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ASSESSMENT OF SEROCONVERSION AND PROTECTION LEVEL AGAINST VIRAL DISEASES IN COMMERCIAL LAYER CHICKENS IN BISHOFTU TOWN, ADA'A AND LUME DISTRICTS, CENTRAL ETHIOPIA

MSc THESIS

BY:

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DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

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ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH DEPARTMENT

ASSESSMENT OF SEROCONVERSION AND PROTECTION LEVEL AGAINST VIRAL DISEASES IN COMMERCIAL LAYER CHICKENS IN BISHOFTU TOWN, ADA'A AND LUME DISTRICTS, CENTRAL ETHIOPIA

A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science Poultry health and management

BY

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June, 2025

Bishoftu, Ethiopia

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Program: MSc. in Poultry Health and Management

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As a member of the Board of Examiners of the MSc. Thesis open defence, we certify that we have read and evaluated the Thesis prepared by Nabon Debela entitled "Assessment of Seroconversion and Protection Level Against Viral Diseases in Commercial Layer Chickens in Bishoftu town, Ada'a and Lume Districts, Central Ethiopia'' and examined the candidate. We recommend that the Thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Science in Poultry Health and Management

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DEDICATION

I dedicate this piece of work to my whole family who committed their lives with strong prayers for the betterment, patience, love and success of my life and with memory of my beloved father Debela Wayima.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that the thesis is my own work and all material used for this thesis has been given acknowledged through citation. I solemnly declare that it has not been previously submitted to any other University or institution for the award of any academic degree, diploma, or certificate. This thesis is submitted in partial fulfilment of the requirements for a Master of Science degree (MSc.) at the Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library.

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LIST OF ABBREVIATIONS

ADOLDF	Ada'a District Office of Livestock Development and Fisheries
AGID	Agar gel immunodiffusion
AGP	Agar gel precipitation
CI	Confidence interval
Classic LB	Classic Lomas Brown
CV	Coefficient of variance
DL	Deep Litter
ELISA	Enzyme linked immunosorbent assay
EU	European Union
HI	Hem agglutination inhibition
IBDV	Infectious bursal disease virus
IBV	Infectious bronchitis virus
IFT	Immune fluorescent test
IHA	Indirect hem agglutination
IHPT	Immune histopathology test
KAP	Knowledge, Attitude and practices
Max	Maximum
Min	Minimum
NCDV	Newcastle disease virus
OD	Optical density
OIE	Office International des Epizooties
PAs	Peasant associations
PCR	Polymerase chain reaction
SD	Standard deviation
S/P	Sample ratio to positive
VN	Virus neutralization
WOAH	World organization animal health

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ABSTRACT

Chickens receive live attenuated or inactivated vaccines to control Newcastle disease (NCD), Infectious bursal disease (IBD) and Infectious bronchitis (IB) virus, with humoral antibodies typically developing within 6–10 days in serum and mucosal surfaces. The efficacy of vaccinations can be estimated best with experimental challenges but the procedure is usually expensive and time consuming. Therefore, the current study aimed to assess the seroconversion and protection level against viral diseases including NCD, IB and IBD in commercial layer farms found in Bishoftu town, Ada'a and Lume districts from November 2024 to June 2025. For this purpose, a cross-sectional study and questionnaire survey were conducted to assess risk factors, and serological investigation to detect the protection level of vaccines in chickens found in 19 sampling sites in three study areas making a total of 384 if chickens during the entire study period. Simple random sampling was employed to obtained proportional number of chickens from each of small-scale, medium scale and large-scale farms during the study period. Data recorded during the study period was entered into a Microsoft Excel spreadsheet and descriptive statistics and chi square test were performed using STATA Version 17 software. Out of the 384 chickens tested, 380 achieved protective antibody levels for NCD, resulting in a seroconversion prevalence of 99%. IBV achieved 377 protective antibody levels resulting in a seroconversion prevalence of 98%, whereas 363 of the chickens developed protective antibodies against IBD, resulting in a seroconversion prevalence of 94.5%. Ventilation, hygiene, post-vaccination, flock size and location showed significant difference ($P < 0.05$), however, age, breed and housing were not showed statistically significant difference ($P > 0.05$) with the occurrences of the aforementioned diseases. The study identified key risk factors associated with the development of protective antibodies after vaccination against viral diseases and help to develop evidence-based recommendations for effective management, handling, storage and transportation of the poultry vaccines in the area.

Keywords: *Chicken, Protection level, Seroconversion, Vaccine, Risk factors, East Shewa*

1 INTRODUCTION

The poultry sector in Ethiopia plays critical role in food security, nutrition, and economic development. Production practices range from backyard to commercial production systems, where the backyard system takes more share, followed by small-scale farmers. Most Ethiopian farmers raise small number of chickens in the backyard production system, where women primarily manage chickens, giving them control over the income generated (Tolasa, 2021). As a vital agricultural sector, poultry production has seen rising demand for meat and eggs in rapidly growing urban areas, leading to the expansion of commercial poultry farms in and around cities.

Poultry production sector has expanded substantially in recent years, driven by population growth, changing dietary preferences, rising per capita income, and the sector's profitability, which encourages investments from farmers and entrepreneurs (Asfaw *et al*., 2020; Birhan *et al.*, 2022; Jilo *et al.*, 2022; Mamo and Yimer, 2021). Moreover, the Ethiopian government initiated the program known as Bounty of the Basket "Yelemat Tirufat" has opened doors to boost production and productivity of chicken meats and eggs. However, the sector is hampered by a high prevalence of poultry diseases, which reduce productivity and cause significant economic losses.

The lack of organized poultry health services and limited access to veterinary assistance, including both diagnosis and vaccination, are inevitably linked to high disease occurrence and mortality rates in birds (Assefa, 2024). The rapid expansion of the poultry sector has not been matched by improvements in veterinary services or disease surveillance frameworks. In addition, the implementation of biosecurity measures and control system is not well established. As a result, poultry production systems are frequently affected by various rampant diseases, continue to cause significant losses and pose public health risks.

Disease is widely recognized as one of the most significant constraints to the poultry industry. Poultry diseases are responsible for several adverse economic effects that cause direct financial losses through mortality, morbidity, and reduced productivity, as well as indirect

losses via increased costs, trade restrictions, and negative impacts on livelihoods and public health (Markos *et al.,* 2024). The high prevalence of infectious and parasitic diseases in Ethiopia presents significant challenges to both backyard and commercial poultry farming systems. Diseases such as Newcastle disease (NCD), infectious bursal disease (IBD), and coccidiosis are among the high prevalent and affects the sector's production and productivity (Aliye *et al.,* 2022). Disease outbreaks, particularly NCD and IBD, can cause mortality rates up to 50%, with 40–60% of chicks dying before maturity, especially in backyard systems (Worku *et al.,* 2022).

Poultry disease outbreaks remained a major constraint to poultry producers (Mesele *et al*., 2023). The impact of these diseases extends beyond economic losses, as they threaten food security and rural livelihoods. The high mortality and reduced productivity caused by diseases like NCD, IBD and Infectious bronchitis hinder the ability of smallholder farmers and commercial farms rely on poultry for income generation and nutrition. In nutshell, challenges are persistent to the sector due to inadequate vaccination, poor biosecurity, and limited disease monitoring, all of which contribute to the continued prevalence and impact of these diseases on the poultry industry (Alemu *et al.,* 2024; Wayou *et al.,* 2021; Hassan *et al.,* 2025).

Vaccines have significant role in disease prevention and control worldwide. Vaccinations against the poultry diseases help to avoid disease outbreaks and considered as effective preventive means (Alemu *et al.,* 2024; Birhane and Fesseha, 2020). Despite the intensive application of various commercial vaccines against the viral pathogens in Ethiopia, many outbreaks are still reported in chickens due to a combination of vaccine-related challenges (Birhane and Fesseha, 2020). Vaccination failure in poultry occurs when birds don't develop adequate immunity after vaccination, either because they don't develop sufficient antibody levels or are susceptible to a field disease outbreak (Abdullahi *et al.,* 2009). Various factors can contribute to vaccination failure, including improper vaccine handling, storage, incorrect administration, vaccine quality, age and maternal antibodies, stress and environmental factors, immune suppression, inappropriate vaccination schedules, improper formulation of vaccine, exposure to direct sunlight, use of expired vaccines (Bosha *et al.,* 2012; Birhane *et al.,* 2020).

Proper vaccination also used to develop a very high antibody levels in hens to increase the maternally derived antibodies (passive immunity) in the hatched offspring to protect the chicks against different infectious diseases during the early phase of life (Endale *et al.,* 2022). Seroconversion refers to the development of detectable level of antibodies in the blood or tissue in response to exposure or vaccination, indicating immunity. Protection level, determined by antibody titers, reflects the strength of this immunity. Vaccination strategies, including live and inactivated vaccines, play a significant role in inducing seroconversion and protection (Demeke *et al.,* 2024).

Several previous studies were conducted in different parts of Ethiopia on the seroprevalence of major viral pathogens of poultry, however, data on post-vaccination seroconversion rates in commercial layer chickens remain scarce. Therefore, this study aimed to assess the seroconversion and antibody titers induced by the commonly used vaccines and to determine the level of protection in egg Hub farms and poultry production farms in Bishoftu town, Ada'a and Lume districts, Oromia Regional state, Ethiopia.

General Objectives:

The general objective of this study is to assess the post-vaccination seroconversion and protection level in layer chickens and its associated risk factors in Bishoftu town, Ada'a and Lume district of East Showa Zone Oromia, Ethiopia.

Specific Objectives:

- To determine the seroconversion following vaccination against viral diseases (NCD, IB and IBD).
- To identify antibody titer status influencing post-vaccination seroconversion and protection levels in commercial layer chickens.
- To assess the contribution of farm vaccination and management practices in the development of protective antibody.

