997). Segment A can also encode VP5, a short 17kDa protein, from a short, partially overlapping ORF (Mundt *et al*., 1995). The smaller genome segment B (approximately 2800bp) encodes VP1, the viral RNA polymerase of 90kDa (Figure 1) (M¨uller & Nitschke, 1987; Spies *et al*., 1987). The IBDV VP2 hypervariable region (HVR) is commonly used to differentiate IBDV strains (Jackwood, 2004).

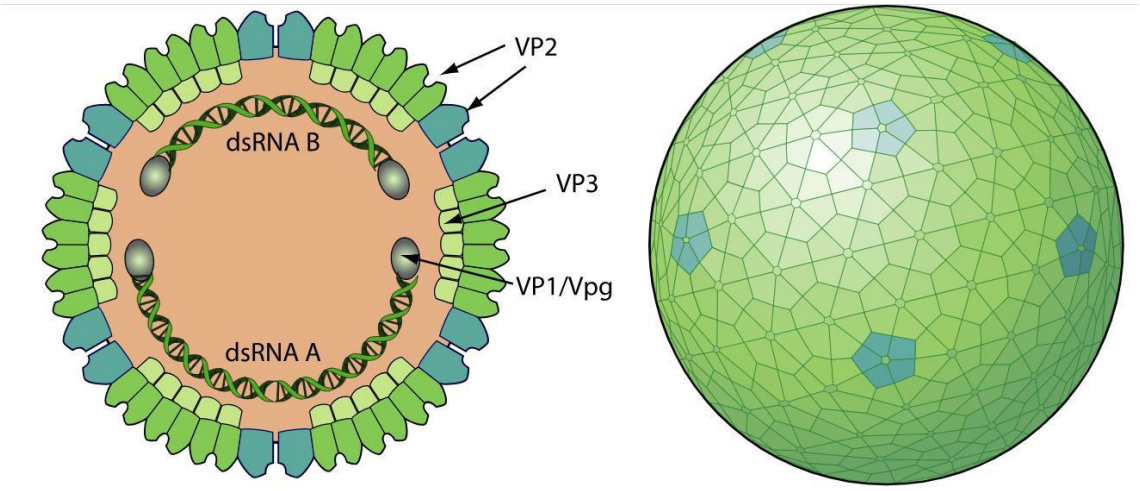


Figure 1 *IBDV genome organization*

Non enveloped, single-shelled T=13 icosahedral symmetry capsid of about 70 nm in diameter, composed of 260 trimers of VP2 that form spikes projecting radially from the capsid. The peptides derived from pre-VP2 C-terminal cleavages remain associated within virion. VP3 forms a ribonucleoprotein complex with the genomic RNA. Minor amounts of VP1 are also incorporated in the virion.

Source: Viral Zone, (2015)

The major capsid protein VP2 plays a pivotal role in antigenic variation by which the virus can escape neutralizing antibodies. Most of the amino acid (aa) changes among the antigenically different IBDVs are clustered in the hypervariable region of VP2 (hVP2). Thus, this hypervariable region of VP2 is the obvious target for IBDV detection, evolution, and pathogenic variation. The hVP2 region has two major hydrophilic domains, namely major hydrophilic peak A (aa 212–224) and peak B (aa 314–325) that form hairpin loops PBC (aa 219–224) and PHI (aa 316–324),

respectively. The minor hydrophilic peak 1 (aa 248–254) and peak 2 (aa 279–290) of hVP2 form loops PDE (aa 249–254) and PFG (aa 279–284), respectively. Either single or combined mutations in the hVP2 region affect the virulence pattern of the virus. (Coulibaly *et al*., 2010).

IBDV is naturally susceptible to varying degrees of genomic mutation or recombination because of its distinctive bi-segmented double-stranded RNA genome and the high error rate of viral RNA-dependent RNA polymerase (RdRp) (Von Einem *et al*., 2004). This predisposition causes the emergence and spread of new mutant or recombinant strains in chickens (Wang *et al*., 2022).

Although serotype 1 has two main antigenic groups known as classical and variant, antigenic drift has also played a role in the emergence of several subtypes within these groups. The hypervariable sequence area of VP2 (hvVP2), specifically the amino acids at the top of loop structures with the designations PBC, PDE, PFG, and PHI, determines the antigenic phenotype of IBDV (Coulibaly *et al*., 2010). It has been discovered that even single-point mutations in these areas might cause antigenic drift in IBDV (Letzel *et al*., 2007), which might make the available IBD vaccines useless. Despite its close antigenic relation to serotype I classical strains, vvIBDV has the ability to break through high antibody titres induced by IBD vaccines (Van den Berg *et al*., 1991).

2.3 Epidemiology of IBD

2.3.1 Host Range

IBDV is known to exhibit host-specificity, particularly towards chickens and turkeys, which serve as the disease primary hosts. Moreover, instances of IBDV infection have also been documented in free-living wild birds such as ostriches, pigeons, Baltic ducks, Herring gulls, and sparrows (Müller *et al*., 1979). IBDV infection has similarly been identified in canine excrement two days following oral administration, indicating the potential of dogs to serve as carriers of the virus. (Jackwood and Sommer-Wagner, 2011). Furthermore, instances of avian species harboring IBDV encompass village weavers (Ploceus cucullatus), speckled pigeons (C. guinea), as well as a variety of raptors and passerines in Japan (Nandhakumar *et al*., 2020).

2.3.2 Transmission

Chickens infected with IBDV release the virus in their feces for at least 14 days (Mazengia, 2012; Rashid *et al*., 2013). Contamination of a breeding location by the IBDV facilitates substantial horizontal transmission among groups of poultry through the consumption of feed and water that have been tainted by virus-infected feces. There has been no reports of vertical transmission of IBDV (Alkie and Rautenschlein, 2016), however, IBDV can persist on the surface of eggshells, acting as a source of surface contamination (McLachlan and Dubovi, 2001). In wild birds living freely, indirect transmission of IBDV is probable, often through scavenging carcasses of infected chickens, ingestion of polluted water, or exposure of respiratory and conjunctival membranes to contaminated poultry dust (Gilchrist, 2005).

2.3.3 Disease distribution

The initial documentation of Infectious bursal disease, a specific ailment impacting the Bursa of Fabricius in chickens, was first identified. The initial instances were noted in the locality of Gumboro, situated in Delaware within the Bunting farm of the United States, hence giving rise to the disease's nomenclature (Cosgrove, 1962). Infectious Bursa Disease occurs in every country and varies in prevalence from 8 to 100%. Even with strict vaccination, IBD outbreaks happen often. vvIBDV first surfaced in Europe at the end of the 1980s, and then it spread to South America, Asia, and the Middle East (Eregae *et al*., 2014; Zachar *et al*., 2016; Khan *et al*., 2017; Moryani *et al*., 2020; Sajid *et al*., 2021; Kapoor *et al*., 2021; Omer and Khalafalla, 2022; Parveen *et al*., 2022; Pikuła *et al*., 2023; Hishamund *et al*., 2023). IBD was initially identified in Beijing and Guangdong in 1979, and it quickly expanded throughout the nation's major poultry regions (Deshan and Zhiqiang, 1991; Zhang *et al*., 2022). The disease has been evaluated to possess notable socioeconomic significance on a global scale, as it affects over ninety-five percent of the countries that are part of the international community (Eterradossi, 2000).

2.4 Pathogenesis

After 16 hours of infection, the occurrence of viremia represents a subsequent phase that initiates the manifestation of clinical disease and the demise of affected tissues, or potentially results in the elimination of the virus within the lymphoid follicles located in the bursa of Fabricius, as well as

in circulating B-cells situated in the cecal tonsils, BALT, CALT, and GALT (Trapp and Rautenschlein, 2022). Furthermore, the spread of the virus to various other lymphoid organs including the bone marrow, thymus, spleen, Peyer's patches, and Harderian glands has been documented. IBDV undergoes initial replication in the gut-associated lymphoid tissue (GALT), leading to the onset of primary viremia. Within a short period, the virus localizes in the bursal lymphoid cells (Eterradossi and Saif, 2013). IBDV particles were also detected in intra-bursal T-cells (Mahgoub, 2012). Additionally, Infiltrating T-cells in the bursa show markers of activation such as upregulated IL-2, major histocompatibility complex (MHC) class II molecules, and IFN-γ mRNA expression (Rauf *et al*., 2011).

The virulence factors of the IBDV carry out various functions within host cells to promote viral replication. VP1 functions as the RNA-dependent RNA polymerase of IBDV and is crucial for screening cellular targets that modulate viral replication. The carboxy-terminal region of eukaryotic initiation factor 4AII involved in translation is the initial host factor identified to interact with VP1 (Tacken *et al*., 2004). The capsid protein VP2 serves as an inducer of apoptosis, virus protease VP4 serves as type I interferon expressions inhibitor by binding to GILZ. On the contrary, VP5 prevents apoptosis in host cells by interacting with the p85α subunit of PI3K soon after IBDV infection, thus creating a conducive environment for viral replication. Moreover, VP3 hinders the recognition of IBDVs double-stranded RNA by MDA5, aiding the virus in evading the hosts immune responses against IBDV infection (Qin and Zheng, 2017).

IBDV induces a significant increase in apoptosis in chicken peripheral blood lymphocytes, particularly targeting B-lymphocyte progenitors within the bursa of Fabricius. This apoptotic response is mediated by caspase activation, ultimately resulting in the depletion of B cells and contributing to the pathogenesis of IBDV infection. (Lombardo *et al*., 2000). IBDV also inhibits glycoprotein release, leading to B cell apoptosis (Oláh *et al*., 2022).

2.5 Disease description, clinical signs and pathological lesions

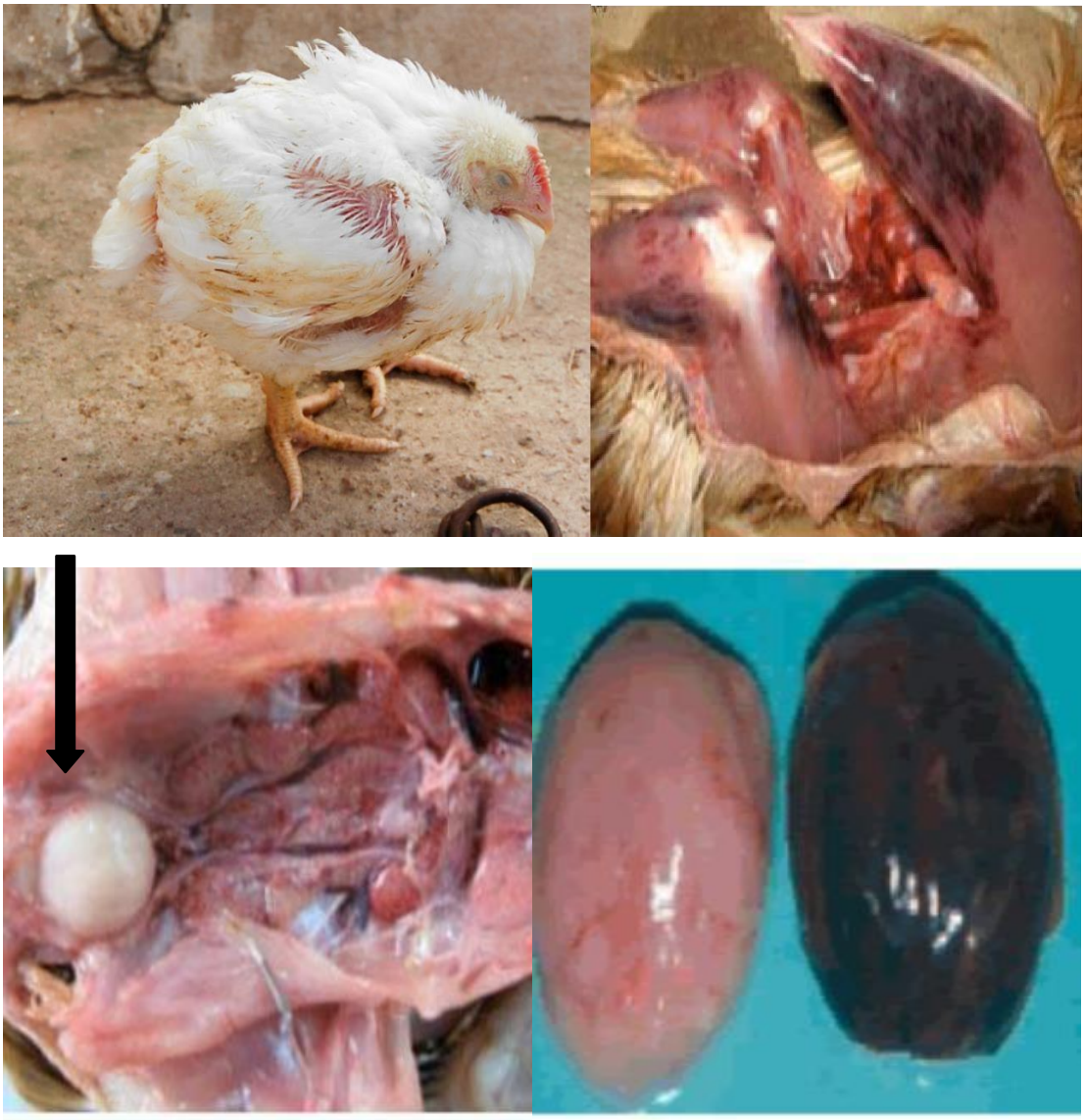
Acute clinical outbreaks of classical IBDV are characterized by sudden onset, high morbidity, spiking mortality curves, and a rapid recovery time of about 5–7 days post-clinical signs (Lukert and Saif, 2003). The incubation period of IBD ranges from 2 to 4 days and the most severe clinical

signs are seen in chickens infected between 3 and 6 weeks of age (Elankumaran *et al*., 2002). The non-specific clinical signs during the acute phase of IBD include distress, ruffled feathers, anorexia, and depression, possibly associated with diarrhea and dehydration (Ingrao *et al*., 2013).

The age, genetic make-up of the chicken, route of infection and nature of the infecting viruses, the presence of maternally derived antibodies, and the vaccination history influence the severity of the infection and virus shedding (Iván *et al*., 2005). IBD severity varies between layer and meat chickens. Recent studies show that vvIBDV strains cause high mortality rates in layer chickens, with over 80% mortality, severe bursal damage, and significant viremia (Li *et al*., 2023). In contrast, meat chickens infected with vvIBDV exhibit lower mortality rates, around 40%-60%, and less severe lesions in the bursa of Fabricius (Adino and Bayu, 2022). Additionally, variant IBDV strains in layer chickens induce severe immune organ damage and persistent infection, emphasizing the need for effective vaccines (Aliyu *et al*., 2022).

Chickens that died of acute IBD exhibit atrophied bursa containing a yellowish fluid, dehydration, and hemorrhage at the junction of proventriculus and gizzard, pectoral and thigh muscles, subcutaneous fascia, and on the serosal surface and plica of the bursa Fabricius are often seen (Mahgoub, 2012; Orakpoghenor *et al*., 2020;), and more or less lesion can also be produced in other organs such the spleen, thymus, and kidneys (Eterradossi and Saif, 2013). The hemorrhages are believed to occur and speculated due to the cytokine storm (Ingrao *et al*., 2013). Weight reduction and enhanced food conversion rate (FCR) have been documented as consequences of immunodeficiency associated with IBDV, leading to vulnerability to subsequent infections (Wagari, 2021). Changes in the B cell genomic methylation and loss of genome integrity during the infection process may contribute to B cell death (Ciccone *et al*., 2017).

A **B**



C **D**

Figure 2*: Clinical sign and different pathological lesions of IBD*

A) Picture showing a depressed chick, B) arrow hemorrhage on the thigh and pectoral muscle,

C) Arrow indicating enlarged Bursa D) serous hemorrhagic to severe hemorrhagic inflammation of Bursa

Source: Musa *et al*., (2012), Lucien Mahin, (2016)

2.6 Host-pathogen interaction in infectious bursal disease

To counteract pathogens, the host gradually adapts to establish an intricate and elaborate antiviral immune reaction (Akira *et al*., 2006). Investigation of the host factors involved in initiating translation by the virus represents a promising initial step in identifying cellular proteins influencing viral replication. The virus exhibits a preference for lymphoid tissue and a specific attraction to immature B lymphocytes in the bursa of Fabricius (BF) for extensive replication (Mwenda *et al*., 2018). The distinct affinity displayed by IBDV towards maturing B cells within the bursa of Fabricius has been extensively recorded, with a significant preference for B cells that express immunoglobulin M (IgM+) (Sharma *et al*., 2000).

2.6.1 Innate immune response

The innate immune system serves as the primary defence mechanism of the host against IBDV (Akira *et al*., 2006). Identification of the invading pathogen by the host represents the initial and most crucial stage in triggering the innate immune response; this process hinges on the recognition of pathogen-associated molecular patterns (PAMPs) by the host's pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), RIG-I-like receptors (RLRs), among others (Yoneyma *et al*., 2004). Concurrently, pathogens have devised various strategies to evade the host's immune response. Accordingly, the persistent battle between the virus and the host continues (Ye *et al*., 2022).

The innate immune response in chickens serves as the first line of defence against pathogens. It includes physical barriers, such as the skin and mucous membranes, as well as cellular and molecular components, such as macrophages and natural killer cells. These elements play a critical role in recognizing and initiating a response to IBDV upon entry into the host. For instance, macrophages can engulf and destroy IBDV-infected cells, while natural killer cells can directly target and eliminate virus-infected cells. This innate immune response is essential in limiting the initial spread of IBDV within the host (Smith *et al*., 2015).

2.6.2 Adaptive immune response

Transfer of maternal antibodies helps to protect the offspring until adaptive immune response becomes fully effective in the newly hatched chick, whereas optimal adaptive immune responses only develop after the first weeks after hatch (Davison *et al*., 2008). According to breed, the half-life of passive antibodies ranges from three days for broilers to five days for laying chickens (Brandt *et al*., 2001). Thus, if the antibody titre of a chick at hatch is known, then the time of maximum flock susceptibility to the wild or vaccinal virus can be determined. This information is very important when establishing the timing of vaccination programs (Van den Berg, 2000).