2 LITERATURE REVIEW

2.1. General description of common viral disease

2.1.1 Newcastle Disease

Newcastle disease (NCD) is one of the highly pathogenic viral diseases of avian species (Yang *et al.,* 2024). Newcastle disease is caused by viruses of the Avian Paramyxovirus type 1 (APMV-1) serotype of the genus *Avulavirus*, family *Paramyxoviridae* (Behboudi, 2023). Avian paramyxoviruses (family Paramyxoviridae; also known as avian avulavirus) are a highly diverse group of zoonotic, negative-sense, single-stranded RNA viruses detected in a variety of domestic and wildlife species. The pathogenicity of Newcastle Disease Virus ranges from lentogenic (low virulence), mesogenic (moderate virulence) to velogenic (highly virulent). The velogenic NCD can either be viscerotropic or neurotropic depending on its predilection site (Suarez *et al.,* 2020). Lentogenic strains (e.g., LaSota) are widely used in vaccines, while velogenic strains trigger international reporting due to their high mortality which may goes up to100%in poultry leading to significant impact on trade restrictions and embargoes in the regions of its outbreak (Yang *et al.,* 2024).

Newcastle Disease Virus (NCDV) affects various poultry species with varying degrees of susceptibility, influenced by viral strain, host species, and environmental factors. Chickens are the most susceptible, experiencing high morbidity and mortality from virulent strains, while ducks and waterfowl often act as reservoirs with minimal clinical signs (Ul-Rahman *et al.,* 2022; Zeng *et al.,* 2024). Newcastle disease is a viral infection of birds, characterized by respiratory, digestive, and nervous system symptoms, with varying degrees of severity depending on the viral strain (Zeng *et al.,* 2024).

Clinical signs vary depending on the pathotype. Severe forms (velogenic) can cause respiratory distress, nervous signs (paralysis, tremors), diarrhea, and a sharp drop or cessation of egg production. Less severe forms (mesogenic) may present with respiratory problems and decreased egg production. NCDV is spread through direct contact with bodily fluids of

infected birds, including respiratory secretions and feces. Indirect transmission can occur through contaminated feed or objects (Dzogbema *et al.*, 2021).

2.1.1.1 Global Distribution of Newcastle Disease Virus

Newcastle Disease Virus remains a significant global concern, affecting poultry populations worldwide. As of 2025, the virus continues to pose challenges to the poultry industry in many regions. NCDV is present in various parts of the world, with different genotypes circulating in different geographic locations. Globally, NCDV has been reported from 46 OIE members in 2019. In line with this, in 2019 in Asia, 21 OIE members reported NDV, including china and Indonesia, indicating they are where still experiences endemic outbreaks in both free-range and commercial chicken farm (WOAH, 2020; Dharmayanti *et al.*, 2023).

From Africa NCDV has been reported from 17 countries in 2019 (WOAH, 2020). A recent study identified 15 different genotypes across 26 African countries, with genotype VII being the most prevalent (Amoia *et al.,* 2024). From Europe, two countries (Bulgaria and Switzerland) have reported NCDV in 2019 (WOAH, 2020). Western Europe experienced a marked increase in outbreaks during the early 1990s, with 239 outbreaks in EU countries in 1994. Three countries namely United States, Haiti, and Mexico reported NCDV in 2019 to WOAH. However, the disease has been largely controlled in Canada and the United States.

In South America NCDV has been reported in 2019 from three countries namely Colombia, Ecuador, and Peru (WOAH, 2020). Newcastle Disease Virus has a global distribution affecting domestic and wild birds (**Figure.1**). While some Western European countries and North America have made progress in controlling NCDV, it remains a significant threat to poultry production worldwide, particularly in developing regions. The total number of NCD outbreaks reported to the WOAH by these 26 countries from 2005 to 2022 was 15,970, with 5,503,385 cases that led to 3,475,377 domestic chicken deaths (Amoia *et al*., 2024).

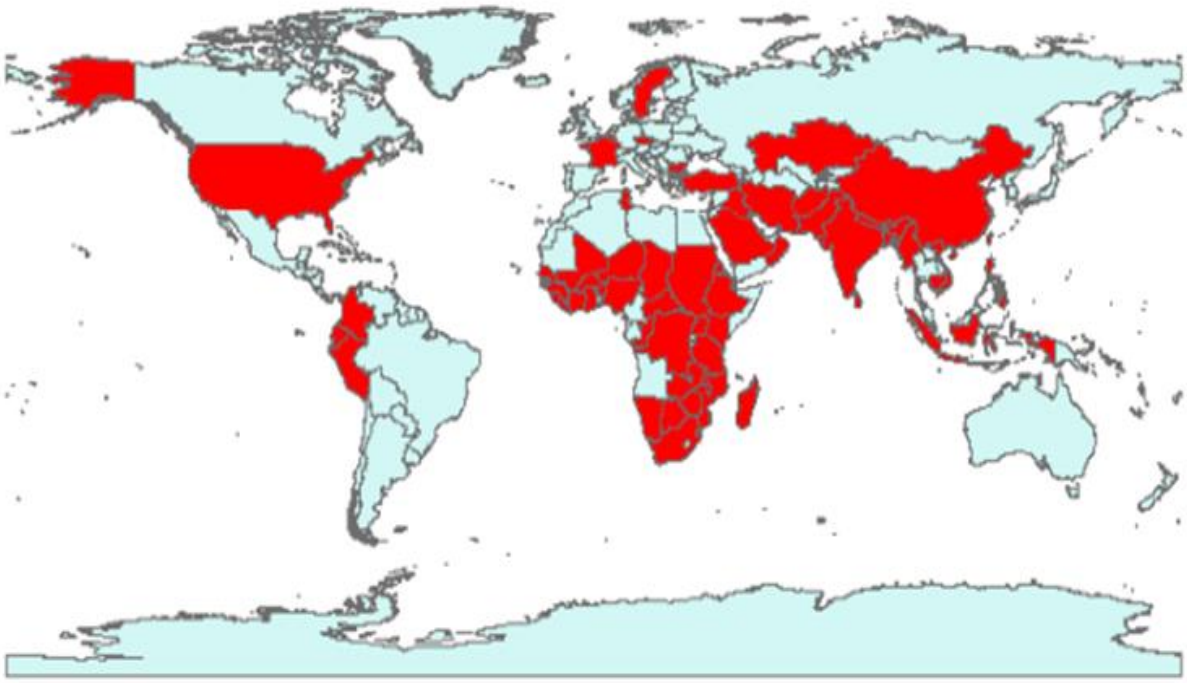

Figure 1: Global distribution of NCDV. Data source: OIE (https://www.oie.int) accessed on 23 June 2019.

In Africa, genotypes I, II, III, IV, V, VI, VII, VIII, XI, XIII, XIV, XVII, XVIII, XX, and XXI have been reported from different countries (**Figure. 2**). With eight different genotypes, Nigeria is the country with the greatest genotypic diversity of NCDV among African countries. Genotype VII is the most prevalent group of NCDV in Africa, which was reported in 15 countries (Amoia *et al*., 2024).

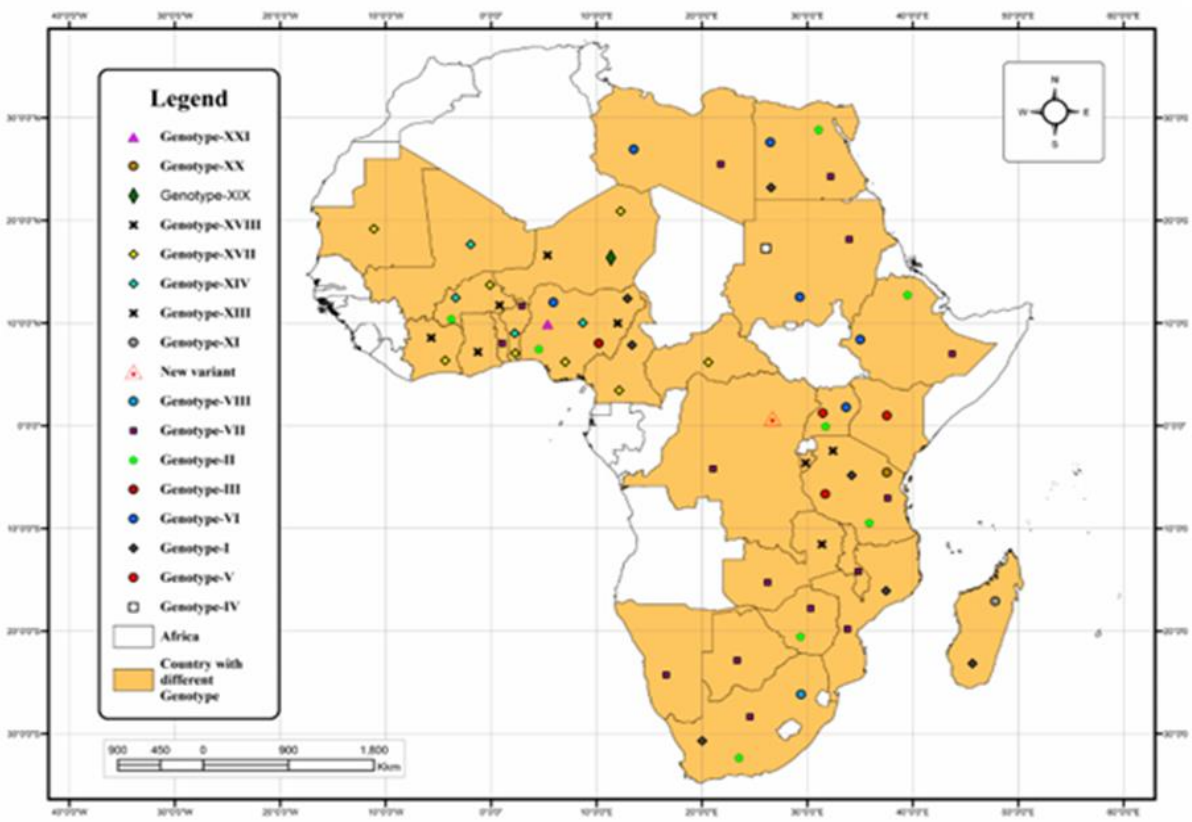

Figure 2: The distribution of NCDV genotypes circulating in African countries by December 2022. *Source: (Amoia* et al., *2024).*

2.1.1.2 Epidemiology of Newcastle Virus in Ethiopia

Newcastle disease virus remains endemic in Ethiopia, posing a significant threat to both commercial and backyard poultry production. Several studies have indicated that the disease is increasing at high seroprevalence rates and frequent outbreaks, particularly affecting village chickens and commercial farms (Sori *et al.*, 2016; Wodajo *et al.*, 2023; Tibebu *et al.*, 2024). The overall pooled seroprevalence of Newcastle disease in chickens in Ethiopia is estimated to be 21.47% (95% CI: 19.54-23.4%) (Zegeye *et al.*, 2022). However, individual studies have reported varying prevalence rates across the country.

A recent study in Melokoza district found an overall seroprevalence of 68.8% (95% CI: 64%, 73%), with the highest seroprevalence of 86% recorded in Gazar kebele of Melokoza district (Betela *et al.*, 2023). Mazengia *et al*. (2009) recorded 21.7% at Bahir Dar; Chaka *et al*. (2012)

reported 5.14% and 7.12% from ATGK and Adea, respectively. More recently, the review and meta-analysis of twenty-four years NCD publication indicated overall NCD prevalence was 22%, with the highest rates in the South (45%) and lowest in Oromia (17%) (Tibebu *et al.*, 2024).

Regarding circulating NCDV, reports indicated that presence of three different genotypes of NCDV in Ethiopia, namely genotypes II, VI, and VII, with a close genetic similarity of virulent isolates with those from Sudan and Egypt (de Almeida *et al.*, 2013; Fentie *et al*., 2014; Damena *et al.*, 2016; Bari *et al*., 2021).

2.1.1.3 Diagnosis, Prevention and control of Newcastle disease

Diagnosis is typically based on clinical signs and confirmed by laboratory testing for NCDV. Poultry respiratory pathogens such as avian influenza, infectious bronchitis, and infectious laryngotracheitis viruses are all considered differential diagnoses that can easily be confused with NCDV based on their clinical presentation (Abd Elfatah *et al.*, 2021). As a result, it requires laboratory confirmation due to the non-specific nature of clinical signs and gross lesions.

Newcastle disease diagnosis is made by virus isolation from tracheal or cloacal swabs together with blood testing to demonstrate high antibody levels. Infectious bronchitis or infectious laryngotracheitis can give similar clinical signs, but lesions, blood tests, and virus isolation tests are decisive. In chickens NCD is characterized by lesions in the brain or gastrointestinal tract. More specific serological techniques most notably monoclonal antibody based serology, have shown the existence of considerable antigenic variation between the different strains of NCD (Brou *et al.*, 2020).

Prevention and control measures include vaccination, good hygiene practices and quarantine of infected flocks. Vaccination with modified live vaccine is primary methods of prevention (Hu *et al.*, 2022). As there is no treatment for NCD, the culling of infected birds combined with strict biosecurity and aggressive vaccination protocols are the most suitable measures to control Newcastle disease outbreaks (Absalón *et al.*, 2019). NCD vaccines are available as

inactivated or live vaccines. For prophylactic use, the lentogenic strains of NCDV of chick embryo origin, such as B1, La Sota, and F, are commonly used as live vaccines (Puro & Sen, 2022). Live vaccines are the most commonly preferred vaccines used in poultry production because of their broader advantages.

2.1.2 Infectious bursal disease

Infectious bursal disease (IBD), also known as Gumboro disease, is a highly contagious, immunosuppressive disease of young chickens (Zhang, *et al.,* 2022). It is caused by IBD virus (IBDV) which is responsible for major economic losses in the poultry industry worldwide. IBDV is a double-stranded RNA virus belongs to the genus *Avibirnavirus* of the family *Birnaviridae* (*C*ampbell *et al.,* 2020). It is a highly contagious viral infection affecting chickens, particularly young birds between 3 to 6 weeks of age.

Gumboro disease is caused by the Infectious Bursal Disease Virus (IBDV), a non-enveloped, double-stranded RNA virus belonging to the Birnaviridae family. The virus primarily targets the bursa of Fabricius, an essential organ for the development of the chicken's immune system, leading to immunosuppression and increased susceptibility to other infections (Zhang *et al.,* 2022).

IBDV has two recognized serotypes, namely serotypes 1 and 2 and both serotypes of the virus can naturally infect chicken, turkey, duck, guinea fowl, ostriches; the pathogenicity being reported only in chicken by serotype 1 (Zhang *et al.,* 2022). Serotype 1 IBDV strains have varying antigenicity and pathogenicity, and are classified as classical, very virulent, and antigenic variants based on their pathogenicity in chickens (Souillard *et al.,* 2024).

Clinical signs usually appear sudden drop in feed and water consumption and may include: Ruffled feathers, diarrhoea (sometimes bloody), dehydration, trembling and weakness, vent pecking, sudden death and infected birds often recover, but their immune system remains compromised or Secondary infections due to immunosuppression. Transmission was highly

contagious, spread through; contaminated feed, water, equipment, and litter, direct contact between birds and fomites (e.g., clothing, tools) (Tilli *et al.,* 2022).

2.1.2.1 Global Distribution of Infectious Bursal Disease

Infectious Bursal Disease (IBD) has a widespread global distribution, affecting most major poultry-producing areas worldwide. The disease is present in approximately 95% of the member countries of the World Organisation for Animal Health (WOAH) (Du *et al.,* 2023). There have been reports of outbreaks in different regions across the world including North America, Europe, Asia, and Africa (Zhang *et al.,* 2022). In Asia, countries like India and Indonesia have reported endemic strains, impacting local poultry industries significantly (Du *et al.*, 2023).

Additionally, studies indicate varying prevalence and strains across countries within Europe, highlighting an intricate pattern of viral genotype circulation. Recent outbreaks continue to be reported worldwide, despite vaccination regimes (Nour *et al.,* 2023). In similar fashion recent study conducted in 2022-2023 in Algeria revealed the persistent circulation of IBDV and indicated as a significant concern for the poultry (Messaï *et al.,* 2024).

Today, the very virulent form is predominant in most countries, and variant strains of IBD are present in several countries (**Figure.3**), leading to sub-clinical forms of the disease. Currently, vvIBDVs have been isolated in Russia, Central Europe, Asia, South America, and the Middle East (Behboudi, 2023). In line with this, it has been proposed that, worldwide, about 60–76% of IBDV isolates are vvIBDV (Fan *et al.,* 2020).

Figure 3: Global distribution of different strains of IBDV

Source: https://poultrycontent.ceva.com/infectious-bursal-disease (OIE, 2017)

2.1.2.2 Epidemiology of Infectious Bursal Disease in Ethiopia

Infectious bursal disease is the most important disease with wide distribution all regions and agro-ecological zones of the country, affecting both commercial farms and backyard poultry production systems (Abey *et al*., 2025). A country-wide recent meta-analysis study in 2025 reported an overall prevalence rate of about 69.4% (95% CI 30.7-96.2) (Abey *et al.*, 2025). It was first reported in Ethiopia in 2005 involving 20–45-day old broiler and layer chickens from commercial farms (Zeleke *et al.*, 2005). Since then, its prevalence has been increasing with geographical expansion.

Subsequently, IBD has become a priority problem in commercial and backyard poultry production systems despite regular vaccination practices (in some cases) using attenuated infectious bursal disease virus (IBDV) D78 vaccine and improved biosecurity measures (Abdeta *et al.*, 2022). In the study conducted by Jenbreie *et al* (2012) on 2597 chickens a prevalence of 83.1% was recorded at Mekelle and Gondar districts using ELISA test. Similarly recent study in western Oromia found a seroprevalence of 66.93% in both intensive and backyard production systems using indirect ELISA (Abdeta *et al*., 2022).

2.1.2.3 Diagnosis, prevention and control of Infectious Bursal Disease

The clinical diagnosis of the acute forms of IBD is based on disease evolution (a mortality peak followed by recovery in five to seven days), and relies on the observation of the symptoms and post-mortem examination of the pathognomonic lesions, in particular of the bursa of Fabricius (Legnardi *et al.,* 2022). However, this can be mistaken to other confusing conditions including avian coccidiosis, Newcastle disease in some visceral forms, stunting syndrome, chicken infectious anaemia, mycotoxicoses and nephropathogenic forms of infectious bronchitis.

Several diagnostic techniques have been used in the detection of IBDV antigen, antibodies, and conserved genes. Serological assays which have been in use for diagnosis and/or confirmation of Gumboro disease include agar gel immunodiffusion test (AGID),PCR, indirect hem agglutination (IHA) test, passive hem agglutination test, enzyme-linked immunosorbent assay (ELISA), immune histopathology test, immune peroxidase test, and immunofluorescent test.These have variable sensitivity and specificity (Hishamund *et al.,* 2023).

In general, ELISA is the primary method for detecting IBDV antibodies, useful for monitoring vaccination responses and seroprevalence in unvaccinated flocks. Vaccination is the main prevention strategy: live attenuated vaccines (for young chicks) and inactivated vaccines (breeder hens to transfer maternal antibodies), strict biosecurity measures: clean and disinfect poultry houses, control of human and equipment traffic and proper disposal of litter and dead birds (Hayajneh & Araj, 2023).

2.1.3 Infectious bronchitis

Infectious bronchitis virus (IBV) is a highly contagious upper respiratory tract avian *Gamma coronavirus* that affects mainly chickens (*Gallus gallus*) but can circulate in other avian species. IBV causes a substantial threat to the poultry industry, by reducing egg yield, growth and mortality levels that can vary in impact. (Falchieri *et al.,* 2024).

It is caused by the infectious bronchitis virus (IBV), an avian gamma coronavirus. IBV possesses a single-stranded, non-segmented RNA genome about 27.6 kb in length that encodes several non-structural and structural proteins (Quinteros *et al.,* 2022). IBV non-structural proteins primarily manipulate host immune responses and replication machinery, while structural proteins mediate virion assembly and infection. These mechanisms collectively enable IBV to evade defences and cause widespread poultry infections (Song *et al.,* 2021; Peng *et al.,* 2022).