Cellular immune response

The elevated quantity of T lymphocytes and the synthesis of associated cytokines and chemokines in the bursa of chickens exposed to IBDV indicate that the cell-mediated immune response can also be triggered by IBDV infection (Kim *et al*., 2000). An important role of CMI is suggested by several groups (Yeh *et al*., 2002). Infiltrating T-cells in the bursa show markers of activation such as upregulated IL-2, major histocompatibility complex (MHC) class II molecules, and IFN-γ mRNA expression (Rauf *et al*., 2011).Type I interferons play a crucial role as anti-IBDV cytokines, underscoring the significance of IBDV as a stimulant of type I interferons and its substantial influence on cellular immune reactions (O'Neill *et al*., 2010). IBDV infection triggers the activation of the inflammasome and leads to an increase in the expression of Th1-like or pro-inflammatory cytokines like IL- 12, IFN-γ, IL-1β, IL-6, iNOS, and IL-18 in bursae tissues; however, the precise mechanisms responsible for this phenomenon are yet to be fully understood (Rasoli *et al*., 2015).

Results of investigations on the role of cell-mediated immunity (Yeh *et al*., 2002) and the significance of virus-specific antibodies (Rautenschlein *et al*., 2002) indicate that antibody alone is not adequate in inducing protection against IBDV and that T-cell involvement is critical for protection. The release of these cytokines was suggested to be tightly regulated by NF-κB, whereby its expression was found to be elevated in the bursa during the early phase of IBDV infection (Guo *et al*., 2012). Additionally, the downregulation of CD132+ and CD8+, upregulation

of CD132+ and CD25+ T cells in the bursa, and altered secretion and function of cytokines were also observed in the thymus (Wang *et al*., 2014).

Humoral immune response

Humoral immunity is an essential part of the protection against IBDV. There is a close relationship between titres of neutralizing antibodies and protection. Immunosuppression is accompanied by high levels of anti-IBDV antibodies as a result of mature B-cell stimulation and susceptibility of the immature lymphocytes to the virus (Van den Berg *et al*., 2000). Upon exposure to the virus, antigen-presenting cells such as macrophages and dendritic cells engulf the viral particles. These cells then present viral antigens to B lymphocytes in the bursa of Fabricius. This interaction activates the B cells, leading to their differentiation into plasma cells (Farzi *et al*., 2022). Activated B cells differentiate into plasma cells, which are specialized in producing antibodies. These antibodies are specific to the viral antigens present in Infectious Bursal Disease. The primary antibody produced in response to IBDV is IgM, followed by IgY (Ratcliffe and Härtle, 2022). The antibodies produced by plasma cells play a crucial role in neutralizing the virus and preventing its spread within the chicken's body. They bind to viral particles, marking them for destruction by other components of the immune system (Neurath, 2008).

IBDV infection has been shown to enhance IgA secretion in the bursa, with IgY+ and IgA+ cells potentially playing a role in generating antibodies against IBDV (Shah *et al*., 2021). Following the reduction of immature B cells in the bursa of chickens as a result of IBDV infection, there will be an expansion of mature specific B cells upon exposure to IBDV, triggering a robust humoral immune response in chickens who have recuperated from the acute phase of IBD (Aliyu *et al*., 2022).

2.7 Immunosuppression

If surviving chickens are infected very early in life, they may suffer lifelong immunosuppression due to incomplete recovery of the B cell compartment (Ingrao *et al*., 2013). It has been noted that infection by IBDV leads to a reduction in the functionality of dendritic cells derived from chicken bone marrow, resulting in diminished expression levels of essential co-stimulatory molecules such as CD40 and CD86 (Liang *et al*., 2015).

This extensive reduction in B cells resulting from IBDV-triggered apoptosis serves as the primary factor leading to immune system impairment, a phenomenon partly attributed to the reduced phagocytic capability of monocytes/macrophages (Sharma *et al*., 2000) and the weakened reaction to T cell mitogen activation (Rauw, 2012). Moreover, the virus has the ability to invade dendritic cells derived from the bone marrow of chickens, resulting in diminished expression levels of essential co-stimulatory molecules such as CD40 and CD86 (Liang *et al*., 2015). The recruitment of CD4 and CD8 T lymphocytes also promotes damage in the BF by releasing cytotoxic cytokines, responsible for prolonged immune suppression after IBDV infection (Wang *et al*., 2009). Although B-lymphocyte re-population in the bursa occurs, the chickens display a poor primary antibody response until seven weeks post-infection (Mahgoub, 2012).

2.8 Diagnosis of IBD

The history of the flock, the clinical symptoms, and the postmortem deformities are all taken into consideration while making the diagnosis of infected bursal disease. Naturally, chickens older than 3 weeks exhibit clinical signs of disease, whereas those younger than 3 weeks do not (Kegne and Chanie, 2014). Although postmortem findings in infected chickens and clinical symptoms may aid in disease diagnosis, laboratory confirmation is necessary for a diagnosis to be made (Hasan *et al*., 2010).

Similar to the majority of RNA viruses that adopt the "bite and run" tactic (Vidalain and Tangy, 2010), the acute IBDV infection causing clinical manifestations lasts for only 3 to 4 days, after which the surviving chickens recover quickly. Age, virus strain, maternal antibody titer, vaccine type, and chicken breed are a few factors that affect clinical presentation. After a 2- to 3-day incubation period, infected chickens exhibit symptoms of discomfort, depression, ruffled feathers, anorexia, diarrhea, and a dirty vent (Dey *et al*., 2019). Since the IBD clinical sign is very similar to coccidiosis, muscular hemorrhages and bursa lesion examination should be done properly since it best differentiates from coccidiosis (Damairia *et al*., 2023). In the acute phase of the infection, IBDV could be detected within the first 3 days post-infection in the bursa. Confirmation of clinical disease or detection of subclinical form is best done by immunological assays (Gul *et al*., 2023).

2.8.1 Virus Isolation

During the acute phase of the disease, the bursa is collected from the susceptible chicks and a 20% bursal homogenate in PBS solution is prepared from the pooled bursa. The isolation of IBDV strains is done by infecting the embryonated chicken eggs following inoculation onto the chorio-allantoic membrane (CAM). IBDV isolation in chicken primary bursal cells from the BF has also been reported (Dulwich *et al*., 2018). However, isolation of very virulent form of the virus is not recommended in cell culture. Death due to IBDV infection begins 3 days post-inoculation (dpi) with typical signs of hemorrhages and edema in the embryos. The best method for virus isolation is done by inoculating bursal homogenate to SPF chickens and the virus is then isolated from the bursal tissue 3 dpi. The virus is then titrated and the endpoint titers are calculated based on specific deaths by inoculating 10-day-old chicken embryos through the CAM route (Dey *et al*., 2019).

2.8.2 Molecular Methods

Molecular virological techniques have been developed that allow IBDV to be identified more quickly than by virus isolation. The most frequently used molecular method is the detection of IBDV genome by the reverse-transcription polymerase chain reaction (RT-PCR) (Wu *et al*., 1992; Lin *et al*., 1993;). This method can detect the genome of viruses that do not replicate in cell culture, because it is not necessary to grow the virus before amplification. RT-PCR is performed in three steps: extraction of nucleic acids from the studied sample, reverse transcription (RT) of IBDV RNA into cDNA, and amplification of the resulting cDNA by PCR. The two latter steps require that the user selects oligonucleotide primers that are short sequences complementary to the virus-specific nucleotide sequence. Different areas of the genome will be amplified depending on the location from which the primers have been selected (Nouen *et al*., 2006). Conventional RT-PCR is the "gold standard" method for detecting IBDV; however, one drawback is the requirement for a thermal cycling system as well as a complex technique for identifying the product after amplification (Lee *et al*., 2011).

2.8.3 Serological Diagnosis

Serological methods are commonly used for diagnosing IBD. For serological investigations, usually blood can be collected from the wing vein, allowed to clot and serum separated by

centrifugation and stored at -20 °C until tested (Chowdhury *et al*., 2011). One of the routine techniques is serological evaluation using enzyme-linked immunosorbent assay (ELISA). ELISA is a sensitive and specific test that detects antibodies specific to IBDV in poultry samples. Another serological test used is the agar gel immunodiffusion (AGID) test, which is less sensitive compared to ELISA. These tests help in the detection of antibodies produced by the host in response to the IBDV infection, aiding in the diagnosis of the viral disease in poultry (Martínez *et al*., 2003). Virus neutralization (VN) testing and the agar-gel precipitin (AGP) or antigen capture enzyme- linked immunosorbent assay (AC-ELISA) are the two most often utilized procedures in IBD diagnosis. The most popular method is ELISA because it is rapid, affordable, and adaptable to computer software automation (Minta *et al*., 2006).

2.9 Economic importance of IBD

One significant barrier to the growth of the chicken industry is infectious bursal disease, which causes enormous financial losses. Under natural conditions, IBDV can infect all breeds of chickens, causing huge economic losses to the poultry industry worldwide (Du *et al*., 2023). The most economic devastation associated with IBD is due to its immunosuppressive effect that leads to poor vaccination response, secondary bacterial, viral, protozoan infection and poor performance and poor economic return (Cereno, 2008) and also from the dramatic decrease in overall flock performance. Cellular immunity is also compromised during an IBDV infection (Rautenschlein *et al*., 2002). IBDV-mediated immunosuppression impedes the effectiveness of other vaccination strategies and worsens secondary infections, some of which have zoonotic implications (Reddy *et al*., 2023).

Furthermore, it has been noted that chickens infected with IBDV may be more vulnerable to coccidiosis, gangrenous dermatitis, and salmonellosis (Phillips and Opitz, 1995). The chickens exhibit a weak primary antibody response until seven weeks after infection, despite the fact that B-lymphocyte re-population occurs in the bursa (Mahgoub, 2012). Additionally, the avian influenza virus, subtype H9N2, and Newcastle disease virus are examples of poultry respiratory viruses whose pathogenicity is thought to be exacerbated by IBDV co-infection (Taifebagherlu *et al*., 2022).

2.10 History and current status of IBD in Ethiopia

Infectious bursal disease (IBD) is a globally prevalent condition that impacts all major regions of poultry production, presenting a significant challenge to efficiency and financial gain in the poultry sectors of both developing and developed nations (Sainsbury, 2000). In Ethiopia, there was no recorded occurrence of IBD cases until 2003. In 2005, a case of outbreak in commercial farms with high mortality (22,437 Broiler chickens and 2508 layer chickens in 20-45 day old) was reported to the National Veterinary Institute (NVI). (Zeleke *et al*., 2005) reported the first report of IBD in Ethiopia.

According to studies, infectious bursal disease affects a wide range of production strategies, breeds of chickens, and geographical areas in Ethiopia. The dissemination of better chicken breeds from contaminated breeding and multiplication facilities is linked to the disease's spread among native chicken populations (Mossie *et al*., 2021). The investigation carried out by the AHI into Gumboro disease in various regions, revealed an overall prevalence rate of approximately 77.48% from the analysis of 706 collected samples (Animal health yearbook, 2011).

Table 1: Prevalence of IBD in different areas of Ethiopia

Study area	Prevalence	Authors
Gondar and west Gojjam	73.50%	Kassa and Molla, 2012
Southwest showa of Ethiopia	76.64%	Hailu *et al., 2009*
Mekelle town	45.05%	Zegeye *et al.,* 2015
Bishoftu	82.20%	Tesfaheywet and Getnet, 2012
Andassa poultry farm	98.90%	Solomon and Abebe 2007
Eastern Ethiopia	83%	Tadesse and Jenbere 2014
Bishoftu	80%	Kassa *et al.,* 2019
Adama	66.67%	Kassa *et al.,* 2019
Addis Ababa	66.67%	Kassa *et al.,* 2019

2.11 Prevention and control measures for IBD

The poultry industry has long been very concerned about IBD especially over the last few decades. In fact, its "reemergence" in different or extremely virulent forms has resulted in enormous financial losses. Before 1987, the viral strains were successfully controlled by vaccination, had minimal virulence, and caused less than 2% specific fatality. However, failures to vaccinate were reported in various parts of the world in 1986 and 1987 (Berg, 2000). Exploration of cellular elements and/or mechanisms that disrupt virus replication either directly or indirectly could offer significant insights in formulating innovative strategies for effectively managing IBD (Zhang and Zheng, 2022). The virus is environmentally stable and resistant to many chemical and physical agents. Its spread between flocks can be restricted through implementation of strict biosecurity measures. The use of therapeutic treatment has been reported to have no effect on the course of infection (Eterradossi and Saif, 2013). Vaccination of chickens with inactivated and live attenuated vaccines are commonly-used clinical methods to control IBD (Müller *et al*., 2012). However, the high mutation rate of IBDV is likely to be the reason for the emergence of mutant viral strains whose antigenicity differs from that of the current commercially-available vaccines (Hou *et al*., 2022).

In addition to mutations, gene reassortment and recombination are important genetic factors in IBDV that cannot be ignored. Genome reassortment of different segments in IBDV has been reported in various regions across the globe and the newly emerging variant strains are becoming a major threat to the poultry industry (Legnardi *et al*., 2022). As the IBDV genome has subgroups of segments A and B, the widespread use of live attenuated vaccines has increased the occurrence of reassortment between vvIBDV and attenuated strains. These re- assortment viruses may exhibit virulence comparable to that of classical or attenuated IBDV or may still inherit the high virulence of vvIBDV (Gao *et al*., 2023).

Therefore, a comprehensive understanding of the patterns of genomic variations in IBDV is critical for the prevention and control of IBD. In recent years, reverse genetics has emerged as a useful technic to combat IBDV infection. This approach can make true the precise mutations or substitutions in the IBDV genome from a genetic perspective, and many advances have been made in this aspect to explore pathogenesis and develop novel vaccines (Yang and Ye, 2020).

Advances in technology have enabled the development of new vaccines that are able to escape the neutralizing effect of maternally derived antibodies (MDA) and hence are eligible for hatchery application. Two types of IBD vaccines are currently available for hatchery application, an immune-complex vaccine in which a live attenuated IBD vaccine strain has been in vitro complexed with a high titre polyclonal serum in specific quantities and alternatively, a vaccine using the herpesvirus of turkeys (HVT) vector technology as a carrier expressing IBDVs VP2 antigen (Kelemen *et al*., 2000).

There is empirical support indicating that optimal management protocols, grounded in the "all in/all out" methodology, in conjunction with stringent cleaning and disinfection procedures within poultry housing, have the capacity to regulate viral spread between production cycles. Nevertheless, these strategies are inconclusive in the absence of a selection of appropriate vaccination initiatives tailored to the specific epidemiological circumstances of the given geographical region (Franciosini and Davidson, 2022).

2.11.1 Available vaccines against IBD

As level of protective antibodies decreases, chicks become susceptible to infection with virulent field strains (Vrdoljak *et al*., 2018). It has been shown that determination of the optimal timing for vaccination owing to interference with MDA in broiler progeny is crucial for effective vaccination (Lambrecht *et al*., 2000). The epitopes responsible for the induction of neutralizing and protective antibodies are located on the VP2 protein (Abdel-Alim *et al*., 2001). Interference of maternally derived antibodies with vaccine uptake still remains a major problem in vaccination against IBD using live vaccines (Block *et al*., 2007). In order to help prevent IBD more effectively, new technologies and next-generation vaccines have been developed and introduced into the market (Meeusen *et al*., 2007).

Conventional live and inactivated IBD vaccines

Live viral vaccines mimic infection in the target host. They can replicate and induce both cellular and humoral immunity. They do not require an adjuvant to be effective and are suitable for mass administration to the chicken, but they may also have undesirable side effects. These include but not limited to reversion to virulence and vaccine reactions that may result in disease or production

loss. In general, the live IBD vaccines in use by the poultry industry have been attenuated by serial passage in tissue culture, eggs or embryo-derived tissues, with the aim of maintaining the immune response induced by the parent virus whilst attenuating the ability of the vaccine virus to cause clinical disease or significant immunosuppression (Schijns *et al*., 2014).

Inactivated IBD vaccines are mostly formulated as water-in-oil emulsions, usually combining several antigens. It has been observed that inactivated IBD vaccines were also able to induce IBDV-specific T-cell and inflammatory responses in chickens (Rautenschlein *et al*., 2002). Interference of maternally derived antibodies with vaccine uptake still remains a major problem in vaccination against IBD using live vaccines (Block *et al*., 2007). Depending on the degree of attenuation, live vaccines are classified as mild, intermediated, intermediate plus, and hot IBD vaccines (Müller *et al*., 2003);Whereas mild and intermediate vaccines are less likely to damage the bursa than intermediate plus and hot vaccines, but they are still shortly neutralized by high quantities of maternally derived antibodies (MDAbs) (Sedeik, *et al*., 2019).

Genetically engineered live IBD vaccines

Determining the complete nucleotide sequences of both segments of the IBDV double-stranded RNA genome (Mundt & Mu¨ller, 1995) allowed development of a reverse genetics system (Mundt & Vakharia, 1996). Meanwhile this approach has been widely used with the aim of generating attenuated IBDV potentially applicable as vaccine. Among others, attenuated mutant IBDV was generated from vvIBDV by site-directed mutagenesis of nucleotide sequences encoding specific amino acids in IBDV structural protein VP2 (Islam *et al*., 2001). Due to technological progress, modern vaccines have been created to combat MDAbs, and are now being sold commercially. An example is the IBD vector vaccine, which utilizes turkey herpes virus (HVT). The composition involves chicken embryo fibroblasts that have been infected with recombinant HVT in order to produce VP2 of IBDV within a living organism. The absence of VP2 expression on the infected cells' surface or in the recombinant HVT vectors plays a role in its capacity to surpass MDA (Bublot *et al*., 2007).

IBD immune complex vaccines

These vaccines consist of a mixture of a certain amount of IBDV-specific antibodies obtained from the sera of hyperimmunized chickens and infectious IBD vaccine virus (Whitfill *et al*., 1995). In addition it has been shown that these vaccines were effective in the presence of maternally derived antibodies (Haddad *et al*., 1997; Giambrone *et al*., 2001). Most remarkable was the low level of depletion of bursal and splenic B lymphocytes in chickens vaccinated this vaccine (Jeurissen *et al*., 1998). Their main benefit is that the Immune complex vaccines can be administered subcutaneously on hatchery as early as day one and are acceptable for in ovo vaccination on day eighteen of incubation using commercial egg injection equipment (Ivan *et al*., 2005).

3. MATERIALS AND METHODS

3.1 Study area

The study area is Bishoftu, since this area is a major poultry production site and frequent IBD outbreaks are reported. Bishoftu is located 45 Km south east of Addis Ababa. The area is located at 9oN latitude and 40oE longitude at an altitude of 1850 maximum above sea level with annual rainfall of 866 mm of which 84% is in the long rainy season (June to September)(NMSA, 2010).