Infectious bronchitis primarily occur in young chicken, but it is susceptible toward chickens of all age. The disease is characterized by respiratory signs, kidney damage, and reproductive problems in chickens (Song *et al.,* 2021). Symptoms of IBV; respiratory signs (mainly): coughing, sneezing, nasal discharge (clear to mucous) gasping, swollen sinuses**,** conjunctivitis (eye inflammation), reproductive issues (in laying hens): drop in egg production, poor egg quality (soft-shelled or misshapen eggs), eggshell colour changes (from normal to pale or speckled), renal (Kidney) issues: kidney damage can lead to kidney failure, causing water belly (ascites) in severe cases (Song *et al.,* 2021).

Additionally, the virus has been reported in pheasants and peafowl, these birds can be sub-clinically infected without showing signs of disease (Albayati *et al.,* 2023). However, the severity of IB is influenced by factors such as the virus strain, chicken breed, immune status, environmental conditions, and presence of secondary infections (Ali *et al.,* 2024). Transmission: Highly contagious through: aerosol droplets from infected birds, fomites like equipment, clothing, and feed, contaminated water and feed, contact with other infected birds. The virus can spread rapidly in flocks, particularly in high-density housing (Ali *et al.,* 2024).

2.1.3.1 Global Distribution of Infectious Bronchitis

Infectious bronchitis (IB) is indeed recognized as a major poultry disease across multiple continents, including North America, Europe, Asia, and Africa (Rafique *et al.,* 2024). Infectious. However, the circulating strains may vary in different regions (Lee *et al.,* 2023). Multiple genotypes and serotypes of IBV strains now co-exist in many countries due to the virus's rapid variation and spread (Zhao *et al.,* 2023). Avian infectious bronchitis (IB) was indeed first identified in the United States in the 1930s and has since become a globally distributed disease affecting poultry worldwide.

The disease was initially described in North Dakota as a novel acute respiratory disease in young chickens in the late 1930s by Schalk and Hawn (Khataby *et al.,* 2020; Zhao *et al.,* 2023). Infectious bronchitis virus exhibits significant variability in prevalence and distribution, influenced by geographic regions, poultry production systems, and circulating genotypes. Studies highlight distinct patterns in seroprevalence rates, viral genotypes, and risk factors across different contexts. In Asia, GI-19 (QX-like) dominates in Thailand and Vietnam, alongside GI-16 (Q1-like) and GI-13 (4/91-like) variants (as shown in **Figure. 4**). China reports GI-19 and GI-7 as predominant, linked to evolutionary origins in Taiwan (Zhao *et al.,* 2023; Rafique *et al.,* 2024). IBV has been circulating in Africa since the 1950s, with reports from countries like Egypt, Morocco, Nigeria, In Ivory Coast, South Africa, and Kenya.

Hence, IBV is endemic across Africa continent, with Nigeria and Ivory Coast as hotspots. In Nigeria about 84% and 49.1% seroprevalence of IBV has been recorded in commercial chickens and indigenous chickens, respectively (Bali *et al.,* 2022; Ekiri *et al.,* 2021). Molecular studies detected IBV RNA in 81.8% of tested farms, with strains including Massachusetts, variant02, 4/91, and Q1 variants (Ekiri *et al.,* 2021). In Ivory Coast, there were regular IBV outbreaks in commercial layers with a 72.3% seroprevalence and 14.6% PCR positivity (Bali *et al., 2022).*

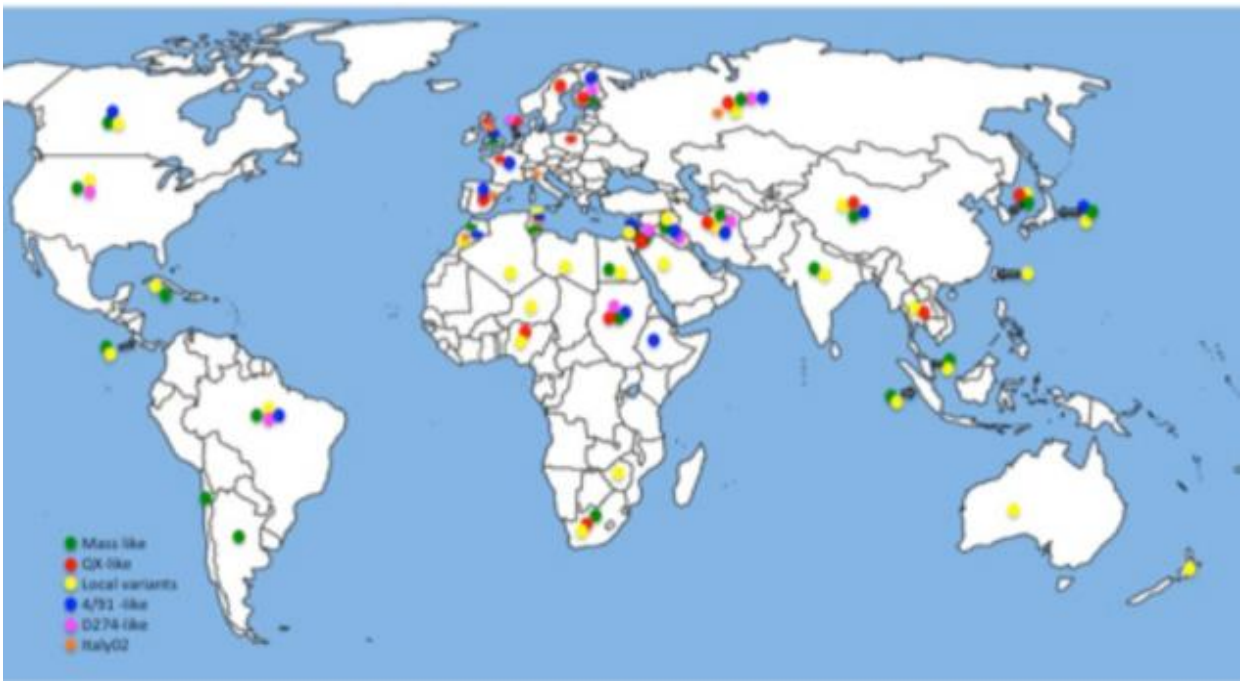

Figure 4: Distribution of major IBV serotypes around the world.

(Bande *et al.*, 2017).

2.1.3.2 Epidemiology of Infectious Bronchitis in Ethiopia

In Ethiopia, infectious bronchitis (IB) poses a significant threat to poultry production, with studies revealing high seroprevalence rates and diverse viral strains. A 2022–2023 study in the Central Gondar Zone found 92.19% seroprevalence using ELISA and 11.54% molecular test using RT-PCR which indicates widespread of exposure and active infections (Mihret *et al.*, 2023). Similarly, research in central Ethiopia reported 97.46% seropositivity in commercial and smallholder farms (Shiferaw *et al.*, 2022). However, earlier studies in Northwest Ethiopia showed lower prevalence rate (23.96%) (Birhan *et al.*, 2021), suggesting presence of regional or temporal variations in IBV circulation.

2.1.3.3 Diagnosis, Prevention and Control of Infectious Bronchitis virus

Although IBV is a virus that causes considerable economic losses in poultry, the clinical signs of IB are not specific. Therefore, it is critical to use different diagnostic test to rule out the disease. The most widely used diagnostic tests include viral isolation, serological (e.g., ELISA) to detect antibodies in the blood and molecular assays (Legnardi *et al.,* 2020). The choice of one test over another is guided by type of sample, availability of test materials and facilities, test reporting time, purpose of the test, and whether the test is carried out in the field or at the laboratory (Samad *et al.,* 2021).

A range of serological tests is available, including agar gel precipitation (AGP), enzyme-linked immunosorbent assay (ELISA), virus neutralization (VN) and hemagglutination inhibition (HI) (Legnardi *et al*., 2020). In the past, serological assays such as virus neutralization (VN) and haemagglutination inhibition (HI) were used widely for detecting and serotyping IBV strains. These tests also have been used to measure flock protection following vaccination (Anaraki *et al*., 2022).

Serotype-specific antibodies usually are detected using HI, PCR (Polymerase Chain Reaction) to identify the specific IBV serotype (Legnardi *et al.,* 2020). Currently, biomolecular assays are the most used tools for IBV detection, because of their sensitivity and quick response time. Besides viral RNA detection, they allow to characterize the detected strains from a genetic standpoint, allowing to properly plan and evaluate vaccination protocols and assess the presence of specific field strains (Ameen *et al*., 2022; Zhao *et al*., 2025; Legnardi *et al.*, 2020).

The remarkable economic impact of IBV on poultry production encourages the implementation of massive vaccination strategies, Live vaccines for primary prevention, especially for young birds, killed vaccines for more sustained immunity and in breeder flocks, vaccination should be carefully matched to local IBV serotypes, timing of vaccination is crucial, with birds typically vaccinated at 1 day old and again at 3-4 weeks (De Wit *et al*., 2022).

Biosecurity Measures: strict hygiene practices (disinfection of equipment and clothing), control of movement into and out of the poultry house, quarantine of new birds or equipment, rodent and insect control to prevent transmission, management: isolation of affected birds to prevent further spread, supportive care for infected birds (e.g., hydration, antibiotics for secondary bacterial infections), ensure proper ventilation in poultry houses to help reduce airborne transmission and control can be eased by good management, correct bird density, air quality, all-in/all-out period duration (Anaraki *et al.*, 2022).

3 MATERIALS AND METHODS

3.1 Study area

The study was conducted from November 2024 to June 2025 in Bishoftu town, Ada'a and Lume Districts of the East Shewa Zones of the Oromia regional state, Ethiopia. Ada'a district is located about 45 km southeast of Addis Ababa, capital of the country. It is found at 08°44′E latitude and 38°58′N longitude. About 90% of the district belongs to the subtropical agro climatic zone having an altitude ranging from 1500 to over 2000 m a. s. l. The district covers an area of about 96,680 hectares. Bishoftu is the town of the study district with a human population of 245,544 (based on a recent household survey) (Kefalew *et al.,* 2015).

The ada'a district receives an annual rainfall of 851 mm with the annual minimum and maximum temperature of 11 and 29°C, respectively. Though the district is most known for cereal crops (mainly teff and wheat) and legumes, livestock production is an integral part of the system. Cattle, small ruminants, poultry, and equines are the major livestock species kept with fast-growing smallholder dairy production. The district has a total livestock population of 264,310 and a total poultry population of 107,554, of which 72,541 are exotic breeds and the remaining 16,700 and 18,313 are local breeds and crossbreeds, respectively (ADOLDF, 2017). Poultry farming in Ada'a District is primarily based on traditional scavenging systems, although there are also commercial farms that contribute to the overall poultry production (Mekonnen *et al.*, 2010).

Lume district is found in East Showa zone of Oromia regional state The district is located 70km South-East of Addis Ababa and cover 75,220.32 ha of land, viz: lowland (Kolla) representing 25%, midland (Weynadega) 45% and highland (Dega) 30% of land coverage of district, with the district having 117,415 total populations. Its geographical location ranges from1450 to 2300 m.a.s.l, annual rainfall ranges from 500 to 1200 mm while the temperature ranges from 18 to 28°C (Guteta and Ameha, 2020).

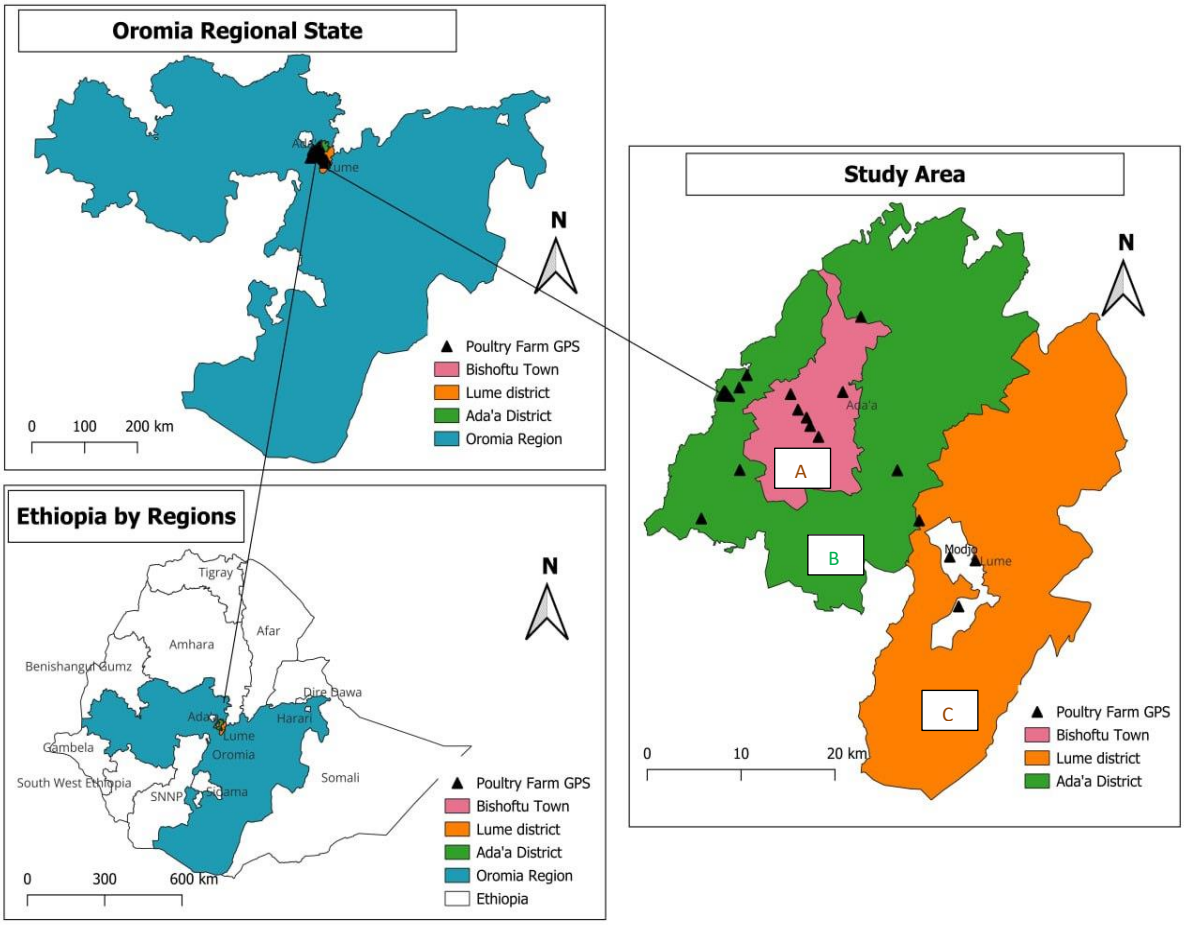

Figure 5: Map of the study area A) Bishoftu town B) Ada'a District C) Lume District

Source: (Abunna *et al*., 2018). By author using QGIS version 3.42.1.

3.2 Study population

The study involved layer chickens from small, medium and large scale production systems, including Egg Hub–affiliated farms, in Bishoftu town, Ada'a, and Lume districts. In the current study, randomly selected chickens irrespective of their breed that were vaccinated against NCD, IB, and IBD within the last three months of period were included.

3.3. Study Design

A cross-sectional study was conducted to assess the serological status (antibody titers) post-vaccination of chicken selected from poultry production system in Egg Hub project and commercial farms in the study area.

3.4. Study Methods

3.4.1. Questionnaire Survey

Questionnaire survey was utilised to collect data from Bishoftu town, Ada'a and Lume districts farmers to gather information on management practices, biosecurity measures, disease history and the knowledge, attitudes and practices (KAP) of owners regarding using vaccine and common viral diseases in poultry. The questionnaires was initially be developed to in English, then translated into the local language, "Afaan Oromo "and pre-tested to enhance understandability and improve reliability (Annex I).

A pretested semi-structured questionnaire was prepared, and smallholder poultry farm managers and employees was interviewed to assess risk factors associated with common poultry diseases, as well as gather information on vaccination history, handling of vaccine, storage of vaccine, transportation of vaccine, recent outbreaks and disease symptoms, the impact of disease, the local name of the disease, predisposing factors, measures taken etc. Respondents were briefed on the study's purpose, and their responses were kept confidential.

The questionnaire comprised key information's such as poultry profiles (age, sex, breed, and type), management systems, housing systems, health and hygiene practices (vaccination, disinfection, and footbath usage), and management strategies, owner knowledge of poultry diseases, egg production performance, flock size and farm record-keeping practices. Oral consent was obtained from participants before administering the questionnaire, which was conducted over a specific period to ensure comprehensive data collection.

In addition, a focused group discussion among the farmers was facilitated to identify common diseases, their understanding, and the associated risk factors. There are a total of 16 project-targeted egg hub-affiliated farms in the study areas, and we planned to include about 25% of those farmers were included in the study population. Therefore, considering these 4 egg hub farms and random sample selection was applied to select the sample population and farms.

3.4.2. Sample size Determination

There is no previous study that showed the seroconversion and protection level of the vaccinated chicken against major poultry disease vaccines in the study area. Therefore, the present study considered 50% expected seroconversion, 95% confidence level, and 5% absolute precision or marginal error. Based on this assumption, the total number of chickens to be included in the study was determined using a formula given by (M. Thrusfield, 2018).

$$n = \frac{Z^2 * Pexp\ (1 - pexp)}{d2}$$

Where n= the required sample size; Z = 1.96 (for a 95% confidence interval); Pexp = expected seroconversion prevalence for each vaccine used in the area, which is 50%; and d = desired absolute precision (5%). Based on the above formula, 384 chickens from the target commercial and egg- hub farms were selected and tested for seroconversion of NCD, IB and IBD vaccines.

3.4.3. Sample Collection

Prior to sampling, a comprehensive physical observation was conducted to assess the health of individual chickens, vaccinated, and identifying any visible signs of illness or abnormalities. Additionally, an overall flock assessment was performed to evaluate the general health and condition of the group. To complement this, detailed flock history regarding any past disease outbreaks was collected through interviews with farm attendants and systematically recorded. This combined approach was ensuring a thorough understanding of flock-level health status.

A total of 384 blood samples were collected from 19 poultry farms (10 small scale, 4 medium scale and 5 large scale) following strict disinfection of the Vena ulnaris (wing vein/brachial vein). Using 3 ml syringes with 23-gauge needles, 2 ml of blood was drawn and transferred to labelled plain vacutainer tubes. To avoid double sampling, each sampled chicken was labeled with a permanent marker on its leg. These tubes were as placed in a nearly horizontal position in a cool environment to allow serum separation from the whole blood. Within 24 hours, the serum was extracted into labelled 1.8 ml cryovials. The samples were transported to the SAL-Alema poultry research center laboratory, where they were stored at −20 °C until testing or further processing OIE (2012).

In addition to the serum samples, a survey questionnaire encompassing open-ended and semi-open-ended questions was administered to commercial poultry farmers. This questionnaire aimed to gather information regarding the age of the chickens, vaccination dates for each disease, records of previous disease outbreaks, and management practices, including feeding and watering.

3.4.4. Indirect ELISA

Sera samples were analyzed for the presence of antibodies against NCDV, IBDV and IBV using commercially available indirect ELISA kits (ID Screen® ND indirect, IBDV and IBV; Veterinary Innovative Diagnostic, 310 rue Louis Pasteur, France) following the manufacturer's instructions. In brief, samples were diluted to a final concentration of 1:100 in a dilution buffer, except for control wells. A total of 100 µL of the negative control was added to wells A1 and B1, while 100 µL of the positive control was added to wells C1 and D1. After adding the samples and controls, each well was washed three times with at least 300 µL of washing solution (1x). The plate was then incubated at 21°C for an additional 30 minutes, followed by the same washing procedure. Next, 100 µL of substrate solution was added to each well and incubated in the dark at 21°C for 15 minutes. To halt the enzymatic reaction, 100 µL of stop solution was added to each well.