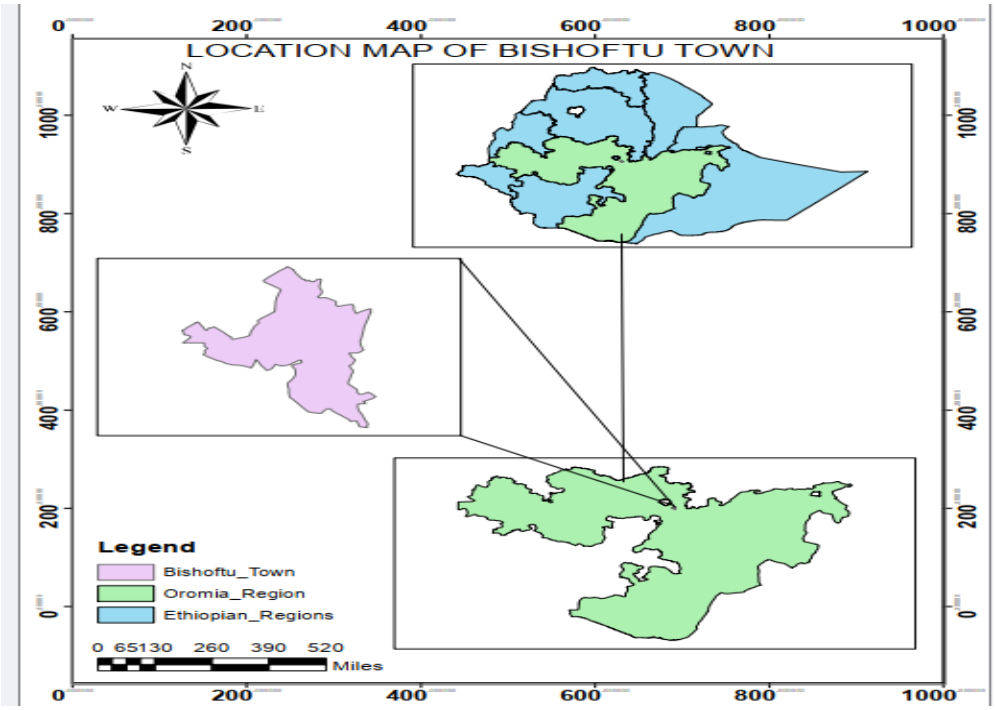


Figure 3*:* Map of the Bishoftu Town.

This map was developed using ArcGIS from Ethiopian shape files.

3.2 Study population

The experimental study was conducted using Cobb 500 species of chickens. The Cobb 500 is a breed of broiler chicken recognized for its quick growth and feed efficiency. Their genetic homogeneity lowers variability and guarantees reliable result outcomes. Furthermore, their

excellent feed efficiency makes them appropriate for studies on the effectiveness of vaccines. For the questionnaire survey, small- and medium-scale commercial poultry farms established by private, micro, and small-scale enterprises and cooperatives that raise exotic breeds. The farms were included to the study, despite of production type.

3.3 Study design

An experimental study over controlled environment was conducted from November, 2023 to May, 2024 to study to investigate the efficacy of infectious bursal disease vaccines (IBD). On top that, a semi-structured survey was conducted among chicken farm owner and /or attendants of Bishoftu town, to thoroughly gather understanding regarding infectious bursal disease and the usage of vaccines against the IBD.

3.4 Study methodology

3.4.1 Questionnaire survey

A semi-structured questionnaire survey was conducted November, 2023 to May, 2024 mainly aiming on comprehensively capturing insights on the understanding of infectious bursal disease and the utilization of vaccines against the disease. A pre-test of the questionnaire was conducted before the main survey to enhance its clarity and comprehensiveness. Subsequently, farm owners were engaged in face-to-face to explore the subject matter. Then, the data was collected from those farm owners who were willing to provide the necessary data based on informed consent by dropping those unwilling farm owners and operators.

The sample size for this study was determined based on the formula given by Arsham (2020) as follows;

$$N= \frac{0.25}{(SE)\ 2}$$

Where N= number of population

SE= Standard Error, which is estimated 0.05, with 95% CI and 5% error

From the formula, our population size was calculated one hundred (100). During the survey, Seven people withdraw from the total population. Hence, ninety three(93) individuals were surveyed

3.4.2 Experimental study

Chickens, housing and feeding conditions

For the experimental study, one hundred day-old COB-500 broiler chickens sourced from Alema Farms P.L.C were acquired and utilized. Upon their arrival, the chickens were housed in AAU-CVMA poultry house with floor pens that were lined with straw. They were provided with a brooder for warmth, ensuring their comfort and well-being. The chickens had access to food and water ad libitum. Additionally, the lighting conditions and temperature within the pens were carefully regulated based on the guidelines provided by the breeder, tailored to meet the specific requirements of broiler chickens. The chickens were then randomly assigned to four groups: group 1, group 2, group 3, and group 4 with 25 chickens per group. Group 1 was vaccinated with CEVAC® TRANSMUNE, vaccine; Group 2 was vaccinated with CEVAC® GUMBO L (which both of them are imported from Ceva Sante Animale, France). Group 3 was vaccinated with IBD VIRUS LC – 75 (locally produced) and Group 4 was kept as a control. The vaccines are purchased directly from their sells office.

Vaccines

CEVAC® TRANSMUNE IBD vaccine contains the Winterfield 2512 strain of Infectious Bursal Disease live virus in complex with IBD immunoglobulins (VPI: Virus Protecting Immunoglobulins) in freeze-dried form. CEVAC® GUMBO L contains the LIBDV intermediate strain of Infectious Bursal Disease virus in live, freeze-dried form. IBD virus LC – 75 intermediate vaccine strain freeze-dried with suitable stabilizer. It contains an Intermediate Standard live virus strain and is presented in freeze-dried form.

Table 2: Summary of experimental design

Group	Vaccine type	Date of vaccination	Route of vaccination
A	CEVAC® TRANSMUNE IBD	Day old chick	Subcutaneous
B	CEVAC® GUMBO L	14th day old	Drinking water
C	IBD virus LC – 75 NVI	14th day old and 21stold (booster)	Drinking water
D	CONTROL	None	None

3.5 Laboratory analysis of samples

Blood samples of 2 ml per chick were collected on the 7th, 14th, 21st, 28th, 35th, and 42nd days post-vaccination using a 3 mL sterile disposable syringe from the wing vein. Each collected blood sample was appropriately labelled with the individual chicken number. The sera were then harvested by centrifuging at 3000 rpm for 5 minutes into labelled cryovials. Subsequently, the cryovials containing the sera were stored at -20 °C until the laboratory procedure was conducted at Animal Health Institute (AHI).

3.5.1 ELISA

The collected serum sample were evaluated for antibody response against each vaccine using a commercial indirect ELISA kit (IDvet, Grabels, France) in Animal Health Institute (AHI), Sebeta Town. In a pre-dilution plate, wells A1, B1, C1 and D1 were kept as controls, and 5 µl of each sample to be tested and 245 µl of Dilution Buffer 14 was added. In the ELISA microplate, 100 µl of the Negative Control (A1 and B1), 100 µl of the positive control (C1 and D1) and 90 µl of Dilution Buffer 14 to the wells of each sample were added. After that the plate was covered and incubated at 21°C. After incubation the plates were emptied and the wells were washed 3 time

with 300 µl of the Wash Solution 1X then 100 µl of the Conjugate 1X were added to each well. Later the plated were covered and incubated at 21°C for 30 min. After incubation the wells were washed 3 times with 300 µl of the Wash Solution 1X. Then substrate solution was added to each well and after covering the wells, the plate was incubated at 21°C for 15 min in the dark. After incubation was done, 100 µl stop solution was to added each well. Finally, the results were read at 450 nm (ANNEX 3). Absorbance was determined using an ELISA microplate reader at 450 nm wavelength. S/P values were calculated using the formula provided by the manufacturer as follows.

$$SP = \frac{\text{OD sample} - \text{ODNC}}{\text{ODPC} - \text{ODNC}}$$

Where OD is Optical density, NC is Negative control and PC is Positive control

Determination of antibody titre from the S/P ratio is as follows

Log10 (titre) = 0.97 * $\log_{10}$(S/P) + 3.449

Titre = $10^{\log10\ (titre)}$

After calculation it was interpreted as positive if antibody titer is greater than 875 (> 875) and negative if antibody titer is less than or equal to 875 (≤ 875).

3.6 Ethical clearance

The present study underwent a thorough review by the Animal Welfare and Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture at Addis Ababa University (ANNEX 1), with consideration of all pertinent ethical and animal welfare considerations. The approval was duly accorded and endorsed, accompanied by a reference number VM/ERC/04/29/16/2024. In order to ensure the ethical integrity of the study, due diligence was exercised in obtaining the necessary ethical clearances and consents from all relevant participants, as well as adherence to relevant principles in public health and animal welfare ethics. The community's ethical, cultural, and religious principles were dutifully upheld. The humane sacrifice of severely ill chickens was carried out in accordance with established animal welfare protocols.

3.7 Data management and statistical analysis

The results of indirect ELISA and the responses obtained from the questionnaire survey were entered into the Microsoft Excel version 16.0 program for organization and initial processing. Survey responses were systematically coded using numerical system. The coding scheme utilized symbols ranging from 0 to 10 to represent different categories of responses. Subsequently, the statistical analysis was conducted using STATA version 14 software. A one- way analysis of variance (ANOVA) was employed to compare the values indirect ELISA. The criterion for establishing statistical significance was set at a p-value less than 0.05. Subsequently, the survey data underwent thorough analysis to determine the frequency distribution of responses for each question included in the questionnaire.

4. RESULTS

4.1 Serology results

The indirect ELISA results showed a mean maternal antibody titre of 3097.21, four day after their arrival. When the overall mean antibody titre each vaccine was evaluated throughout the study, it was found to be 3497.94, 2995.11, 4518.72 and 49.45 for immune complex, Gumbo L, IBD LC-75 vaccinated and control group respectively (Table 3).

Table 3: Summary of Mean OD, Mean, SP and mean antibody-titre with respect to Standard deviation (SD)

Groups	Mean OD	Mean SP	Mean Ab-titre	Age in Days
ICX	1.02±0.87	1.27±1.13	3497.99±3074.59	42
Gumbo L	0.87±0.61	1.08±0.79	2995.11±2161.74	56
IBD LC-75	1.31±0.82	1.64±1.07	4518.72+2909.98	56
Control	0.06±0.02	0.02±0.03	49.45±90.01	56

Upon analysis of the mean antibody titre of each vaccine, it was observed that the vaccines have demonstrated varying titres on same and at different time. For example, on the 7th day post vaccination, it was found that the mean antibody titre of immune complex (Group 1) was higher than all other groups (Table 4).

Table 4: Summary of group result of 7th, 14th, 21st, 28th, 35th and 42nd day post vaccination

	MEAN ANTIBODY TITRE (MEAN AB ± SE) POST-VACCINATION					
GROUP	**7 day**	**14 day**	**21 day**	**28 day**	**35 day**	**42 day**
ICX	1298.81±89.93	183.87±82.10	38.86± 18.45	6280.42±1341.50	4424.76± 594.57	6129.62± 546.23
GUMBO L	21.46± 6.50	2581.28± 1140.00	3363.25±873.84	3156.71±458.54	3400.04± 310.70	5973.53± 889.88
IBD LC-75	22.92± 11.00	642.14± 390.55	5270.40±951.41	5653.07±349.90	5793.22± 665.37	6520.84± 963.25
CONTROL	145.62± 65.27	32.80± 11.10	17.14± 10.67	2.29±0		

All vaccines have induced protective immune response from 7 days post-vaccination onwards. Besides, the protective level of each vaccine is different at different time post-vaccination owing to the potency of the vaccines (Figure A and B, below).

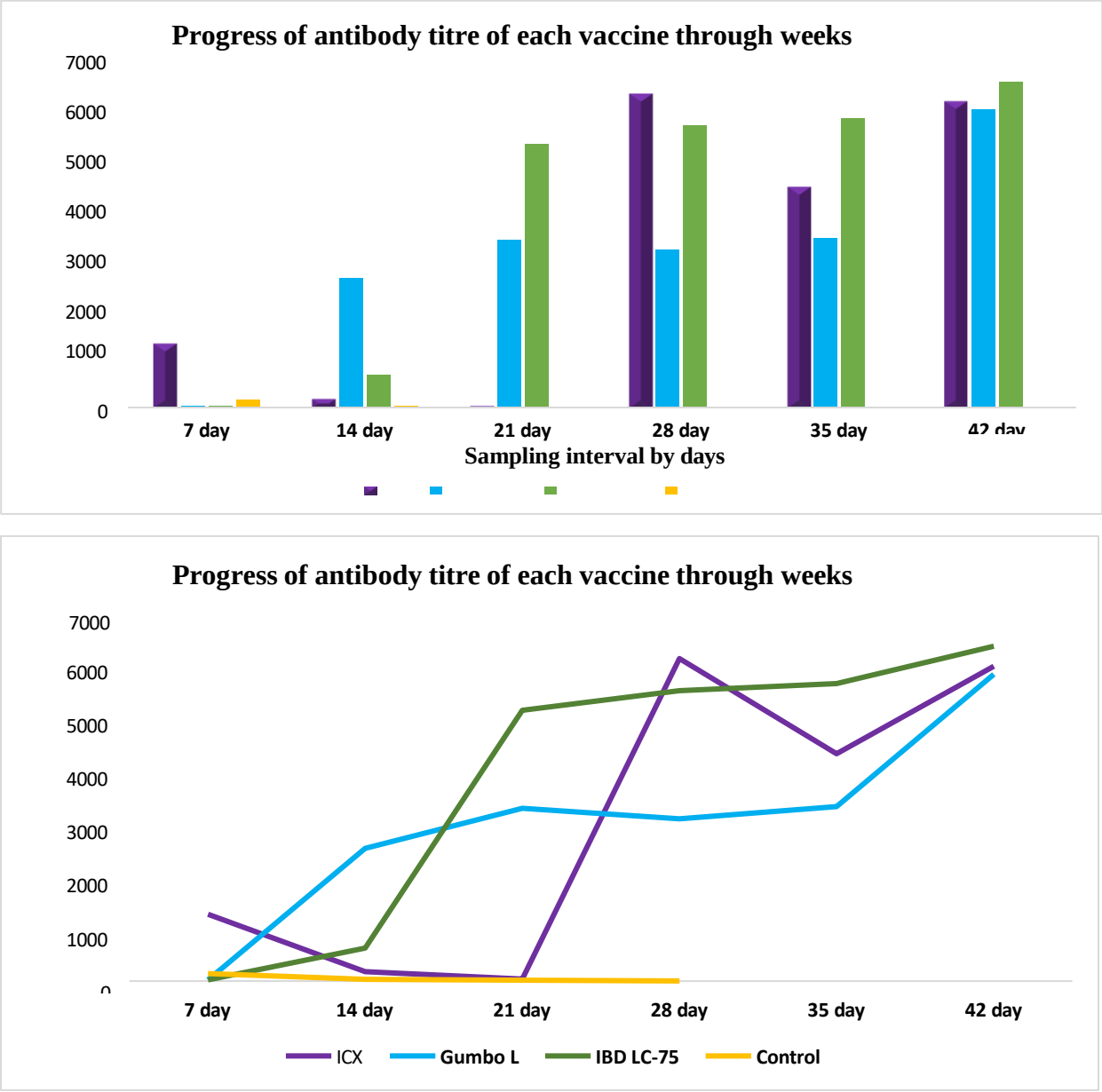


Figure 4: Bar and Line graph of the serology result

A): Bar graph representing the progress of antibody titre of each vaccine through weeks

B) Line graph representing the progress of each vaccine through weeks

4.2 Questionnaire survey

Demographic and General Information on the respondents and farms

From the personnels involved in the current survey female and male, were 28% and 72% respectively. The years of experience in poultry farming was 3.23% (<1 year), 64.52% (>1 and<5) and 32.26% (>5 years). Regarding the respondents educational level; 27.96% can read and write, whereas 32.26% has attended higher education (Table 5).

Table 5: Summary of demographic and general information of respondents and farms

Variables	Category	Number (%)
Gender	Female	26 (28)
	Male	67 (72)
Years of experience	< 1 year	3 (3.23)
	>1 year < 5 years	60 (64.52)
	> 5 years	30 (32.26)
Educational level:	Read and write	26 (27.96)
	Elementary school	7 (7.53)
	High school	30 (32.26)
	Higher education	30 (32.26)
Role on the farm	Farm owner	82 (88.17)
	Chicken farm attendant	11 (11.83)

Information on flock history and management

From all the farms 65.59% were layer farming, 25.81% were broiler production, and 8.61% practiced dual-purpose farming. The majority (76%) of farms relied on pipe water, and 17%, utilized well water as their source of water. It was found that 98.92% of the respondents acknowledged keeping records of their inventory. Among these respondents, 32.61% reported using manual tracking methods, 19.57% utilized electronic tracking systems, and 47.83% employed both method. Regarding farm size, 31.18% of the respondents had less than 1000 chickens on their farms, 41.94% had between 1000 and 2500 chickens, and 26.88% had more than 2500 chickens (Table 6).