The optical density (OD) values of both samples and controls were measured using an ELISA reader (ELX800 ELISA Plate Reader, Biotech Instruments, USA) at a wavelength of 450 nm. Sample-to-positive (S/P) ratios were calculated using the following formula: S/P= (ODsample−ODnegative control) (ODpositive control−ODnegative control) ×100 S/P= (ODpositive control−ODnegative control) (ODsample−ODnegative control)×100. S/P values less than 0.3 were considered negative, while values greater than 0.3 were regarded as positive. For the three tested kits, the antibody titer was further calculated using the formula:

log10 (titer) =1.00 × log10 (S/P) +3.520 log10(titer) =1.00× log10 (S/P) +3.520. Antibody titers were interpreted as negative when the titer was less than or equal to 993, and positive when the titer greater than 993.

3.4.5. Ethical Clearance

All procedures were conducted in accordance with the experimental practices and standards approved by the Animal Research Ethical Review Committee of Addis Ababa University, College of Veterinary Medicine and Agriculture, aligning with international guidelines for animal welfare. The research received approval from the committee under certificate reference number VM/ERC/04/58/17/2025.

3.5. Data analysis

The collected data were entered into a Microsoft Excel spread sheet, edited and coded for analysis. Descriptive statistics, including mean, standard deviation (SD), and CV, were calculated for the seroconversion rates of chickens from each farm. Additionally, chi square test was performed using STATA Version 17 software. A 95% confidence interval (CI) and a 5% level of precision were utilized, with p-values < 0.05 considered statistically significant.

4. RESULTS

The minimum standard protective level of NCD, IB and IBD vaccine based on the manufacturer recommendation was set in the antibody titers. Out of the total of 384 sera of chickens tested, 380 achieved protective antibody levels for Newcastle disease virus (NCDV), resulting in a seroconversion prevalence of 99%. Similarly, out of the total of 384 chickens tested, 377 achieved protective antibody levels for Infectious bronchitis virus (IBV), resulting in a seroconversion prevalence of 98% and whereas 363 of the chickens developed protective antibodies against IBDV, resulting in a seroconversion prevalence of 94.5% (**Figure 6).**

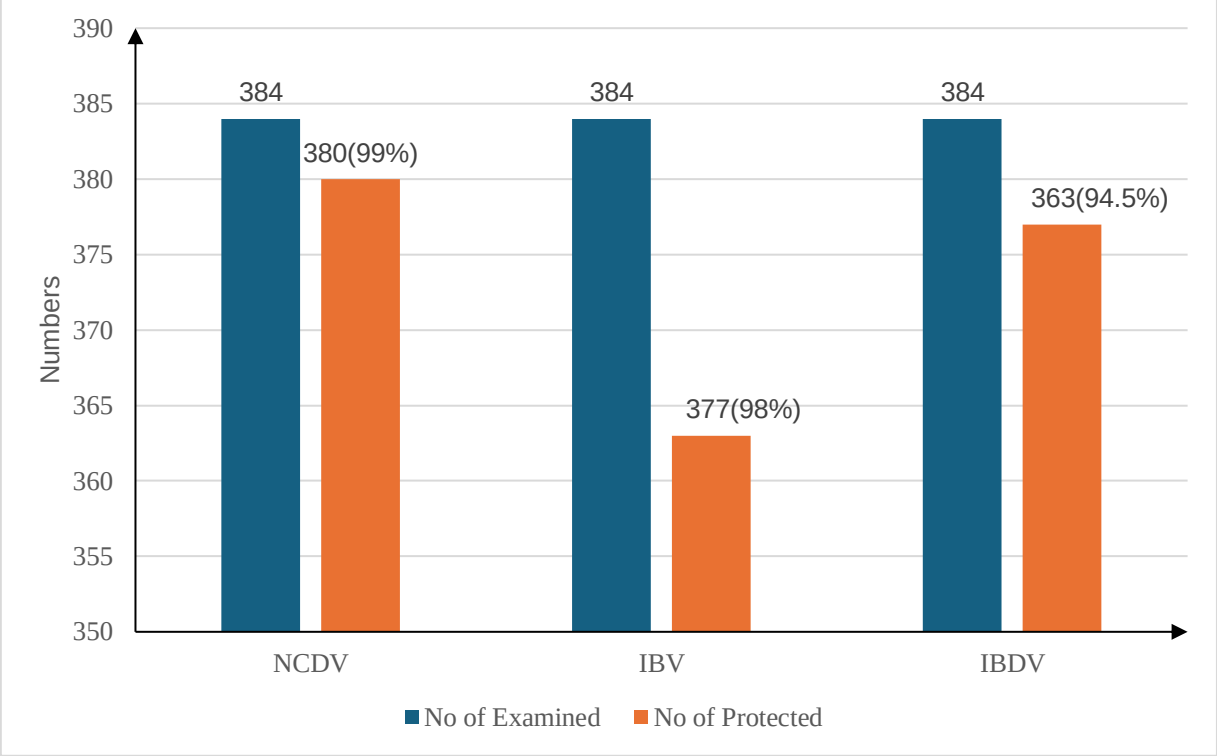

Figure 6: Overall protective antibody levels against NCD, IBV and IBDV in vaccinated chickens during the study period

4.1. Mean Titer Value and Percentage of chickens with Protective Titer for Newcastle Disease

The minimum standard protective level of NCD vaccine as indicated on the manufacturer recommendation was 994 antibody titers (iD.Vet, ID screen NCD Indirect conventional vaccines, 2024/03, batch no/N de lot: 015, France). Interestingly, results of the present study showed the total percentage of vaccine protectiveness in 19 flock size was 99% (380/384), suggesting the presence of good protective immunity in chickens from NCDV.

Based on age categories, chickens with age of 3months induced an average mean antibody titer of 27396.9. However, better level of immunity was induced in birds aged between 4-6 months (titer = 39141.8) followed by chickens of age 7-10 months (titer=33942.8), but chickens with the age of >10 months showed lower antibody titer (34301.8) which may suggest the decline of antibody production at older age. The overall average minimum, maximum and mean antibody titer for this study was 520, 68434 and 34608.8, respectively. The highest antibody mean titer was recorded in flock 16 (68057), while the lowest was observed in flock 6 (13134.8) (**Table 1).**

Table 1: Mean titer value of each flock using indirect ELISA test and percentage of chickens with protective titer per flocks vaccinated against Newcastle disease.

Flock Number	Number of Samples	Breed	Age (Month)	Period since last vaccination	Min titer	Max titer	Mean	SD	CV %	% of chickens per flock with protective titer
1	24	Lohman Brown	2	1 month	6049	33056	28719.25	8206.7	28.6	24/24 (100)
2	20	Classic LB	8	2 month	3225	33026	32401	7787.1	24.03	20/20 (100)
3	20	Lohman Brown	8	1 month	7483	32954	27920.65	8338.8	29.9	20/20 (100)
4	20	Bovans Brown	9	1 month	5900	32858	26855	8111.5	30.2	20/20 (100)
5	20	Bovans Brown	5	3 month	4261	32745	23485.75	10599.2	45.1	20/20 (100)
6	20	Lohman Brown	11	3 month	980	32136	13134.85	11234.6	85.5	19/20 (95)
7	20	Lohman Brown	8	1 month	599	32854	20217.35	9873.2	48.8	19/20 (95)
8	20	Lohman Brown	5	1 month	1254	30540	19215.15	10573.1	55.02	20/20 (100)
9	20	Lohman Brown	3	1 month	857	32791	22537	9208.6	40.8	19/20 (95)
10	20	Lohman Brown	3	1 month	3241	42891	30934.6	13338.6	43.2	20/20 (100)
11	20	Cockek	8	2 month	19053	42209	34693.1	8075.7	23.3	20/20 (100)
12	20	Lohman Brown	8	1 month	16689	42752	34286.5	8252.4	24.06	20/20 (100)
13	20	Lohman	1.2 Year	2 month	6311	42454	32474.7	11431	35.2	20/20 (100)

		Brown								
14	20	Lohman Brown	6	1 month	520	68325	38730.8	17277.4	44.6	19/20 (95)
15	20	Lohman Brown	6	1 month	20357	68047	56452.1	15459.9	27.4	20/20 (100)
16	20	Lohman Brown	5	1 month	18066	68246	68057	16263.5	23.9	20/20 (100)
17	20	Bovans Brown	1.1 Year	2 month	10457	68434	57316.8	16169.1	28.2	20/20 (100)
18	20	Bovans Brown	8	1 month	33980	67752	61226.2	8821.6	14.4	20/20 (100)
19	20	Lohman Brown	5	1 month	26513	67544	28909.9	16235.3	56.2	20/20 (100)

4.2. Mean Titer Value and Percentage of chickens with Protective Titer for Infectious bronchitis virus

The minimum standard protective level of IBV vaccine as per the manufacturer recommendation was 1625 antibody titers (Id.Vet, ID screen IB indirect 2.0, IBVARSV2 Ver 0223 EN, February 2023, France). Interestingly, results of the current study indicated the total percentage of vaccine protectiveness in 19 flock size was 98% (377/384), suggesting the presence of good protective immunity against IBV in chickens.

Based on age categories, chickens with age of 3months induced an average mean antibody titer of 13841.1. However, better level of immunity was induced in birds aged between 4-6 months (titer = 17062.3) followed by chickens of age 7-9 months (titer=16673.6), but chickens with the age of >10 months showed lower antibody titer (16837.7) which may suggest the decline of antibody production at older age. The overall average minimum, maximum and mean antibody titer detected was 517, 26223 and 16375, respectively. The highest antibody mean titer was recorded in flock 17 (22157), while the lowest was observed in flock 9 (11638.2) (**Table 2).**

Table 2: Mean titer value of each flock using indirect ELISA test and percentage of chickens with protective titer per flocks vaccinated for Infectious bronchitis virus.

Flock Number	Number of Samples	Breed	Age (Month)	Period since last vaccination	Min titer	Max titer	Mean	SD	CV %	% of Animals per flock with protective titer
1	20	Lohman Brown	2	1 month	10120	19140	16647	2769.9	16.6	20/20 (100)
2	20	Classic LB	8	2 month	3924	19039	16074.3	3794.7	23.6	20/20 (100)
3	21	Lohman Brown	8	1 month	4803	19189	17218.6	3202.7	18.6	21/21 (100)
4	20	Bovans Brown	9	1 month	12452	19156	17243.8	2171.5	12.6	20/20 (100)
5	21	Bovans Brown	5	3 month	825	19156	14532	5715.4	39.3	20/21 (95.2)
6	20	Lohman Brown	11	3 month	635	18435	12057.1	5659.4	46.9	18/20 (90)
7	20	Lohman Brown	8	1 month	1034	18987	13196.7	5790.9	43.9	19/20 (95)
8	20	Lohman Brown	5	1 month	517	18777	13131.6	5160.8	39.3	19/20 (95)
9	20	Lohman	3	1 month	595	18797	11638.2	6132.5	52.7	19/20 (95)

		Brown								
10	20	Lohman Brown	3	1 month	1437	20354	13238.2	6105.6	46.1	20/20 (100)
11	20	Cockek	8	2 month	3119	20801	16390.9	5054.3	30.8	20/20 (100)
12	20	Lohman Brown	8	1 month	6603	20972	17604.3	3876.9	22	20/20 (100)
13	21	Lohman Brown	1.2 Year	2 month	2489	20911	16299.2	5595.6	34.3	21/21 (100)
14	20	Lohman Brown	6	1 month	743	23140	17005.3	6375	37.5	19/20 (95)
15	21	Lohman Brown	6	1 month	6064	26223	20395.3	6555.1	32.1	21/21 (100)
16	20	Lohman Brown	5	1 month	5398	25984	20529.3	6939.6	33.8	20/20 (100)
17	20	Bovans Brown	1.1 Year	2 month	3782	25995	22157	5618.2	25.4	20/20 (100)
18	20	Bovans Brown	8	1 month	5395	25684	18986.3	6348.1	33.4	20/20 (100)
19	20	Lohman Brown	5	1 month	7840	25790	16780.4	5630.5	33.6	20/20 (100)

4.3. Mean Titer Value and Percentage of chickens with Protective Titer for Infectious bursal disease virus

The minimum standard protective level of IBDV vaccine as indicated in the manufacturer recommendation was 875 antibody titers (Id.Vet, ID screen IBD indirect, IBDS Ver 0416 EN, September 2021, France). Interestingly, results of the present study demonstrated a total percentage of vaccine protectiveness at 19 flock size was 94.5% (363/384), suggesting the development of good protective immunity against IBDV in chickens.

Based on age categories, chickens with age of 3months induced an average mean antibody titer of 7489.2. However, better level of immunity was induced in birds aged between 4-6 months (titer = 8046) followed by chickens of age 7-9 months (titer=7938.7), but chickens with the age of >10 months showed lower antibody titer (6819.3) which may suggest the decline of antibody production at older age. The overall average minimum, maximum and mean antibody titer recorded was 517, 26223 and 16375, respectively. The highest antibody mean titer was recorded in flock 17 (22157), while the lowest was observed in flock 9 (11638.2) (**Table 3).**

Table 3: Mean titer value of each flock using indirect ELISA test and percentage of chickens with protective titer per flocks vaccinated for Infectious bursal disease virus.

Flock Number	Number of Samples	Breed	Age (Month)	Period since last vaccination	Min titer	Max titer	Mean	SD	CV %	% of Animals per flock with protective titer
1	20	Lohman Brown	2	1 month	1270	26231	9284.5	7523.3	81	20/20 (100)
2	20	Classic LB	8	2 month	467	26338	8386.9	7253.5	86.5	19/20 (95)
3	21	Lohman Brown	8	1 month	330	24045	11706.8	7189.8	61.4	20/21 (95.2)
4	20	Bovans Brown	9	1 month	3059	26974	10869.5	6618.2	60.9	20/20 (100)
5	21	Bovans Brown	5	3 month	1832	24721	9646.5	6159.5	63.9	21/21 (100)
6	20	Lohman Brown	11	3 month	1577	12030	7156.9	2658.3	37.1	20/20 (100)
7	20	Lohman Brown	8	1 month	809	11761	5717.2	2441.4	42.7	20/20 (100)
8	20	Lohman Brown	5	1 month	3266	10776	7128.5	2320.6	32.6	20/20 (100)

9	20	Lohman Brown	3	1 month	3018	14427	7468.4	3197.7	42.8	20/20 (100)
10	20	Lohman Brown	3	1 month	72	13204	5714.6	3727	65.2	16/20 (80)
11	20	Cockek	8	2 month	109	13342	5795.8	3748.2	64.7	17/20 (85)
12	20	Lohman Brown	8	1 month	162	11181	6389.3	4115	64.4	16/20 (80)
13	21	Lohman Brown	1.2 Year	2 month	41	10760	4816.9	3712.4	77.1	17/21 (80.9)
14	20	Lohman Brown	6	1 month	41	12733	6462.5	3610.2	55.9	16/20 (80)
15	21	Lohman Brown	6	1 month	3486	16894	7559.4	3881.9	51.4	21/21 (100)
16	20	Lohman Brown	5	1 month	939	16329	7789.9	4410.9	56.6	20/20 (100)
17	20	Bovans Brown	1.1 Year	2 month	2915	19371	8484.2	4354.5	51.3	20/20 (100)
18	20	Bovans Brown	8	1 month	1312	13838	6705.7	3286.4	49	20/20 (100)
19	20	Lohman Brown	5	1 month	3759	17472	9689.7	3819.4	39.4	20/20 (100)

The overall mean antibody titers were 34,608.8 for NDV, 16,375 for IBDV, and 7,724.9 for IBV (**Figure 7**).

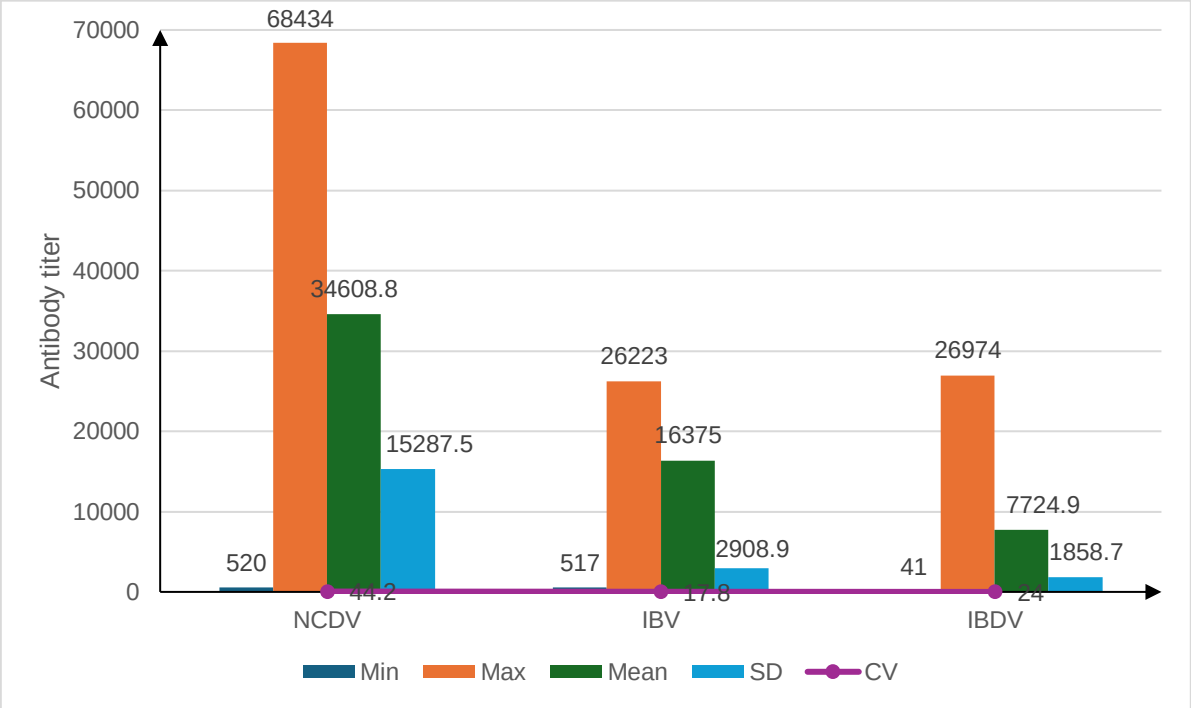

Figure 7: Overall mean antibody titers against NDV, IBDV, and IBV recorded during the study period

4.4. Association of Risk Factors with the development of antibody levels against Newcastle diseases

In the current study, 380 out of 384 (99%) vaccinated layer chickens reached protective antibody levels, indicating the development of strong immunity against NCD. All chickens in Ada'a district (108, 100%) exceeded the protective threshold, followed by Bishoftu (176, 99.4%) and Lume (96, 97%). The level of protection varied significantly across the study areas ($p < 0.05$). The conversion of different breeds of chickens was calculated between Bovans brown, Classic Lomas Brown, Cockek and Lohman brown. The seroconversion and protection level of Loman brown was relatively lower (98.5%), whereas all the other breeds of

chickens have demonstrated. above the protection level for NCD. But the variation among the breed are not statistically significant ($p > 0.05$).