Table 6: Information on flock history and management

Variables	Category	Number (%)
Type of production	Layer	61 (65.59)
	Broiler	27 (29.04)
	Dual purpose	5 (5.38)
Breed of chickens	Cobb 500	14 (14.43)
	Sasso	18 (18.55)
	Lohmann brown	28 (28.87)
	Bovans brown	38 (38.16)
Source of water	Well water	17 (18.28)
	Pipe water	76 (81.72)
Number of chickens	Less than 1000	29 (31.18)
	1001-2500	39 (41.94)
	Greater than 2500	25 (26.88)
Keep a record of vaccination	Yes	92 (98.92)
	No	1 (1.08)
Inventory management	Manual tracking	30 (32.60)
	Electronic tracking	18 (19.57)
	Both	44 (47.83)

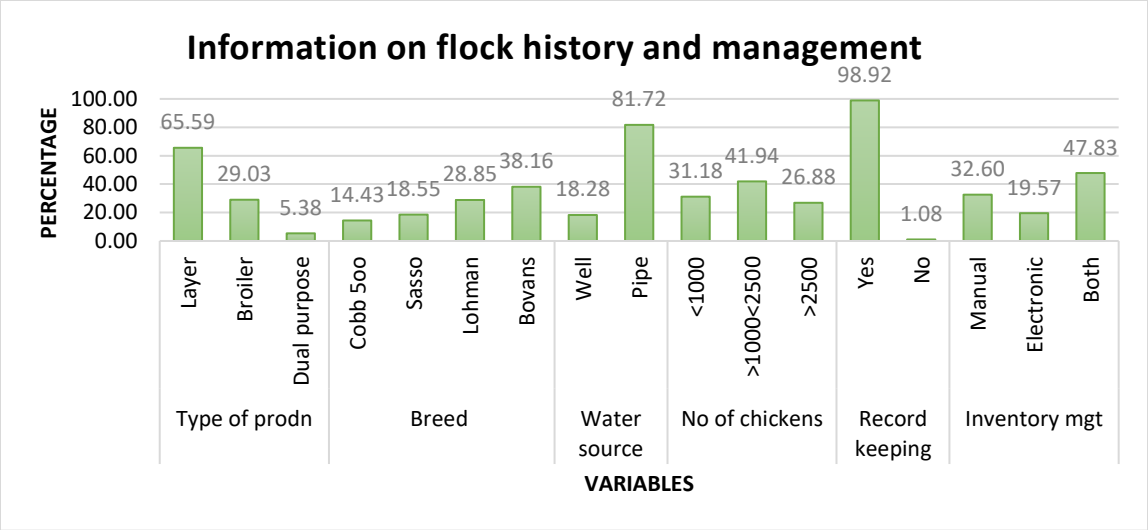


Figure 5: Bar graph of the results on Information on flock history and management

Awareness and experience on Gumboro Disease outbreak and its impact

The level of familiarity with IBD is as follows. Very familiar (50.54%), stated they were somewhat familiar (43.01%), and not familiar (6.45%). Their awareness on current IBD prevalence in their area, 62.36% were aware, 23.66% remained unsure and 13.98% were not aware. During the survey, 91.40%, believed that IBD does have a significant effect on the poultry industry, 4.30%, were unsure and 4.30% respondents did not agree. The responses indicated that 63.44% of the participants answered “no” to encountering an IBD outbreak, while 36.56% had encountered it. Participants responded that IBD outbreak impacted their business through increased mortality (7.69%), experienced profit losses (15.38%), both increased mortality and profit losses (53.85%), and 23.08% of participants indicated that they had encountered all the mentioned problems (Table 7).

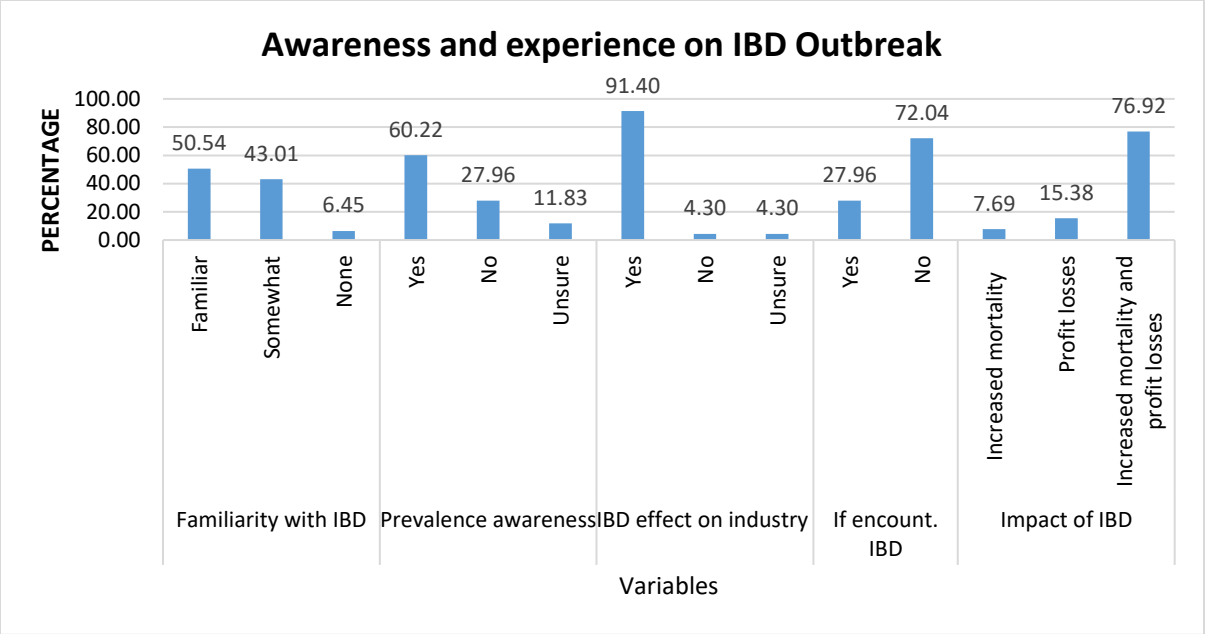


Figure 6: Bar graph of the results on Awareness and experience on Gumboro Disease Outbreak

Table 7: Summary of Awareness and experience on Gumboro Disease outbreak its impact

Variables	Category	Number (%)
Familiarity with IBD	Very familiar	47 (50.54)
	Somewhat familiar	40 (43.01)
	Not familiar at all	6 (6.45)
Awareness of prevalence of IBD outbreak in your area	No	26 (27.96)
	Yes	56 (60.22)
	Unsure	11 (11.83)
Do you believe IBD has effect on poultry industry	Yes	85 (91.40)
	No	4 (4.30)
	Unsure	4 (4.30)
Have you encountered IBD outbreak	Yes	26 (27.9)
	No	67 (72.04)
Impact on your farm	Increased mortality	2 (7.69)
	Profit losses	4 (15.38)
	Increased mortality and profit losses	14 (53.85)
	I have faced all the problems	6 (23.08)

Progress of Gumboro Disease Outbreak

Participants were asked if the use of the IBD vaccine led to a decrease in IBD outbreaks. The results are significant decrease (38.71%), moderate decrease (43.01%). On the other hand, 13.98% stated that they observed no decrease in outbreaks, and 4.30% were unsure of the impact. Participants were asked about their observations regarding the incidence of IBD outbreaks over the past few years. The responses were yes, a significant decrease (29.03%) Yes, a slight decrease (47.31%), No change (4.30%) and Unsure (19.35%). When asked about the reasons behind any observed improvements, better vaccines than before (32.26%), enhanced biosecurity measures

(22.58%), improved vaccination programs (16.13%), better disease monitoring and diagnostics (9.68%) and all factors have attribute (19.35%), were the responses. In response to the question, "Do you believe that increased awareness and education on the Gumboro outbreak and vaccination practices can lead to better disease management in poultry farms?" 63.44% of respondents answered affirmatively, 9.68% responded negatively, and 26.88% were unsure (Table 8).

Table 8: Summary on Progress of IBD Disease Outbreak

Variables	Category	Number (%)
Does the use of the vaccine led to a decrease in outbreaks?	Significant decrease	36 (38.71)
	Moderate decrease	40 (43.01)
	No decrease	13 (13.98)
	Not sure	4 (4.30)
Have you observed a decrease in the incidence of the IBD outbreak over the past few years	Yes, a significant decrease	27 (29.03)
	Yes, a slight decrease	44 (47.31)
	No change	4 (4.30
	Unsure	18 (19.35)
If you have observed a decrease, what factors do you attribute to this improvement	Improved vaccination programs	15 (16.13)
	Better vaccines than before	30 (32.26)
	Enhanced biosecurity measures	21 (22.58)
	Better disease monitoring and diagnostics	9 (9.68)
	All factors	18 (19.35)
Do you believe that the decrease in the IBD outbreak has a positive impact on the overall health and productivity of poultry farms?	Yes	79 (84.95)
	Unsure	14 (15.05)

Do you believe that increased awareness and education on the IBD outbreak and vaccination practices can lead to better disease management in poultry farms?	Yes	59 (63.44)
	No	9 (9.68)
	Unsure	25 (26.88)

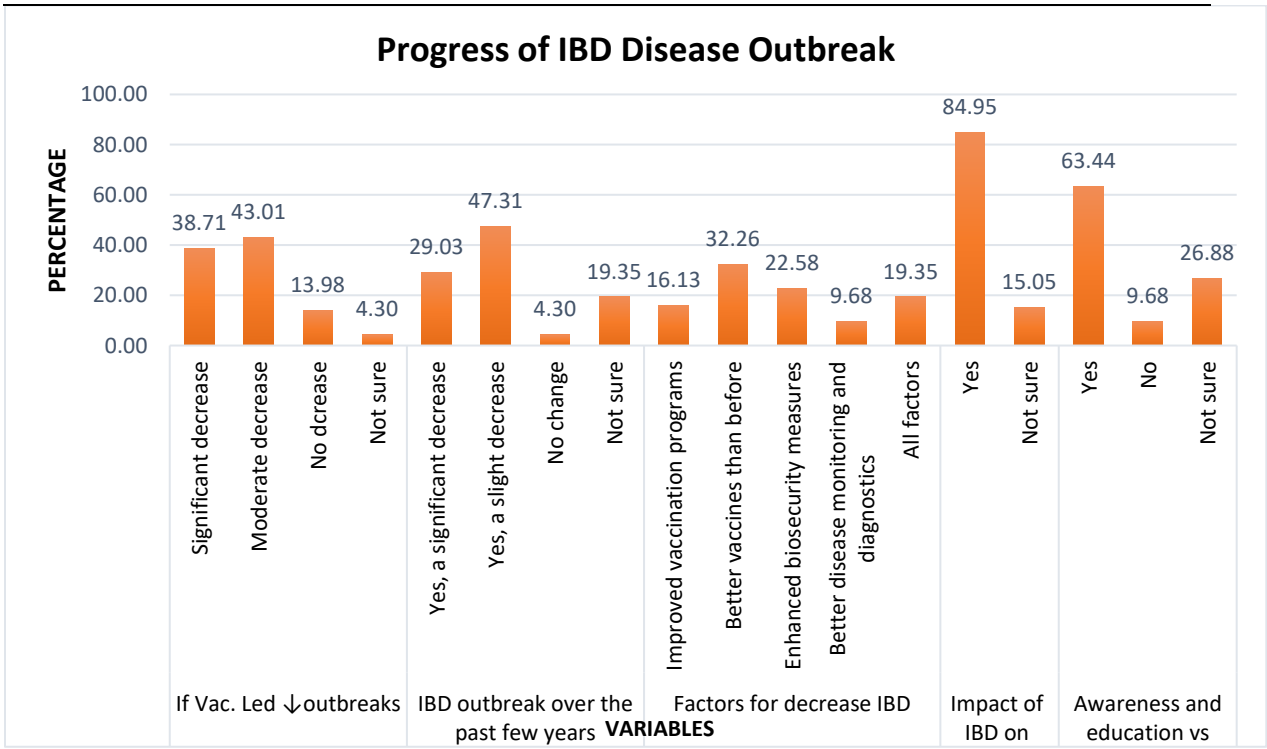


Figure 7: Bar graph of the results on Progress of IBD Disease Outbreak

Questionnaire Survey results on General information on IBD vaccine

The majority (65.59%) use locally produced vaccines; while the rest use the imported one. Almost all (95.70%) of the respondents admitted that they know the recommended storage of the vaccines while the rest (4.30%) don't. From this 97.85% of respondents have enough storage facilities for their farm. Among the responses gathered, vaccine availability (32.26%) proper training for administration (30.11%), vaccine storage (2.15%) is mentioned as a challenge when administering IBD vaccine, and no specific challenges was faced (35.48%). When they were asked if they ensure that the IBD vaccine is maintained within the cold chain during transportation and storage, 97.85% responded positively (Table 9).

Table 9: General information on IBD vaccine

Variables	Category	Number (%)
Source of IBD vaccine for your farm	Local	61 (65.59)
	Imported	32 (34.41)
How frequently is the IBD vaccine administered in your farm	Once	18 (19.36)
	Twice	75 (80.64)
Aware on recommended vaccine storage	Yes	89 (95.70)
	No	4 (4.30)
Do you have adequate storage facility	Yes	91 (97.85)
	No	2(2.15)
Maintaining the cold chain	Yes	91 (97.85)
	N o	2 (2.15)

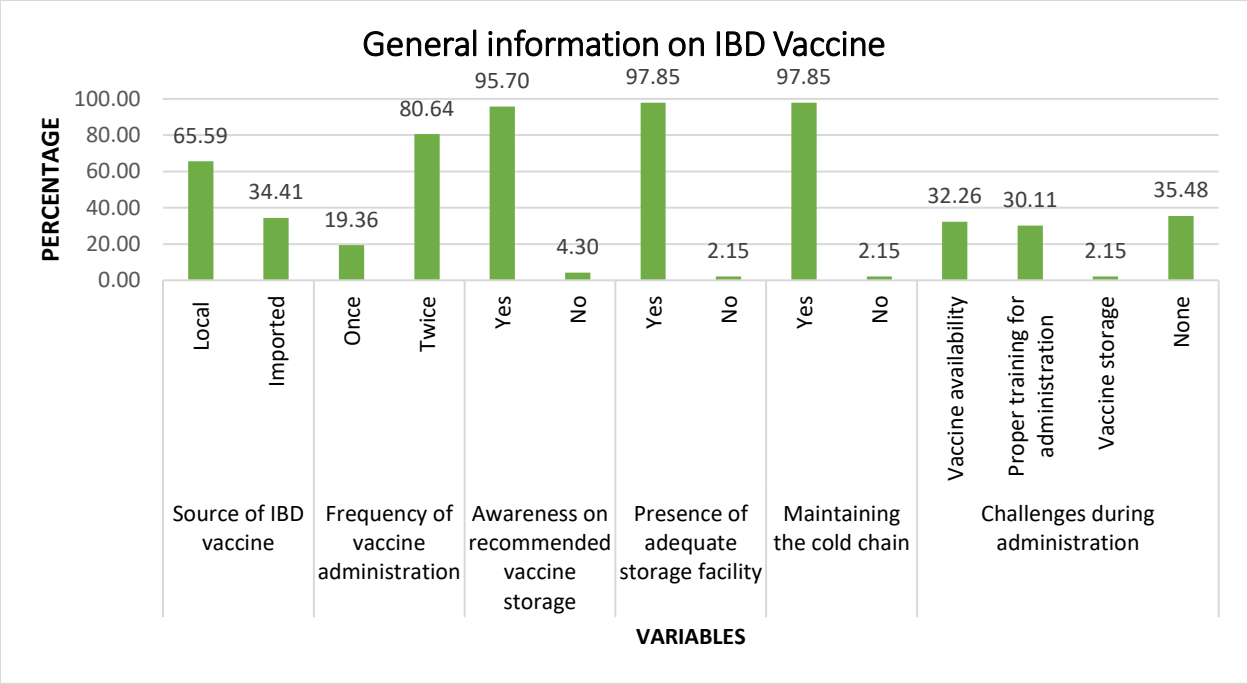


Figure 8: Bar graph of the results on General information on IBD vaccine

Source of IBD related information and vaccination protocol

Participants were asked where they typically seek such information on updates about IBD and its vaccine. The responses indicated that Veterinarians/animal health assistants (58.06%), Scientific journals (4.30%), industry publications (4.30%), training programs (8.60%). Some of used combined source like, Veterinarians/animal health assistants and Training programs (15.05%), Veterinarians/animal health assistants and scientific journals (3.23%), Industry publications and Training programs (3.23%). The rest (3.23%) mentioned obtaining information from friends in the same industry. Regarding the adherence to strictly label instructions when administering the IBD vaccine, 69.89% answered "Yes," 11.83% responded with "No," and 18.28% stated that they followed the instructions to some extent. When administering IBD vaccine, the protocols used were of vaccine manufacturers (65.59%), recommendations from veterinarians or animal health practitioners (29.03%), determining vaccination protocols independently (3.23%) and (2.15%) mentioned relying on suggestions from friends (Table 10).

Table 10: Summary on Source of information and vaccination protocol

Variables	Category	Number (%)
Information source	Veterinarians/animal health assistants	54 (58.06)
	Scientific journal	4 (4.30)
	Industry publications	4 (4.30)
	Training programs	8 (8.60)
	Veterinarians/animal health assistants and Training programs	14 (15.05)
	Veterinarians/animal health assistants and Scientific journals	3 (3.23)
adhere to the label instructions during vaccination	Yes	65 (69.89)
	No	11 (11.83)
	To some extent	17 (18.28)
Vaccination protocols you use	Based on vaccine manufacturer's protocol	61 (65.59)
	Friend suggestion	2 (2.15)
	By veterinarian/animal health practitioner/ recommendation	27 (29.03)
	By Myself	3 (3.23)

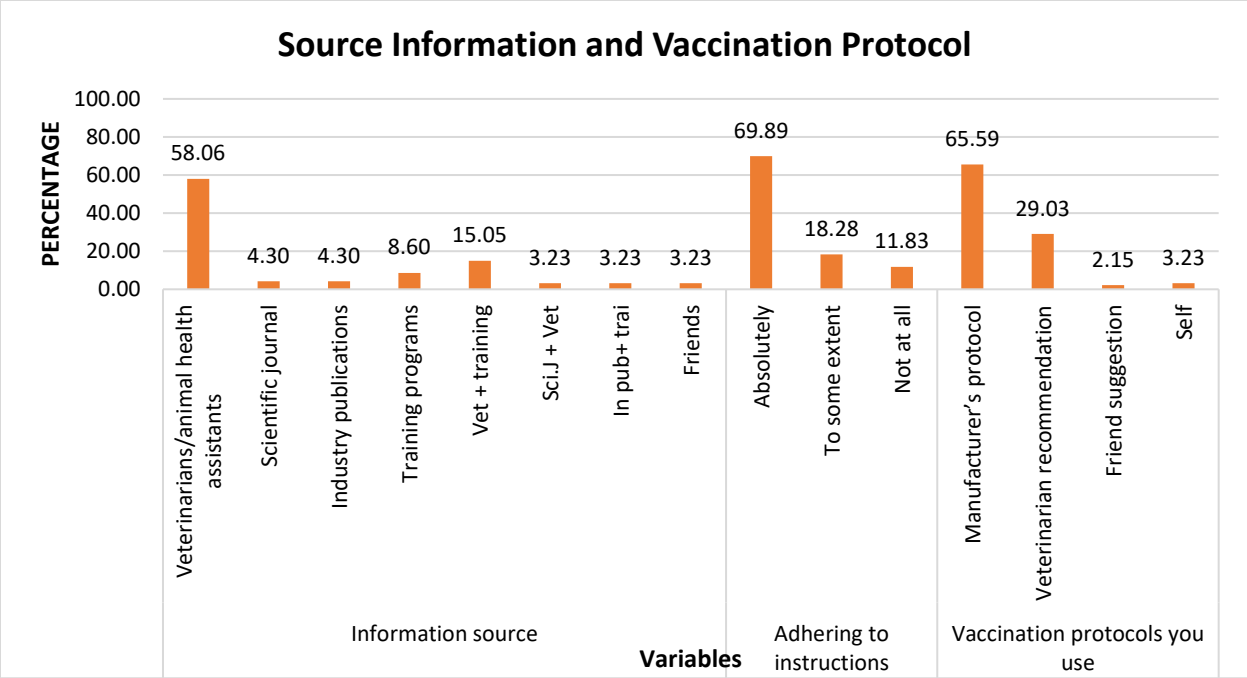


Figure 9 Bar graph of the results on Source of IBD related information and vaccination protocol

Information on IBD vaccine efficacy

In a survey conducted to assess the factors considered when selecting IBD vaccine, the reasons selected were, efficacy (45.16%), availability (19.35%), cost (18.28%), reputation of the manufacturer (10.75%), ease of administration(4.39%) and cost and efficacy (2.15%). From all participants, 36.56% conduct follow-up assessments to assess long term efficacy of IBD vaccine; while, 63.44% of participants don't. When asked about methods on measuring the vaccines efficacy, the respondents reported using clinical signs observation (18.28%) and laboratory testing (1.08%). And 55.91% state that they have never measured the efficacy of the vaccine while 24.73% never thought about measuring it. When the respondents are asked if they have ever provided feedback on the vaccine's efficacy to the vaccine supplier or manufacturer, only 29.03% said yes while most of the respondents, 70.97%, said they haven't. Participants were asked whether they communicated the efficacy of the vaccine to their customers or friends. The results indicated that 32.26% of respondents answered "Yes," 47.31% answered "No," and 20.43% responded with "Sometimes" (Table 11).

Table 11: Summary Information on IBD vaccine efficacy

Variables	Category	Number (%)
Factors you consider when selecting an IBD vaccine	Cost	17 (18.28)
	Efficacy	42 (45.16)
	Ease of administration	4 (4.30)
	Manufacturer's reputation	10 (10.75)
	Availability	18 (19.35)
	Cost and efficacy	2 (2.15)
Conducting follow-up assessments	Yes	34 (36.56)
	No	59 (63.44)
How do you currently measure the efficacy	Clinical signs observation	17 (18.28)
	Laboratory testing	0(0)
	Both	1 (1.08)
	Not measured	52 (55.91)
	Never thought about that	23 (24.73)
Providing feedback on efficacy	Yes	27 (29.03)
	No	66 (70.97)
Communicating the efficacy	Yes	30 (32.26)
	No	44 (47.31)
	Some times	19 (20.43)

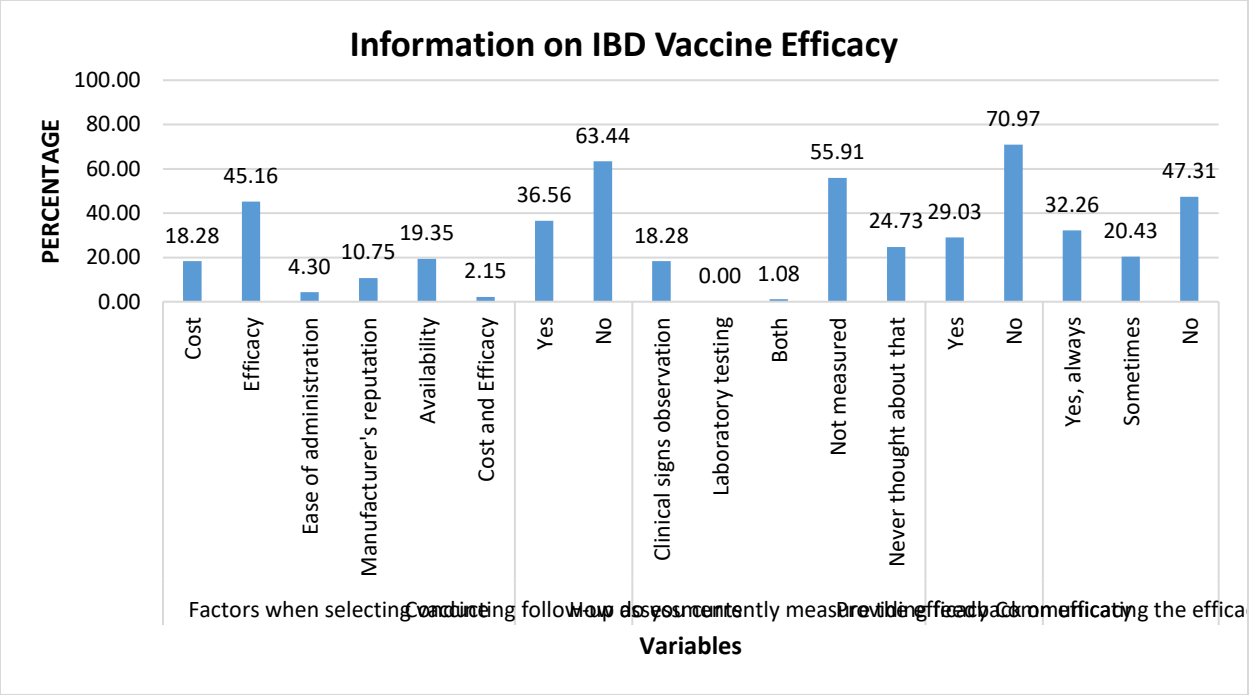


Figure 10: Bar graph of the results on Source of IBD related information and vaccination protocol

Awareness on IBD vaccine efficacy

The response to the question on awareness was on the efficacy of the vaccine preventing IBD, they responded as, highly aware (27.96%), somewhat aware (60.22%) and (11.83%) were not aware. When questioned about how effective they think IBD vaccine is against the disease, respondents provided the following breakdown: Highly effective (25.81%), moderately effective (56.99%), Ineffective (16.13%), and I don't know (1.08%).To the question, have you encountered instances of IBD vaccine resistance? The responses were yes (26.88%), no (61.29%) and unsure (11.83%). To the question what do you recommend to improve the efficacy of the Gumboro vaccine in preventing IBD outbreaks? They responded as Enhanced training programs (16.13%), improved vaccine storage facilities (2.15%), enhanced monitoring systems (30.11%), Vaccine matching and efficacy testing (34.48%) and Enhanced biosecurity (16.13%). The respondents were asked whether they believed in the necessity of enhanced vaccines. The results indicated that 29.03% (believed), 44.09% (unsure) and 26.88% responded negatively to the idea of altering the current vaccine (Table 12).

Table 12: Summary on IBD vaccine efficacy awareness

Variables	Category	Number (%)
Awareness on the efficacy	Highly aware	26 (27.96)
	Somewhat aware	56 (60.22)
	Not aware	11 (11.83)
How effective do you think is the vaccine	Highly effective	24 (25.81)
	Moderately effective	53 (56.99)
	Ineffective	15 (16.13)
	I don't know	1 (1.08)
Have you encountered any instances of vaccine resistance	Yes	25 (26.88)
	No	57 (61.29)
	Unsure	11 (11.83)
Do you believe there is a need for improved vaccines	Yes	27 (29.03)
	No	25 (26.88)
	Unsure	41 (44.09)
Recommendation to improve the efficacy	Enhanced training programs	15 (16.13)
	Improved vaccine storage facilities	2 (2.15)
	Enhanced monitoring systems	28 (30.11)
	Vaccine matching and efficacy testing	33 (35.48)
	Enhanced biosecurity	15 (16.13)

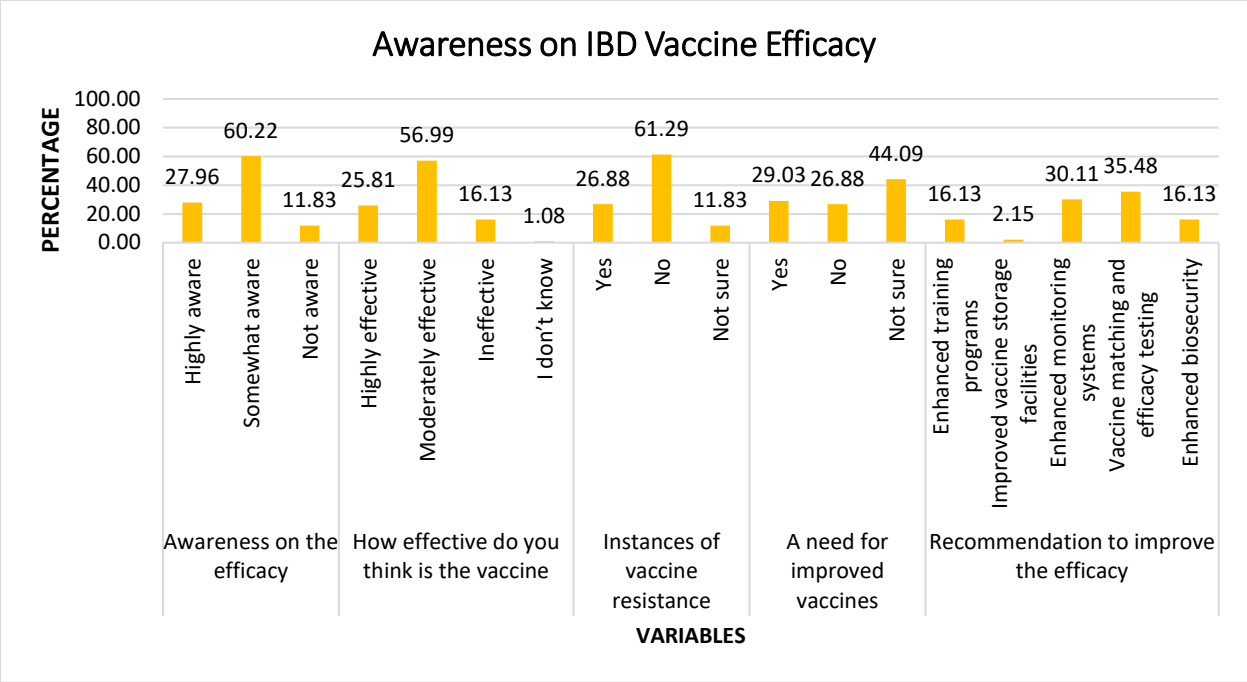


Figure 11: Bar graph of the results on Awareness on IBD vaccine efficacy

5. DISCUSSION

In the poultry sector, protection against IBD is achieved through the use of commercially available live, inactivated, or genetically engineered IBD vaccines. The nature of the vaccine plays a crucial role in determining and controlling the effectiveness of IBD vaccination (Müller *et al*., 2012). The increasing prevalence of hatchery vaccination is a suitable trend due to the growing capacity of poultry production industries, as well as the dedication to improving the management of vaccination procedures through automated techniques, whether through subcutaneous injection on the first day of life or in ovo vaccination (Jacquinet and Gardin, 2011). Vaccination of parent flock is done routinely whereby newly hatched chickens are immunologically shielded through the transfer of maternal-derived antibodies (MDAs) that are passed from the parents through the yolk sac (Abadalla, 2005). The ELISA technique is often used as a standard serological tool for determining antibodies against IBD in poultry farming. (Dey *et al*., 2019).

Maternal antibodies play a crucial role in determining the optimal timing for Gumboro vaccine administration in chickens. Research indicates that maternal antibody levels decline significantly within the first few weeks of life, affecting the efficacy of live vaccines against infectious bursal disease. Studies have also shown that maternal antibody levels decrease rapidly, reaching negative levels by around day 20 after hatching (Mymensing *et al*., 2002). In the current study the mean maternal antibody titer was recorded as 3097.21 four day after their arrival. The mean antibody titers of the control group were recorded as 145.616 on the 7th day, 32.766 on the 14th day, 17.142 on the 21st day, and 2.29 on the 28th day after their arrival. These values suggest that there was no maternal antibody protection present in these chickens by the given time they were tested for IBD antibodies except the first few days. Skeeles *et al*. (1979) also reported that the MDA was decreasing from 7 to 28 days of age in the subjects of the study. The last serological examination was performed when the subjects were at 28 days of age; and there was no detectable antibody titer. This reduction in the MDA levels can be correlated with the established half-life of MDA that is believed to range 3-5 days (Zorman *et al*., 2011).

On the 7th day post vaccination, it was found that the mean antibody titer of immune complex (Group 1) was higher than all other groups, with a statistical significance level of $P < 0.05$. A low antibody titer in the results 7 days post-vaccination is part of the normal immune response trajectory. The time lag until the peak antibody levels is due to the kinetics of the replication of viruses, antigen presentation, and subsequent antibody production (El-Mahdy *et al*. 2013). Moving forward to the 14th day, Group two (the ones vaccinated with Gumbo L) exhibited a higher antibody response compared to the other groups. By the 21st day, group three (the one vaccinated with IBD virus LC – 75) showed a significantly higher antibody titer compared to the rest, while group one (immune complex) displayed the lowest response; this result is also reported by (Sedeik *et al*., 2019). Variations between the different groups may be attributed to the existence of a virus neutralizing factor (VNF) that may be still attached to the virus in the vaccine, hence lowering its active immunization effect (Bose *et al*., 2003). This disparity of vaccination rate, could have a profound effect on the immune reaction elicited by the vaccines. Administration of the live vaccine (IBD LC -75) two times may have given the immune system more chance to identify the antigens that were present in the vaccine making the immunity stronger and long lasting as compare to the administration of the immune complex.

As the study progressed to the 28th day, it was noted that immune complex vaccine produced a higher antibody response compared to the live and this is different with the result of (Chansiripornchai and Sasipreeyajan, 2009) but aligns with (Sedeik *et al*.,2019). The results post 21 day suggest that all vaccines induced strong and persistent immune responses against IBD, as evidenced by the substantial increase in mean antibody titers over time until there is no significant difference ($P>0.05$) by the day forty-two. In contrast, the control group exhibited much lower antibody levels, emphasizing the importance of vaccination in conferring protection against infectious diseases like IBD.

The results of this survey are a resourceful indicator of the level of knowledge on IBD among participants, awareness of its prevalence, and perception about its effects on the poultry industry, and experience with outbreaks and consequences thereof. From the survey result, the majority had experience in the poultry farming industry and this plays a significant role in disease control by enhancing knowledge, improving disease management practices and enabling early detection (Dashe *et al*., 2009).

The high proportion of participants who either said they were very familiar or somewhat familiar with IBD gives indications that knowledge on the disease is reasonably good among the surveyed population. The fact that the majority were aware of the current prevalence of IBD in their area is quite commendable since early detection and response are critical in the management of outbreaks. However, the percentage of participants who did not know indicates possible information gaps in dissemination or monitoring systems. The participants that had experienced an outbreak faced different impacts on their lives. A small subset reported increased mortality, profit losses; and a significant portion indicated they had faced both increased mortality and profit losses. While the remaining of participants reported they had encountered all the mentioned problems. The negative consequences ranged from increased mortality to financial losses, with many individuals reporting both issues. This is in line with the findings of Aiyedun (2014). Since these findings underscore the importance of effective prevention and control measures to minimize its impact on poultry populations and related industries, understanding factors contributing to outbreak occurrences can aid in developing targeted intervention strategies (Alkie and Rautenschlein, 2016).

The survey findings indicated that a minority admitted to being oblivious to the recommended vaccine storage conditions. When asked whether they keep the IBD vaccine within the cold chain during transport and storage, the majority said they ensured that they uphold the requirements of the cold chain. Keeping the vaccine at the right conditions of storage and in the cold chain retains its effectiveness, prevents damage, and keeps it safe and potent enough to invoke immunity to IBD in poultry (Sharif and Ahmad, 2018).

When asked about the difficulties they encounter when administering IBD vaccines, the survey revealed that availability and proper training on vaccine administration were major challenges. The percentage of respondents who said that vaccine storage was a challenge was much lower, with the greatest number responding that they did not have any specific challenge during vaccination. The educational training of people in the poultry industry on the vaccination protocol positively impacts flock health, productivity, cost-effectiveness, and industry reputation (Okata and Al-Hassan, 2023).

When administering IBD vaccines, most commonly used protocols are those given by vaccine manufacturers. Recommendations by veterinarians or animal health practitioners were also mentioned. This underlines the importance of expertise in determining vaccination protocols. Vaccination protocols were set independently by a small percentage of respondents. Such instances may arise from situations where no vets or health practitioners could give guidance. Lastly, few respondents alluded to depending on suggestions from friends when giving IBD vaccines. It means that majority of individuals consider expert advice and manufacturer directives important in regard to making decisions about vaccination administration. The value of established protocols cannot be overemphasized as they help minimize possible risks connected with the use of wrong vaccination protocol and ensure maximum protection against IBD (Adino and Bayu, 2022). Understanding consumer preferences- like routes of administration or perceived effectiveness- also plays a part in vaccine selection as seen by La Sota and I-2 Newcastle disease vaccines in Tanzania (Haghighi *et al*., 2005).

According to survey results, only a small number of respondents chose "No" for their answer suggesting that they did not comply with instructions as required, while majority stick to label instruction when vaccinating. Moreover, others indicated some measure of adherence whereas they were not fully compliant with label guidelines. Nonetheless, by sticking to a well-structured immunization plan the producers can enhance disease prevention, flock health improvement and public health protection (Habte *et al*., 2017).

In a survey conducted to assess the factors taken into consideration when selecting a Gumboro vaccine, some key reasons were highlighted. These play an important role in the decision- making process for poultry farmers and veterinarians. Among all these IBD vaccines, efficacy was the most vital. Availability and cost were likewise among these factors. The vaccine manufacturer's reputation is also such one factor that makes sway decision on what vaccine to choose. For instance, poultry producers prefer vaccines from manufacturers with proven track records of producing superior quality and effective products. This kind of preference was also seen in other bacterial poultry vaccines (Islam *et al*., 2023). Information dissemination is a necessary prerequisite to adequate control measures against adverse effect of disease outbreak on farms (Sabitu *et al*., 2022). Understanding where individuals in the veterinary field obtain such information is crucial for improving dissemination strategies and ensuring that accurate and up-

to-date information reaches relevant stakeholders. The majority of participants rely on veterinarians or animal health assistants for information. This underscores the pivotal role that these professionals play in disseminating knowledge within the industry. A small percentage of respondents turn to scientific journals, Industry Publications and Training Programs. This underlines the significance of remaining updated with regards to the most recent changes in veterinary medicine. Lastly, a small number of respondents reported that their colleagues who worked in similar professions are their source of information. However, informal networks may not be able to provide reliable and verified information.

Their perception on the efficacy of the vaccine varied a lot. A majority portion of the respondents reported the vaccine to be moderately and some reported that the vaccine is ineffective while few reported that they were unsure. It is crucial to understand that the survey findings shouldn't be taken as the actual vaccine efficacy of the IBD vaccine. A significant percentage of the respondents reported that they have experienced IBD vaccine resistance cases. This indicates the real field problem that poultry farmers face regarding the vaccine resistant strains of the virus. Vaccine resistance can develop for several reasons including viral evolution, wrong vaccination practices or poor vaccine matching (Knoblich, 2000). Vaccine efficacy testing, regular vaccine matching studies, enhanced monitoring system, implementing robust training programs, enhanced biosecurity and improved vaccine storage facilities were suggested be the respondents to improve vaccine efficacy. Improving biosecurity on poultry farms, controlling visitor and worker traffic onto farms, implementing hygiene protocols upon entry and preventing cross-contamination between different areas of a farm can reduce the risk of disease introduction and spread (Huber *et al*., 2022).

Most respondents believed there was a need for an improved IBD vaccine. They may realize that there is potential to improve the current vaccination in order to boost its effectiveness, increase its protection, or prevent IBD outbreaks. The key will be to understand why they believe this and what information they relied upon to support their opinion. This will help determine the level of understanding and knowledge among the respondents that contributed to their responses. Majority of the respondents said they did not know if an enhanced Gumboro vaccine was needed. This uncertainty could be lack of information, contradicting idea of experts or they are concerned with adverse reactions to the vaccine or the cost of a new vaccine. Examining the causes of this

ambiguity might shed light on areas that may require education and outreach initiatives to correct misunderstandings or knowledge gaps.

It is important to understand the views and experiences of the respondents in relation to the association between the use of IBD vaccine and IBD outbreaks in order to understand the true world effectiveness of this vaccine. The majority of the respondents reported a strong reduction in IBD outbreaks after using the Gumboro vaccine. Which is an encouraging outcome. This finding supports the theory that vaccination can lessen outbreaks of IBD in poultry (Kurukulasuriya, 2017). The fact that a tiny percentage of respondents stated that there was no decrease in IBD outbreaks following vaccination is concerning. This finding raises questions about potential factors contributing to this lack of efficacy or whether there are other variables influencing disease dynamics. Additionally, a small percentage expressing uncertainty about the impact highlights the need for further investigation and clarification regarding the relationship between vaccination and IBD outbreak control.

About observations over the last few years regarding the frequency of Gumboro outbreaks, a small decrease in Gumboro outbreaks was observed by the majority of responders. A small portion of individuals said that the frequency of Gumboro outbreaks had not changed. This answer challenges the adequacy of current prevention and control measures. The different viewpoints on the prevalence of Gumboro outbreaks make it clear how vital it is to engage in continuous monitoring, research as well as implementation of focused interventions to win the fight against the disease.

One of the major announced factors which helped to control incidence rate of IBD outbreak was availability of safer vaccines. Development progress towards vaccine technology has resulted into more safe and efficacious vaccines against infectious bursal disease (Lin *et al*., 2020). Another crucial factor identified in the survey responses is the implementation of enhanced biosecurity measures. By adopting strict biosecurity protocols such as controlling access, disinfection procedures, and hygiene practices, poultry producers can minimize the risk of IBD outbreaks (Huber *et al*., 2022). Effective vaccination strategies, including proper timing, dosage, and administration techniques, are essential for ensuring optimal immune response in poultry flocks. Regular vaccination schedules tailored to specific flock requirements contribute significantly to reducing Gumboro-related morbidity and mortality rates. Furthermore, respondents highlighted

the role of better disease monitoring and diagnostics in managing Gumboro outbreaks. Integrating multiple strategies such as vaccination, biosecurity practices, monitoring programs, and diagnostic capabilities synergistically enhances the overall resilience of poultry operations against Gumboro disease challenges (Swai *et al*., 2011).

Based on the results of the survey, majority of respondents expressed their belief that increased awareness and education on the Gumboro outbreak and vaccination practices can lead to better disease management in poultry farms. Awareness and education play crucial roles in ensuring effective vaccination practices in poultry farms. Proper understanding of the disease transmission dynamics, vaccination schedules, and potential side effects can help farmers make informed decisions regarding vaccination programs. Moreover, regular monitoring of flocks for signs of illness and prompt reporting to veterinary services can facilitate early detection and intervention, reducing the spread of the disease and minimizing economic losses as recommended by Manabe *et al*., (2011).

Limitations of the study

The absence of a challenge virus for assessing the effectiveness of vaccines and treatments in real-world scenarios poses a significant limitation to the study. Another limitation to the study was availability of other vaccines by the time the experiment was conducted.

6. CONCLUSION AND RECOMMENDATIONS

Given the substantial economic ramifications linked to infectious Bursal Disease Virus (IBDV), it is crucial to prioritize the creation and assessment of advanced IBDV vaccines to mitigate the influence of these pathogens and establish effective preventative measures within the expanding poultry sector. Monitoring the antibody response post-vaccination is essential, as maternal antibodies provide protection for a limited period and the comparison of different types of vaccines for Gumboro disease is crucial for optimizing vaccination strategies and disease control in poultry farming. The current study has provided insightful informations on poultry farms personnels awareness and perception on IBD and its vaccine management. It is understood that farms have differences in the type of vaccines being used, awareness on the efficacy and management as well as current knowledge on disease status which has it own impact on overall disease control and prevention. Besides, up on evaluating and comparing the immune response of chickens for different IBD vaccines, it is seen that all tested vaccines have demonstrated varied protection levels at different times evidenced by the substantial increase in mean antibody titers over time though the chickens has not been challenged with local isolate which would have boosted comparison of the protection level of the vaccines.

Therefore based on the above conclusive remarks, the following recommendations are forwarded:

- Continuous and updated surveillance on vaccine types utilized and management is crucial for effective follow-up and control of IBD
- Although it has been noted that all tested IBD vaccines has shown remarkable immune response, all commercially available vaccines should be tested for their efficacy to select the outperforming one from economical and ease administration point of view.
- Further study should be conducted on layer chickens considering their production importance and effect difference of IBD on different production types
- The efficacy and immune response of the IBD vaccines should be evaluated in the presence of challenge virus.

7. REFERENCES

Abadalla, M.A.A., (2005): The Decaying Pattern Of Maternally Derived Antibodies Against Infectious Bursal Disease Virus In Broiler Chicks (Doctoral dissertation, University of Khartoum).

Abdel-Alim, G. A., and Y. M. Saif (2001): Immunogenicity and antigenicity of very virulent strains of infectious bursal disease viruses. *Avian Dis*. **45**, 92-101.

Adino, G.W. and Bayu, M.D (2022): Review of diagnostic and vaccination approaches of infectious bursal disease of poultry. *Vet Med Open J*, **7**, 22-8.

Aiyedun, A., (2014): Predictors of high levels of knowledge of the HIV window period among diverse men: An online study that includes evaluation of an avatar video intended as e-health on the HIV window period. Teachers College, Columbia University.

Akira, S., Uematsu, S. and Takeuchi, O (2006): Pathogen recognition and innate immunity. *Cell*, **124**(4), 783-801.

Aliyu, H.B., Hamisu, T.M., Hair Bejo, M., Omar, A.R. and Ideris, A (2022): Comparative pathogenicity of Malaysian variant and very virulent infectious bursal disease viruses in chickens. *Avian Pathology*, **51**(1), 76-86.

Alkie, T.N. and Rautenschlein, S. (2016). Infectious bursal disease in poultry: current status and future prospects. Veterinary Medicine: Research and Reports, **7**, 9-16.

Animal Health Yearbook (2011): Ministry Of Agriculture Animal and Plant Health Regulatory Directorate Veterinary Epidemiology, Disease Prevention, Control and Eradication, Ethiopia: 22-24.

Asfaw, Y.T., Ameni, G., Medhin, G., Gumi, B., Hagos, Y. and Wieland, B., (2021) Poultry disease occurrences and their impacts in Ethiopia. Tropical Animal Health and Production, 53, 1-10.

Azad, A.A, Jagadish, M.N., Brown, M.A. & Hudson, P.J. (1987): Deletion mapping and expression in E. Coli of the large genomic segment of a birnavirus. *Virology*, **161**, 145–152.

Azad, A.A., Barrett, S.A. & Fahey, K.J. (1985): The characterization and molecular cloning of the double-stranded dsRNA genome of an Australian strain of infectious bursal disease virus. *Virology*, **143**, 35–44.

Bari, F.D., (2021): Genogrouping of infectious bursal disease viruses circulating in Ethiopian chickens: proposal for assigning very virulent strains in the country into new sub genogroup 3d. *Veterinary Medicine: Research and Reports*, 43-52.

Berg, T.P.V.D., (2000): Acute infectious bursal disease in poultry: a review. *Avian pathology*, **29**(3), 175-194.

Block, H., Meyer-Block, K., Rebeski, D.E., Scharr, H., de Wit, S., Rohn, K. and Rautenschlein, S., (2007): A field study on the significance of vaccination against infectious bursal disease virus (IBDV) at the optimal time point in broiler flocks with maternally derived IBDV antibodies. *Avian Pathology*, **36**(5), 401-409.

Bose, R.K., Hossain, K.M., Sil, B.K., Taimur, M., Pugliese, C. and Franci, O., (2003): Comparative sero evaluation of live and killed Gumboro vaccine in broilers. Italian Journal of Animal Science, 2(2), 157-162.

Böttcher, B., Kiselev, N.A., Stel'Mashchuk, V.Y., Perevozchikova, N.A., Borisov, A.V. and Crowther, R.A., (1997): Three-dimensional structure of infectious bursal disease virus determined by electron cryomicroscopy. *Journal of virology*, **71**(1), 325-330.

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 infectious bursal disease of chickens in the face of high-titred maternally derived antibody. *Journal of comparative pathology*, **137**, 81-S84.

Cazaban, C., Swart, W.B.F., Rietema, R.M.W., Wit, J., Palya, V. and Gardin, Y., (2018): Field assessment of an immune-complex infectious bursal disease vaccine in chicks born to non-hyperimmunized broiler breeders. Journal of Veterinary Science and Animal Husbandry, 6, 302.

Central Statistical Agency (CSA) (2021): TheFederal Democratic Republic of Ethiopia, Central Statistical Agency, Agricultural Sample Survey (2014 E.C.), Volume II Report on Livestock and LivestockCharacteristics (Private Peasant Holdings). Tatistical Bulletin 589 Addis Ababa, Ethiopia.

Cereno, T.N., (2008): Infectious Bursal Disease (IBD): Causative agent, diagnosis and prevention.

Chansiripornchai, N. and Sasipreeyajan, J., (2009): Comparison of the efficacy of the immune complex and conventionally live vaccine in broilers against infectious bursal disease infection. The Thai Journal of Veterinary Medicine, **39**(2), 115-120.

Chowdhury, M.M.I., Islam, M.T., Aktar, A., Bhuiyan, M.K.J., Kamal, M.M., Haque, M.A., Ara, M.J., Rahman, M.M. and Islam, M.A., (2011): Comparative Performance of In-House and Commercially Available ELISA Kits for the Detection of Antibody Against Newcastle Disease Virus Vaccine. *Progressive Agriculture*, **22**(1-2), 55-64.

Ciccone, N.A., Smith, L.P., Mwangi, W., Boyd, A., Broadbent, A.J., Smith, A.L. and Nair, V., (2017): Early pathogenesis during infectious bursal disease in susceptible chickens is associated with changes in B cell genomic methylation and loss of genome integrity. Developmental & Comparative Immunology, **73**, 169-174.

Collett, S.R., Smith, J.A., Boulianne, M., Owen, R.L., Gingerich, E., Singer, R.S., Johnson, T.J., Hofacre, C.L., Berghaus, R.D. and Stewart-Brown, B., (2020): Principles of disease prevention, diagnosis, and control. Diseases of poultry, 1-78.

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

Coulibaly, F., Chevalier, C., Delmas, B. and Rey, F.A., (2010): Crystal structure of an Aquabirnavirus particle: insights into antigenic diversity and virulence determinism. *Journal of virology*, **84**(4), 1792-1799.

Damairia, B.A., Putri, K., Sutrisno, B. and Wibowo, M.H., 2023. Current Symptoms and Pathological Changes of Bursa Fabricius from Commercial Farming Broilers Led to Infectious Bursal Disease. Jurnal Sain Veteriner, 41(3), pp.297-305.

Dashe, Y. G, Okewole, P, Jwande, D L and Alasa, M. U (2009). Gumboro disease outbreak in a vaccinated pre new pen house. Central Diagnostic Department. NVRI Vom Nigeria

Davison, F., Magor, K.E., Kaspers, B., Fred, D. and Karel, A.S., (2008): Structure and evolution of avian immunoglobulins. *Avian immunology*, **1**, 107-127.

Deshan, L. and Zhiqiang, W., (1991): Isolation and preliminary identification of a supervirulent strain of infectious bursal disease virus. Chinese Journal of Animal and Poultry Infectious Diseases (China), 6.

Dessie, T., Zewdie, Y., Yilma, Z., Ayalew, W. and Haile, A., (2023): Yelemat Tirufat: An Overview of the initiative and lessons of experience from selected livestock development interventions in Ethiopia.

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*, 85-97.

Du, X.X., Ahmad, L., Wang, B.J., Ding, M.S., Elsaid, F.G., Wen, H.M., Yang, J.B. and Khan, A., (2023): Infectious bursal disease: distribution, pathogenesis and pathology. 388-395.

Dulwich, K.L., Asfor, A.S., Gray, A.G., Nair, V. and Broadbent, A.J., (2018): An ex vivo chicken primary bursal-cell culture model to study infectious bursal disease virus pathogenesis. *JoVE (Journal of Visualized Experiments)*, (**140**), 58489.

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.

El-Mahdy, S.S., Farouk, H., Abd El-Wanis, N.A. and Hamoud, M.M., (2013): Comparative studies between different commercial types of live Infectious bursal disease [IBD] vaccine strains in Egypt. American Journal of Research Communication, **1**(10), 113-129.

Eregae, M.E., Dewey, C.E., McEwen, S.A., Ouckama, R., Ojkić, D. and Guerin, M.T., (2014): Flock prevalence of exposure to avian adeno-associated virus, chicken anemia virus, fowl adenovirus, and infectious bursal disease virus among Ontario broiler chicken flocks. *Avian Diseases*, **58**(1), 71-77.

Eterradossi N (2000): Progress in the diagnosis and prophylaxis of infectious bursal disease in poultry.Journal of poultry science **42**: 36.

Eterradossi, N. and Saif, Y.M., (2013): Infectious bursal disease. *Diseases of poultry*, 219-246.

FAO. 2019. Poultry Sector Ethiopia FAO Animal Production and Health Livestock CountryReviews, No. **11** (Rome), 1-60.

Farzi, R., Aghbash, P.S., Eslami, N., Azadi, A., Shamekh, A., Hemmat, N., Entezari-Maleki, T. and Baghi, H.B., (2022): The role of antigen-presenting cells in the pathogenesis of COVID-19. Pathology-Research and Practice, **233**, 153848.

Franciosini, M.P. and Davidson, I., (2022): A Walk through Gumboro Disease. *Poultry*, **1**(4), 229-242.

Gao, H., Wang, Y., Gao, L. and Zheng, S.J., (2023): Genetic Insight into the Interaction of IBDV with Host—A Clue to the Development of Novel IBDV Vaccines. *International Journal of Molecular Sciences*, **24**(9), 8255.

Giambrone, J.J., Dormitorio, T. and Brown, T., (2001): Safety and efficacy of in ovo administration of infectious bursal disease viral vaccines. *Avian Diseases*, 144-148.

Gilchrist, P., (2005): Involvement of free-flying wild birds in the spread of the viruses of avian influenza, Newcastle disease and infectious bursal disease from poultry products to commercial poultry. *World's poultry science journal*, **61**(2), 198-214.

Gul, I., Hassan, A., Muneeb, J.M., Akram, T., Haq, E., Shah, R.A., Ganai, N.A., Ahmad, S.M., Chikan, N.A. and Shabir, N., 2023. A multiepitope vaccine candidate against infectious bursal disease virus using immunoinformatics-based reverse vaccinology approach. *Frontiers in Veterinary Science*, **9**, 1116400.

Guo, X., Wang, L., Cui, D., Ruan, W., Liu, F. and Li, H., (2012): Differential expression of the Toll-like receptor pathway and related genes of chicken bursa after experimental infection with infectious bursa disease virus. *Archives of virology*, **157**, 2189-2199.

Habte, T., Amare, A., Bettridge, J.M., Collins, M., Christley, R.M. and Wigley, P., (2017): Guide to chicken health and management in Ethiopia: For farmers and development agents. ILRI Manual.

Haddad, E.E., Whitfill, C.E., Avakian, A.P., Ricks, C.A., Andrews, P.D., Thoma, J.A. and Wakenell, P.S., (1997): Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. *Avian Diseases*, 882-889.

Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Sanei, B., Parvizi, P., Gisavi, H., Chambers, J.R. and Sharif, S., (2005): Modulation of antibody-mediated immune response by probiotics in chickens. Clinical and Vaccine Immunology, **12**(12), 1387-1392.

Hailu M, Tilahun SB, Negash T (2009): Incidence of infectious bursal disease in village chickens of two districts of Amhara Region, Northwest Ethiopia. Livestock Research for Rural Development **21**(12).

Hasan, A.R., Ali, M.H., Siddique, M.P., Rahman, M.M. and Islam, M.A., (2010): Clinical and laboratory diagnoses of newcastle and infectious bursal diseases of chickens. Bangladesh Journal of Veterinary Medicine, **8**(2), 131-140.

He, X., Wang, W., Chen, G., Jiao, P., Ji, Z., Yang, L. and Wei, P., (2019): Serological study reveal different antigenic IBDV strains prevalent in southern China during the years 2000–2017 and also the antigenic differences between the field strains and the commonly used vaccine strains. *Veterinary microbiology*, **239**, 108458.

Hishamund, P., Emikpe, B.O. and Shyakaa, A., (2023): Seroprevalence and associated factors of infectious bursal disease (IBD) in indigenous chicken in eastern province in Rwanda. *Journal of Immunoassay and Immunochemistry*, **44**(3), 296-308.

Hon, C.C., Lam, T.T.Y., Yip, C.W., Wong, R.T.Y., Shi, M., Jiang, J., Zeng, F. and Leung, F.C.C., (2008): Phylogenetic evidence for homologous recombination within the family Birnaviridae. *Journal of General Virology*, ***89***(12), 3156-3164.

Hou, B., Wang, C.Y., Luo, Z.B. and Shao, G.Q., (2022): Commercial vaccines used in China do not protect against a novel infectious bursal disease virus variant isolated in Fujian. *Veterinary Record*, **191**(10).

Huber, N., Andraud, M., Sassu, E.L., Prigge, C., Zoche-Golob, V., Käsbohrer, A., d'Angelantonio, D., Viltrop, A., Żmudzki, J., Jones, H. and Smith, R.P., (2022): What is a biosecurity measure? A definition proposal for animal production and linked processing operations. One Health, **15**, 100433.

Hudson, P.J., McKern, N.M., Power, B.E. and Azad, A.A., (1986): Genomic structure of tbe large RNA segment of infectious bursal disease virus. *Nucleic acids research*, **14**(12), 5001-5012.

Ingrao, F., Rauw, F., Lambrecht, B. and van den Berg, T., (2013): Infectious Bursal Disease: A complex host–pathogen interaction. Developmental and Comparative Immunology, **41**(3), 429-438.

Isihak, F.A., Ismail, H.K. and Wahid, A.A., (2021): Comparison study between the efficacy of immune complex and conventionally live vaccine against Gumboro disease in broilers. Iraqi Journal of Veterinary Sciences, **35**(4), 627-632.

Islam, M.R., Zierenberg, K., Raue, R. and Müller, H., (2001): June. Molecular cloning and sequence analysis of Bangladeshi strain of vvIBDV and its adaptation in tissue culture by site directed mutagenesis. In *Proceedings of the II. International Symposium on Infectious Bursal Disease and Chicken Anaemia, Rauischholzhausen, Germany* (30-39).

Islam, M.S. and Rahman, M.T., 2023. A Comprehensive review on bacterial vaccines combating antimicrobial resistance in poultry. *Vaccine*s, *11*(3), 616.

Iván, J., Velhner, M., Ursu, K., Germán, P., Mató, T., Drén, C.N. and Mészáros, J., (2005): Delayed vaccine virus replication in chickens vaccinated subcutaneously with an immune complex infectious bursal disease vaccine: quantification of vaccine virus by real-time polymerase chain reaction. *Canadian journal of veterinary research*, **69**(2), 135.

Jackwood, D.H. and Saif, Y.M., (1987): Antigenic diversity of infectious bursal disease viruses. *Avian diseases*, 766-770.

Jackwood, D.J. and Sommer-Wagner, S.E., (2011): Amino acids contributing to antigenic drift in the infectious bursal disease Birnavirus (IBDV). Virology, **409**(1), 33-37.

Jackwood, D.J., (2004): Recent trends in the molecular diagnosis of infectious bursal disease viruses. *Animal Health Research Reviews*, **5**(2), 313-316.

Jacquinet, C. and Gardin, Y., (2011): Monitoring on French farms of the vaccine uptake in chickens vaccinated (in ovo) in the incubator with Cevac Transmune: benefits and monitoring examples compared to traditional vaccination methods.

Jenberie, S., Lynch, S.E., Kebede, F., Christley, R.M., Gelaye, E., Negussie, H., Asmare, K. and Ayelet, G., (2014): Genetic characterisation 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. & Whitfill, C.E. (1998): The working mechanism of an immune complex vaccine that protects chickens against infectious bursal disease. *Immunology*, **95**, 494500

Kapoor, S., Kaur, H., Rehman, A.U., Kumar, V. and Gupta, A., (2021): Infectious bursal disease: Overview. *Journal of Medical Pharmaceutical and Allied Sciences*, **11**(2), 4661-4665.

Kassa SA, Molla W (2012): Seroprevalence of infectious bursal disease in backyard chickens of North West Ethiopia. Scientific *Journal of Crop Sciences* **1**(1): 20-25.

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

Kelemen, M., Forgách, K., Iván, J., Palya, V., Süveges, T., Tóth, B. and Mészáros, J., (2000): Pathological and immunological study of an in ovo complex vaccine against infectious bursal disease. *Acta Veterinaria Hungarica*, **48**(4), 443-454.

Khan, R.S.A., Sajid, S., Habib, M., Ali, W., Shah, M.S.U.D. and Sarfraz, M., (2017): History of Gumboro (infectious bursal disease) in Pakistan. *Saudi Pharmaceutical Journal*, **25**(4), 453-459.

Kibenge, F.S.B. & Dhama, V. (1997): Evidence that virion-associated VP1 of avibirnaviruses contains viral RNA sequences. *Archives of Virology*, **142**, 1227–1236.

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.

Knoblich, H.V., (2000): Pathogenicity and immunogenicity of infectious bursal disease virus. The Ohio State University.

Korver, D.R., (2023): Current challenges in poultry nutrition, health, and welfare. animal, 17, 100755.

Kurukulasuriya, S.N., (2017): Efficacy of vaccines against variant infectious bursal disease viruses to control immunosuppression in the broiler chicken industry in Canada (Doctoral dissertation, University of Saskatchewan).

Lambrecht, B.N., De Veerman, M., Coyle, A.J., Gutierrez-Ramos, J.C., Thielemans, K. and Pauwels, R.A., (2000): Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *The Journal of clinical investigation*, **106**(4), 551-559.

Lee, M.S., Lin, Y.C., Lai, G.H., Lai, S.Y., Chen, H.J. and Wang, M.Y., (2011): One-step reverse-transcription loop-mediated isothermal amplification for detection of infectious bursal disease virus. *Canadian journal of veterinary research*, **75**(2), 122-127.

Legnardi, M., Franzo, G., Tucciarone, C.M., Koutoulis, K., Duarte, I., Silva, M., Le Tallec, B. and Cecchinato, M., (2022): Detection and molecular characterization of a new genotype of infectious bursal disease virus in Portugal. *Avian Pathology*, **51**(1), 97-105.

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.

Li, G., Kuang, H., Guo, H., Cai, L., Chu, D., Wang, X., Hu, J. and Rong, J., (2020): Development of a recombinant VP2 vaccine for the prevention of novel variant strains of infectious bursal disease virus. Avian Pathology, **49**(6), 557-571.

Li, K., Niu, X., Jiang, N., Zhang, W., Wang, G., Li, K., Huang, M., Gao, Y., Qi, X. and Wang, X., (2023): Comparative pathogenicity of three strains of infectious bursal disease virus closely related to poultry industry. *Viruses*, **15**(6), 1257.

Liang, J., Yin, Y., Qin, T. and Yang, Q., (2015): Chicken bone marrow-derived dendritic cells maturation in response to infectious bursal disease virus. Veterinary Immunology and Immunopathology, **164**(1-2), 51-55.

Lin, Z., Kato, A., Otaki, Y., Nakamura, T., Sasmaz, E. and Ueda, S., (1993): Sequence comparisons of a highly virulent infectious bursal disease virus prevalent in Japan. *Avian Diseases*, 315-323.

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.

Lucien Mahin (2016): Infectious bursal disease; Broiler infected with infectious bursitis.

Lukert, P.D. and Saif, Y.M. (2003) Infectious bursal disease. In Saif, Y.M., Glisson, J.R., Fadly, A.M., McDougald, L.R. and Swayne, D.E. (Eds.), Diseases of Poultry, 11th Edition, Iowa State University Press, *Ames*, 161-180.

M¨uller, H. & Becht, H. (1982): Biosynthesis of virus-specific proteins in cells infected with infectious bursal disease virus and signification as structural elements for infectious virus and incomplete particles. Journal of *Virology*, **44**, 384–392.

Maclachlan, N.J. and Dubovi, E.J. (2001): Fenner's th Veterinary Virology, 4 edition. Academic Press, London, UK. 293-300

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

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

Manabe, T., Thuy, P.T.P., Can, V.V., Takasaki, J., Huyen, D.T.T., Chau, N.T.M., Shimbo, T., Ha, B.T.T., Izumi, S., Hanh, T.T. and Chau, N.Q., (2011): Impact of educational intervention

concerning awareness and behaviors relating to avian influenza (H5N1) in a high-risk population in Vietnam. *PloS one*, **6**(8), 23711.

Martinez, J., Tomás, G., Merino, S., Arriero, E. and Moreno, J., (2003): Detection of serum immunoglobulins in wild birds by direct ELISA: a methodological study to validate the technique in different species using antichicken antibodies. *Functional Ecology*, 700-706.

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

Meeusen, E.N.T., Walker, J., Peters, A., Pastoret, P.-P. & Jungersen, G. (2007): Current status of veterinary vaccines. *Clinical Microbiology Reviews*, **20**, 489510.

Minta, Z., Domańska-Blicharz, K., Bartnicka, B. and Śmietanka, K., (2006): Applying immunoperoxidase and Antigen-Capture ELISA methods for rapidly diagnosing infectious bursal disease. Medycyna Weterynaryjna, **62**(3), 335-338.

Moryani, A.A., Rajput, N., Rajput, M.N. and Shah, A.H., (2020): Prevalence of common poultry diseases in chicken and influence of different medicinal herbs on the growth of broiler chicken. *Pure and Applied Biology (PAB)*, **9**(1), 1199-1208.

Mossie, T., (2021): A Comprehensive Review on Infectious Bursal Disease Which Has Serious Threat for Ethiopian Poultry Sector.

Müller, H. and Nitschke, R., (1987): The two segments of the infectious bursal disease virus genome are circularized by a 90,000-Da protein. *Virology*, **159**(1), 174-177.

Müller, 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.

Müller, H., Mundt, E., Eterradossi, N. and Islam, M.R., (2012): Current status of vaccines against infectious bursal disease. *Avian pathology*, **41**(2), 133-139.

Müller, R., Käufer, I., Reinacher, M. and Weiss, E., (1979): Immunofluorescent studies of early virus propagation after oral infection with infectious bursal disease virus (IBDV). *Zentralblatt für Veterinärmedizin Reihe B*, **26**(5), 345-352.

Mundt, E. & Mu¨ller, H. (1995): Complete nucleotide sequences of 5'- and 3'-noncoding regions of both genome segments of different strains of infectious bursal disease virus. *Virology*, **209**, 1018.

Mundt, E. and Vakharia, V.N., (1996): Synthetic transcripts of double-stranded Birnavirus genome are infectious. *Proceedings of the National Academy of Sciences*, **93**(20), 11131-11136.

Mundt, E., Beyer, J. & M¨uller, H. (1995): Identification of a novel viral protein in infectious bursal disease virus-infected cells. *Journal of General Virology*, **76**, 437–443.

Musa LW, Saidu L, Abakaka ES (2012): Economic impact of recurrent outbreak of Gumboro disease in commercial poultry farm Kano, Nigeria, Asia. *Journal of Poultry science* **89**: 525-565.

Mwenda, R., Changula, K., Hang'ombe, B.M., Chidumayo, N., Mangani, A.S., Kaira, T., Takada, A., Mweene, A.S. and Simulundu, E., (2018): Characterization of field infectious bursal disease viruses in Zambia: evidence of co-circulation of multiple genotypes with predominance of very virulent strains. *Avian pathology*, **47**(3), 300-313.

Mymensing, B., (2002): Effect of maternally derived antibody on vaccination against infectious bursal disease (Gumboro) with live vaccine in broiler. Int J Poultry Sci, 1, 98-101.

Nandhakumar, D., Rajasekhar, R., Logeshwaran, G., Ravishankar, C., Sebastian, S.R., Anoopraj, R., Sumod, K., Mani, B.K., Chaithra, G., Deorao, C.V. and John, K., (2020): Identification and genetic analysis of infectious bursal disease viruses from field outbreaks in Kerala, India. *Tropical animal health and production*, **52**(3), 989-997.

Neurath, A.R., (2008): Immune response to viruses: antibody-mediated immunity. Encyclopedia of virology, 56.

NMSA (2010): National Metrological Agency, Addis Ababa, Ethiopia.

Nouën, C.L., Rivallan, G., Toquin, D., Darlu, P., Morin, Y., Beven, V., de Boisseson, C., Cazaban, C., Comte, S., Gardin, Y. and Eterradossi, N., (2006): Very virulent infectious bursal disease virus: reduced pathogenicity in a rare natural segment-B-reassorted isolate. *Journal of general virology*, **87**(1), 209-216.

Okata, E.O. and Al-Hassan, R.M., 2023. Does publishing poultry vaccination schedule increase awareness and compliance among small-scale farmers? Evidence from Eastern Ghana. *Cogent Food & Agriculture*, **9**(1), 2241709.

Oláh, I., Felföldi, B., Benyeda, Z., Kovács, T., Nagy, N. and Magyar, A., (2022): The bursal secretory dendritic cell (BSDC) and the enigmatic chB6+ macrophage-like cell (Mal). *Poultry Science*, **101**(4), 101727.

Omer, M.G. and Khalafalla, A.I., (2022): Epidemiology and laboratory diagnosis of very virulent infectious bursal disease virus in vaccinated chickens in Khartoum, Sudan. *Open Veterinary Journal*, **12**(1), 33-43.

O'Neill, P.M., Badhwar–O'Neill (2010): galactic cosmic ray flux model—Revised. IEEE *Transactions on Nuclear Science*, **57**(6), 3148-3153.

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.

Parveen, S., Mahmood, A., Azad, A., Umar, S., Shoukat, N., Azam, M.M.A. and Malik, N.A., (2022): Prevalence of concurrent infections in broiler population of district Chakwal, Pakistan, 480-488.

Phillips, R.A. and Opitz, H.M., (1995): Pathogenicity and persistence of Salmonella enteritidis and egg contamination in normal and infectious bursal disease virus-infected leghorn chicks. *Avian diseases*, 778-787.

Pikuła, A., Lisowska, A. and Domańska-Blicharz, K., (2023): Epidemiology of infectious bursal disease virus in Poland during 2016–2022. *Viruses*, **15**(2), 289.

Prandini, F., Simon, B., Jung, A., Pöppel, M., Lemiere, S. and Rautenschlein, S., (2016): Comparison of infectious bursal disease live vaccines and a HVT-IBD vector vaccine and their effects on the immune system of commercial layer pullets. *Avian Pathology*, **45**(1), 114-125.

Qin, Y. and Zheng, S.J., (2017): Infectious bursal disease virus-host interactions: multifunctional viral proteins that perform multiple and differing jobs. *International journal of molecular sciences*, **18**(1), 161

Rashid, H., Xue, C., and Islam, T. (2013): Risk factors associated with infectious bursal disease in commercial chickens in Bangladesh. *Preventive Veterinary Medicine*, **111**(1–2), 181–185.

Rasoli, M., Yeap, S.K., Tan, S.W., Roohani, K., Kristeen-Teo, Y.W., Alitheen, N.B., Rahaman, Y.A., Aini, I., Bejo, M.H., Kaiser, P. and Omar, A.R., (2015): Differential modulation of immune response and cytokine profiles in the bursae and spleen of chickens infected with very virulent infectious bursal disease virus. *BMC Veterinary Research*, **11**, 1-14.

Ratcliffe, M.J. and Härtle, S., (2022): B cells, the bursa of Fabricius, and the generation of antibody repertoires. *In Avian immunology* (71-99).

Rauf, A., Khatri, M., Murgia, M.V., Jung, K. and Saif, Y.M., (2011): Differential modulation of cytokine, chemokine and Toll like receptor expression in chickens infected with classical and variant infectious bursal disease virus. *Veterinary research*, **42**(1), 1-11.

Rautenschlein, S., Yeh, H.Y. & Sharma, J.M. (2002): The role of T cells in protection by an inactivated infectious bursal disease virus vaccine. *Veterinary Immunology and Immunopathology*, **89**, 159167.

Rauw, W.M., (2012): Immune response from a resource allocation perspective. Frontiers in genetics, **3**, 32243.

Ravikumar, R., Chan, J. and Prabakaran, M., (2022): Vaccines against major poultry viral diseases: strategies to improve the breadth and protective efficacy. Viruses, **14**(6), 1195.

Reddy, V.R., Nazki, S., Asfor, A. and Broadbent, A.J., (2023): An Infectious Bursal Disease Virus (IBDV) Reverse Genetics Rescue System and Neutralization Assay in Chicken B Cells. *Current Protocols*, **3**(1), 639.

Sabitu, C.A., Olajide, A.O. and Kamila, A.O.K.O., (2022): Effects of gumboro disease on productivity of poultry farmers in Ogun state, Nigeria. *Ethiopian Journal of Applied Science and Technology*, **13**(1), 87-93.

Sainsbury, D., (2000): Infectious bursal disease: poultry health and management 125-126.

Sajid, S., Rahman, S.U., Mohsin Gilani, M., Sindhu, Z.U.D., Ali, M.B., Hedfi, A., Almalki, M. and Mahmood, S., (2021): Molecular characterization and demographic study on infectious bursal disease virus in faisalabad district. *Plos one*, **16**(8), 0254605.

Schijns, V.E., van de Zande, S., Lupiani, B. and Reddy, S.M., (2014): Practical aspects of poultry vaccination. In *Avian immunology* (345-362).

Sedeik, M.E., El-Shall, N.A., Awad, A.M., Abd El-Hack, M.E., Alowaimer, A.N. and Swelum, A.A., (2019): Comparative evaluation of HVT-IBD vector, immune complex, and live IBD vaccines against vvIBDV in commercial broiler chickens with high maternally derived antibodies. *Animals*, **9**(3), 72.

Shah, A.U., Li, Y., Ouyang, W., Wang, Z., Zuo, J., Shi, S., Yu, Q., Lin, J. and Yang, Q., (2021): From nasal to basal: single-cell sequencing of the bursa of Fabricius highlights the IBDV infection mechanism in chickens. *Cell & Bioscience*, **11**, 1-24.

Sharif, A. and Ahmad, T., (2018): Preventing vaccine failure in poultry flocks. Immun-Vaccine Adjuv Deliv Syst Strateg, 10.

Sharma. J., Kim. I., Rautenschlein. S., and H. (2000)*:* Infectious bursal disease virus of chickens: Pathogenesis and immune suppression. *Dev. Comp. Immunol.* **24***:223–235*

Shegu, D., Sori, T., Tesfaye, A., Belay, A., Mohammed, H., Degefa, T., Getachew, B., Abayneh, 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**, 1367-1375.

Smith, J., Sadeyen, J.R., Butter, C., Kaiser, P. and Burt, D.W., (2015): Analysis of the early immune response to infection by infectious bursal disease virus in chickens differing in their resistance to the disease. Journal of virology, **89**(5), 2469-2482.

Solomon W, Abebe W (2007): Infectious bursal disease (Gumboro disease) case report of Andasa poultry farm Amahara region. *Ethiopian Veterinary Journal* **11**: 151.

Spies, U., Müller, H. and Becht, H., (1987): Properties of RNA polymerase activity associated with infectious bursal disease virus and characterization of its reaction products. *Virus research*, **8**(2), 127-140.

Swai, E.S., Kessy, M.J., Sanka, P.N. and Mtui, P.F., (2011): A serological survey for infectious bursal disease virus antibodies in free-range village chickens in northern Tanzania. *Journal of the South African Veterinary Association*, **82**(1), 32-35.

Sze, L.P., Isa, N.M., Rahman, O.A., Ideris, A. and Mohd, H.B., (2016): Vaccines and vaccination against infectious bursal disease of chickens: Prospects and challenges. *Pertanika Journal of Scholarly Research Reviews*, **2**(2).

Tacken, M.G., Thomas, A.A., Peeters, B.P.H., Rottier, P.J. and Boot, H.J., (2004): VP1, the RNA-dependent RNA polymerase and genome-linked protein of infectious bursal disease virus, interacts with the carboxy-terminal domain of translational eukaryotic initiation factor 4AII. *Archives of virology*, **149**, 2245-2260.

Tadesse B, Jenbere S (2014): Sero-Prevalence of Infectious Bursal Disease in Backyard Chickens at Selected Woredas of Eastern Ethiopia. *Journal of Biology*, **4**(17).

Taifebagherlu, J., Talebi, A. and Allymehr, M., (2022): concurrent occurrence of infectious bursal disease and respiratory complex caused by infectious bronchitis and avian influenza (h9n2) in broilers. *Bulgarian Journal of Veterinary Medicine*, **25**(4).

Terefe, S., (2018): Infectious bursal disease: an important challenge in transforming the Ethiopian poultry sector. *Greener Journal of Agricultural Sciences*, **8**, 277-285.

Tesfaheywet Z, Getnet F (2012): Seroprevalence of infectious bursal disease in chickens managed under backyard production system in Central Oromia, Ethiopia. African Journal of MicrobiologyResearch **6**(38): 6736-6741.

Tirfie, F.W., (2021): Review on Poultry Production Status and Economic Contribution of the Sector in Ethiopia.

Trapp, J. and Rautenschlein, S., (2022): Infectious bursal disease virus' interferences with host immune cells: what do we know?. *Avian Pathology*, **51**(4), 303-316.

Vakharia, V.N., He, J., Ahamed, B. and Snyder, D.B., (1994): Molecular basis of antigenic variation in infectious bursal disease virus. *Virus research*, **31**(2), 265-273.

Van den Berg TP (2000): Acute infectious bursal disease in poultry: a review. *Avian Pathology* **29**(1): 175-194.

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

Van den Berg, T.P., Morales, D., Eterradossi, N., Rivallan, G., Toquin, D., Raue, R., Zierenberg, K., Zhang, M.F., Zhu, Y.P., Wang, C.Q. and Zheng, H.J., (2004): Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. *Avian Pathology*, **33**(5), 470-476.

Vidalain, P.O. and Tangy, F., (2010): Virus-host protein interactions in RNA viruses. *Microbes and infection*, **12**(14-15).

Viral zone (2015): infectious bursal disease virus (Gumboro virus) particle.

Von Einem, U.I., Gorbalenya, A.E., Schirrmeier, H., Behrens, S.E., Letzel, T. and Mundt, E., (2004): VP1 of infectious bursal disease virus is an RNA-dependent RNA polymerase. *Journal of general virology*, **85**(8), 2221-2229.

Vrdoljak, A., (2018): Comprehensive evaluation of infectious bursal disease vaccine candidate according to the current industry standards. *Journal of Veterinary Medicine and Research*,1164–1172.

Wagari, A., (2021): A review on infectious bursal disease in poultry. *Health Econ. Outcome Res. Open Access*, **7**(2), 167.

Wang, S., Teng, Q., Jia, L., Sun, X., Wu, Y. and Zhou, J., (2014): Infectious bursal disease virus influences the transcription of chicken γc and γc family cytokines during infection. *PLoS One*, **9**(1), 84503.

Wang, W., He, X., Zhang, Y., Qiao, Y., Shi, J., Chen, R., Chen, J., Xiang, Y., Wang, Z., Chen, G. and Huang, J., (2022): Analysis of the global origin, evolution and transmission dynamics of the emerging novel variant IBDV (A2dB1b): The accumulation of critical aa-residue mutations and commercial trade contributes to the emergence and transmission of novel variants. *Transboundary and Emerging Diseases*, **69**(5), 2832-2851.

Wang, Y., Sun, H., Shen, P., Zhang, X. and Xia, X., (2009): Effective inhibition of infectious bursal disease virus replication by recombinant avian adeno-associated virus-delivered microRNAs. *Journal of general virology*, **90**(6), 1417-1422.

Whitfill, C.E., Haddad, E.E., Ricks, C.A., Skeeles, J.K., Newberry, L.A., Beasley, J.N., Andrews, P.D., Thoma, J.A. and Wakenell, P.S., (1995): Determination of optimum formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal disease antibody with IBDV. *Avian diseases*, 687-699.

Wu, C.C., Lin, T.L., Zhang, H.G., Davis, V.S. and Boyle, J.A., (1992): Molecular detection of infectious bursal disease virus by polymerase chain reaction. *Avian Diseases*, 221-226.

Yang, H. and Ye, C., (2020): Reverse genetics approaches for live-attenuated vaccine development of infectious bursal disease virus. *Current Opinion in Virology*, **44**,139-144.

Ye, C., Jia, L., Sun, Y., Hu, B., Wang, L., Lu, X. and Zhou, J., (2022): Inhibition of antiviral innate immunity by birnavirus VP3 protein via blockage of viral double-stranded RNA binding to the host cytoplasmic RNA detector MDA5. *Journal of virology*, **88**(19), 11154-11165.

Ye, G., Liu, H., Zhou, Q., Liu, X., Huang, L. and Weng, C., (2022): A tug of war: pseudorabies virus and host antiviral innate immunity. *Viruses*, **14**(3), 547.

Yeh. H., Rautenschlein u. J., and Sharma. M. (2002): Protective immunity against infectious bursal disease virus in chickens in the absence of virus-specific antibodies. *Vet Immunol Immunopathol.* **89,** 149-158.

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S. and Fujita, T., (2004): The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nature immunology*, **5**(7), 730-737.

Zachar, T., Popowich, S., Goodhope, B., Knezacek, T., Ojkic, D., Willson, P., Ahmed, K.A. and Gomis, S., (2016): A 5-year study of the incidence and economic impact of variant infectious bursal disease viruses on broiler production in Saskatchewan, Canada. *Canadian journal of veterinary research*, **80**(4), 255-261.

Zegeye S, Yisehak T, Haftay A, Nesibu A (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.

Zhang, S. and Zheng, S., (2022): Host combats IBDV infection at both protein and RNA levels. Viruses, **14**(10), 2309.

Zhang, W., Wang, X., Gao, Y. and Qi, X., (2022): The Over-40-years-epidemic of infectious bursal disease virus in China. *Viruses*, **14**(10), 2253.

Zorman Rojs, O., Krapež, U., Slavec, B., Juršič-Cizerl, R. and Poljanec, T., (2011): Field efficacy of different vaccines against infectious bursal disease in broiler flocks. Acta Veterinaria Hungarica, **59**(3), 385-398.

8. APPENDICES

Appendice 1: Ethical Clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
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Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/04/29/16/2024

Name of Applicant: **Debebe Ashenafi Bekele (MSc, PhD student)**

Address: Department Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Molecular characterization and vaccine-matching studies of circulating strains of Infectious Bursal Disease Virus (IBDV)in Ethiopia for improved diagnostics and vaccination strategies*

Date of application:	**December, 2023**
Nature of the project:	**Filed and experimental study, questionnaire survey**
Target animal species:	**Chicken**
Number of animals involved:	**300**
Study area:	**Ethiopia**

Minutes No. and date of review: **VM/ERC/04/16/024, 16/05/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of **Debebe Ashenafi Bekele.**

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia

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Please quote Our Ref. No. When replying

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Fax 251-11-4339933
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Tel. +251 114338450
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P.o.x. Box}34
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Bishoftu, Ethiopia

Appendice 2: Questionnaire

Introduction

A semi-structured questionnaire administered to interview selected owners of commercial poultry farms to assess potential risk factors for the difference in IBDV vaccine response of chickens.

Thank you for participating in this survey aimed at evaluating the efficacy of the Gumboro vaccine. Your valuable input will contribute to important research in the field of poultry health. Please answer the following questions to the best of your knowledge, practice, and experience. All the information will be confidential.

A. Demographic Information

1. Location (Town/District): _____ kebele_____
2. Age of respondent: < 20 years □ >20 < 30 years □ > 30 years
3. Gender: Male □ Female □
4. Years of experience in poultry farming: < 1 year □ >1 year < 5 years □ > 5 years □
5. Educational level:
    - Read and write
    - Elementary school
    - High school
    - Higher education
6. What is your role in the poultry industry?
    - Veterinarian/animal health practitioner
    - Farm owner
    - Chicken farm attendant
    - Other (specify)

B. General information (Management and flock history)

1. Type of farm/production:

    - Layer
    - Broiler
    - Dual purpose

2. To which production system is the farm categorized?
    - Backyard scavenging (family-owned) for subsistence
    - Semi-intensive commercial farms
    - Intensive commercial

3. Breed of Poultry in your Farm:
    - Cobb 500
    - Sasso
    - Lohmann brown
    - Bovans brown
    - Other (Specify), _____

4. Number of chickens found on your farm currently?
    - Less than 1000
    - 1001-2500
    - Greater than 2500

5. Housing you use for your poultry is a type of?
    - Deep litter system
    - Cage system
    - Other (specify), _____

6. The major feeding system in your flock is a type of:-
    - Scavenging
    - Housed feeding
    - Rationed
    - Scavenging with regular supplementation
    - Scavenging with conditional supplementation

- Other, _____

7. What source of water are you using for chicken on your farm?
   - Well water
   - Pipe water
   - Other (specify), _____
8. Do you keep a record of chickens in and out, vaccination, and treatment?

   - Yes
   - No

C. Awareness and experience of Gumboro Disease, outbreak, and Vaccines

1. How familiar are you with Gumboro disease and the Gumboro vaccine?
   - Very familiar
   - Somewhat familiar
   - Not familiar at all
2. Are you aware of the current prevalence of Gumboro outbreak in your area?

   - Yes
   - No
   - Unsure

3. Do you believe that the Gumboro outbreak poses a significant effect to the poultry industry?

   - Yes
   - No
   - Unsure

4. Have you encountered an outbreak of Infectious Bursal Disease (Gumboro disease) in poultry under your care?
   - Yes

- No

5. If yes, how did the outbreak impact your poultry or patients?
    - Increased mortality
    - Decreased egg production
    - Profit losses
    - Other (please specify): _
    - Increased mortality and profit losses
    - I have faced all the problems

D. Information Gumboro Disease Vaccines

1. Which vaccine types do you use mostly for Gumboro disease?
    - Local
    - Imported

2. How frequently is the Gumboro vaccine administered in your poultry farm/pharmacy?
    - once
    - twice
    - thrice
    - Other (please specify): _____
3. Are you aware of the recommended storage conditions for the Gumboro vaccine?
    - Yes
    - No
4. Are the storage facilities for the Gumboro vaccine adequate for your poultry farm?
    - Yes
    - No
5. Do you ensure that the Gumboro vaccine is maintained within the cold chain during transportation and storage?
    - Yes
    - No
6. What challenges, if any, do you face in administering the Gumboro vaccine?

- Vaccine availability
- Proper training for administration
- Vaccine storage
- Other (please specify):
- None

E. Knowledge on information source and vaccination protocol

1. Where do you usually seek information about the Gumboro vaccine?
    - Veterinarians/animal health assistants
    - Scientific journals
    - Industry publications
    - Training programs
    - Other (please specify): _
    - Veterinarians/animal health assistants and Training programs
    - Veterinarians/animal health assistants and Scientific journals
    - Industry publications and Training programs
    - Friends in the same industry
2. Do you strictly adhere to the label instructions when administering the Gumboro vaccine?
    - Yes
    - No
    - To some extent
3. What vaccination protocols do you follow to prevent IBD outbreaks in your chickens?
    - Based on vaccine manufacturer's protocol
    - Friend suggestion
    - By veterinarian/animal health practitioner/ recommendation
    - By Myself
    - Other (please specify): _____

F. Knowledge on vaccine efficacy assessment

1. What factors do you consider when selecting an IBD vaccine for poultry farms? (Select all that apply)

    - Cost
    - Efficacy
    - Ease of administration
    - Manufacturer's reputation
    - Availablity
    - Cost and efficacy

2. Do you conduct follow-up assessments to evaluate the long-term efficacy of the Gumboro vaccine in your poultry?
    - Yes
    - No
3. How do you currently measure the efficacy of the Gumboro vaccine in your poultry farm?
    - Clinical signs observation
    - Laboratory testing
    - Both
    - Not measured
    - Never thought about that
4. Have you ever provided feedback on the efficacy of the Gumboro vaccine to the vaccine supplier or manufacturer?
    - Yes
    - No
5. Do you communicate the efficacy of the Gumboro vaccine to your customers or superiors?
    - Yes
    - No
    - Some time

G. Awareness on IBD vaccine efficacy

1. How aware are you of the efficacy of the Gumboro vaccine in preventing IBD outbreaks?
    - Highly aware
    - Somewhat aware
    - Not aware
2. How effective do you think current IBD vaccines are in preventing Gumboro outbreak?

    - Highly effective
    - Moderately effective
    - Ineffective
    - Other
    - I don't know

3. Have you encountered any instances of vaccine resistance in Gumboro outbreak management?

    - Yes
    - No
    - Unsure

4. Do you believe there is a need for improved vaccines to combat Gumboro outbreak?

    - Yes
    - No
    - Unsure

5. What do you recommend to improve the efficacy of the Gumboro vaccine in preventing IBD outbreaks?
    - Enhanced training programs
    - Improved vaccine storage facilities
    - Enhanced monitoring systems

- Vaccine matching and efficacy testing
- Other (please specify): _____

H. Progress of Gumboro Disease Outbreak

1. In your experience, does the use of the Gumboro vaccine led to a decrease in IBD outbreaks?
    - Significant decrease
    - Moderate decrease
    - No decrease
    - Not sure

1. In your experience, have you observed a decrease in the incidence of the Gumboro outbreak over the past few years?

    - Yes, a significant decrease
    - Yes, a slight decrease
    - No change
    - Unsure

2. If you have observed a decrease, what factors do you attribute to this improvement? (Select all that apply)

    - Improved vaccination programs
    - Better vaccines than before
    - Enhanced biosecurity measures
    - Better disease monitoring and diagnostics
    - Other (please specify), _____

3. Do you believe that the decrease in the Gumboro outbreak has/will positively impacted the overall health and productivity of poultry farms?

    - Yes
    - No
    - Unsure

4. Do you believe that increased awareness and education on the Gumboro outbreak and vaccination practices can lead to better disease management in poultry farms?

    - Yes
    - No

Thank you for your participation in this questionnaire. Your information and identity will remain confidential. Your input is valuable in understanding the current landscape of Gumboro outbreak management and vaccine efficacy in the poultry industry.

Appendice 3: Indirect ELISA Testing Procedure

Allow reagents to come to room temperature (21°C 15°C) before use. Homogenize all reagents by inversion or vortexing. 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. Samples, however, are tested at a final dilution of 1:500 in Dilution Buffer 14 (1:50 pre-dilution, followed by 1:10 dilution in the microplate).

1. In a pre-dilution plate, set aside wells A1, B1, C1 and D1 for the controls, and add: 5 µl of each sample to be tested, 245 µl of Dilution Buffer 14 to all well EXCEPT to control wells A1, B1, C1 and D1. Note: It is recommended to respect the indicated order of deposit to be able to visually control addition of sample to each well.
2. In the ELISA microplate, add 100 µl of the Negative Control to wells A1 and B1, 100 µl of the Positive Control to wells C1 and D1, 90 µl of Dilution Buffer 14 to as many wells as there are samples to be tested (NOT to control wells A1, B1, C1 and D1), 10 µl of the pre-diluted samples as prepared above.
3. Cover the plate and incubate 30 min + 3 min at 21°C(+5°C).
4. Prepare the Conjugate 1X by diluting the concentrated conjugate 10X to 1:10 in Dilution Buffer 3
5. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash 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 30 min 3 min at 21°C. (±5°C).
8. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash Solution 1X. Avoid drying of the wells between washes,
9. Add 100 ul of the 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 the Stop Solution to each well in order to stop the reaction. The Stop Solution should be added in the same order as in step N°9.
12. Read and record the O.D. at 450 nm.

Appendice 4: Blood collection procedure from wing vein

1. Gently restrain the chicken to ensure safety and minimize stress.
2. Locate the brachial wing vein on the underside of the chickens wing, near the elbow.
3. Place the chicken in lateral recumbency with its feet facing the phlebotomist.
4. The assistant lifts and stretches the top wing to expose the vein for collection.
5. Insert a needle into the brachial wing vein at a shallow angle (10-20°) with the bevel up.
6. Aspirate gently to confirm proper placement in the vein.
7. Collect the desired amount of blood into a syringe or collection tube.
8. Withdraw the needle carefully to avoid causing discomfort or injury to the chicken.
9. Apply pressure using cotton to the site of venipuncture to prevent bleeding.

Appendice 5: Pictures taken during the Experiment