The results showed no statistically significant differences in antibody protection levels among the age groups following Newcastle disease vaccination ($p > 0.05$). Findings of the present study f showed that out of the total of 384 layer chickens sampled, the antibody titer protection level in chickens was significantly ($P < 0.05$) varied by the length of days from the last vaccination. The highest level of antibody protection was detected after 1 month of post-vaccination (99.6 %), followed by 2 months post-vaccination (98.7 %), and 3 months post-vaccination (95), respectively.

Regarding the housing system of the layer birds, sero-conversion status of the vaccine was also assessed and showed that the layers in the full liter (99%) were with above protection level of titer, which was followed by cage housing system (98.7 %) but statistical variation among this housing system was not significant($p > 0.05$). The results of the present study showed that serological antibody levels above the minimum protective threshold were highest in farms with excellent (100%) and very good (100%) ventilation, followed by good (98.1%) and poor (95%) ventilation types suggesting ventilation significantly affects antibody protection levels in layer farms vaccinated against Newcastle disease ($p < 0.05$).

Similarly, farm hygiene had a significant impact ($p < 0.05$) on achieving protective antibody levels. Higher protection level was observed in farms with excellent (100%), very good (99%), good (99%), and poor (96.6%) hygiene conditions (**Table 4**) respectively. The minimum antibody protection level and Sero-conversion against Newcastle disease in vaccinated birds with the a flock size of >10000 layer chickens developed the highest level of antibody titers above the minimum protection level (100%), followed by a flock size of 1500-10000 (99.3%) layer chickens, 500-1500 (98.6%) layer birds and <500 (95%) birds. The results of this study indicate that large poultry farms had significantly ($p < 0.05$) better ability to produce protective level of antibody titer against Newcastle disease as compared to small poultry farms.

Table 4: Association of risk factors with development of antibody titer levels against Newcastle disease virus

Variables	Total sample	Antibody titer ranges (%)								No of protected birds (%)	Chi-square	p-value
		<=993	994-1999	2000-3999	4000-5999	6000-8999	9000-11999	12000-14999	>=15000			
Location											30.9839	0.006
Adea	108	0	0	0	0	2(1.9)	2(1.9)	1(0.9)	103(95.4)	108 (100)		
Bishoftu	177	1(0.5)	1(0.5)	2(1.1)	5(2.8)	5(2.8)	5(2.8)	4(2.3)	154(87)	176 (99.4)		
Lume	99	3(3)	1(1)	5(5.1)	3(3)	3(3)	8(8.1)	5(5.1)	71(71.7)	96 (97)		
Breed											19.1444	0.576
Bovans	80	0	0	0	4(5)	0	3(3.8)	2(2.5)	71(88.8)	80 (100)		
Classic LB	20	0	0	1(5)	0	0	0	0	19(95)	20 (100)		
Cockek	20	0	0	0	0	0	0	0	20(100)	20 (100)		
Lohman	264	4(1.5)	2(0.8)	6(2.3)	4(1.5)	10(3.8)	12(4.5)	8(3)	218(82.6)	260(98.5)		
Age											25.7709	0.215
<=3months	64	1	0	1	1	2	4	3	52	63(98.4)		
4-6months	120	1	1	1	4	3	2	2	106	119(99.1)		
7-9month	140	1	0	1	2	2	4	3	127	139(99.3)		
>10months	60	1	1	4	1	3	5	2	43	59(98.3)		
Last vacc/n											51.8588	0.000
1month	264	1	1	2	4	7	9	6	234	263(99.6)		
2month	80	1	0	1	0	1	0	1	76	79(98.7)		
3month	40	2	1	4	4	2	4	3	20	38(95)		
Housing											16.0941	0.308
Cage syste	80	1	0	0	0	0	0	0	79	79(98.7)		
DL, fully	304	3	2	7	8	10	15	10	249	301(99)		
Ventilation											32.7490	0.037
Poor	20	1	1	1	1	3	1	0	12	19(95)		
Good	160	3	1	4	3	4	7	4	131	157(98.1)		
V/good	40	0	0	0	0	0	1	0	39	40(100)		
Excellent	164	0	0	2	4	3	6	6	143	164(100)		

Hygiene										31.5298	0.039
Poor	60	2	0	0	2	1	4	1	50	58(96.6)	
Good	100	1	2	5	2	5	4	1	80	99(99)	
V/good	104	1	0	1	1	3	3	1	94	103(99)	
Excellent	120	0	0	1	3	1	4	5	106	120 (100)	
Flock size										36.7387	0.018
1-500	20	1	0	0	1	1	2	2	13	19 (95)	
500-1500	148	2	0	0	3	0	3	6	134	146 (98.6)	
1500-10000	152	1	2	6	4	6	8	2	123	151(99.3)	
>=10000	64	0	0	1	0	3	2	2	56	64 (100)	
Egg production										28.9890	0.4724
>85%	60	0	1	1	1	2	3	3	49	60 (100)	
75-84%	80	0	1	0	1	0	1	1	76	80 (100)	
50-74%	120	0	3	4	1	2	6	4	100	120 (100)	
1-50%	80	2	1	1	4	4	3	3	62	78 (97.5)	
Not yet started	44	2	0	1	1	2	2	1	35	42 (95.5)	

4.5. Association of Risk Factors with the development of antibody levels against Infectious bronchitis

The minimum standard protective level of IB vaccine as indicated in the manufacturer recommendation was 1625 antibody titers. Out of the total of 384 sera samples analyzed 377 (98%) of vaccinated layer chickens against Infectious bronchitis virus achieved protective antibody levels for Infectious bronchitis virus (IBV) suggesting the presence of good protective immunity in chickens. Regarding the location of study areas, all chickens demonstrated above the minimum protection level in Ada'a 108 (100%) district, followed by Bishoftu city 174 (98.3%), and Lume district 95(95.9%). The protection level of chickens among the study areas was statistically significant ($p< 0.05$).

The conversion level in different breeds of chickens was compared among Bovans brown, Classic Lohman Brown, Cockek and Lohman brown breeds of chickens. The seroconversion and protection level of Lohman brown was relatively lower (97.7%), whereas all the other breeds of chickens had demonstrated. above the protection level against IBV however, the variation among the different breeds never showed statistically significant ($p >0.05$) variations. The results of the present study didn't showed statistically significant variation ($p > 0.05$). In antibody protection levels among the different age groups of chickens ((≤3 months, 4–6 months, 7–9 months, and >10 months) after vaccination against Infectious Bronchitis (IB).

On the other hand, antibody titers were significantly affected by the time elapsed after the last vaccination ($p < 0.05$). Accordingly, out of the total of the 384 sera of layer chickens tested, the highest proportion of birds with protective antibody levels was detected one month post-vaccination (98.8%), followed by two months (97.5%) and three months (95%). The impact of housing systems on seroconversion was also assessed. Accordingly birds raised in deep litter systems showed 98% protection, while those in cage systems showed 98.7%. Despite these high protection rates, the difference between housing systems was not statistically significant ($p > 0.05$).

Ventilation quality in poultry houses had a significant effect on antibody protection levels ($p < 0.05$). Birds kept under farms with excellent ventilation showed the highest serological protection levels (99.3%), followed by very good (97.5%), good (97.5%), and poor (95%) ventilation types. Similarly, birds kept in farms with excellent hygiene showed significantly highest 99.1% protection of antibody protection ($p < 0.05$) followed by very good (99%), good (97%), and poor hygiene (96.6%) conditions.

Regarding flock size, larger farms (>10,000 birds) had 100% of chickens above the minimum protective antibody titer level followed by farms with 1,500–10,000 birds (98%), 500–1,500 birds (97.9%), and fewer than 500 birds (95%). The difference in protection levels among birds kept in different flock size was statistically significant ($p < 0.05$), indicating that larger flocks tend to achieve better serological protection against Infectious Bronchitis.

The conversion level in different egg production performance was compared among >85%, 75-84%, 50-74%, 1-50% and not yet started egg production of chickens. The seroconversion and protection level of not yet started egg production was relatively lower (95.5%), whereas all the other performance of chickens had demonstrated. Above the protection level against IBV however, the variation among the different egg production performance never showed statistically significant ($p >0.05$) variations.

Table 5: Association of risk factors with development of antibody titer levels against Infectious bronchitis virus

Variables	Total sample	Antibody titer ranges (%)								No of protected birds (%)	Chi-square	p-value
		<=1625	1626-2999	3000-4999	5000-6999	7000-9999	10000-12999	13000-15999	>=16000			
Location											28.16	0.044
Adea	108	0	1(0.9)	2(1.9)	6(5.6)	3(2.8)	3(2.8)	17(15.7)	76(70.4)	108(100)		
Bishoftu	177	3(1.7)	1(0.6)	5(2.8)	5(2.8)	8(4.5)	11(6.2)	30(16.9)	114(64.4)	174(98.3)		
Lume	99	4(4)	2(2)	7(7.1)	7(7.1)	6(6)	10(10.1)	17(17.2)	46(46.5)	95(95.9)		
Breed											13.8	0.878
Bovans	80	1(1.3)	1(1.3)	2(2.5)	4(5)	1(1.3)	3(3.8)	13(16.3)	55(68.8)	79(98.8)		
Classic LB	20	0	0	1(5)	0	0	2(10)	4(20)	13(65)	20(100)		
Cockek	20	0	0	1(5)	0	2(10)	2(10)	1(5)	14(70)	20(100)		
Lohman	264	6(2.3)	3(1.1)	10(3.8)	14(5.3)	14(5.3)	17(6.4)	46(17.4)	154(58.3)	258(97.7)		
Age											26.0	0.091
<=3months	64	2(3.1)	2(3.1)	4(6.3)	2(3.1)	2(3.1)	7(10.9)	15(23.4)	30(46.9)	62(96.9)		
4-6months	120	3	0	2	6	7	7	13	82	117(97.5)		
7-9month	140	1	1	2	5	6	7	16	102	139(99.2)		
>10months	60	1	0	2	3	4	1	8	41	59(98.3)		
Last vacc/n											31.6	0.039
1month	264	3	2	8	14	12	15	49	161	261(98.8)		
2month	80	2	0	4	1	1	5	7	60	78(97.5)		
3month	40	2	0	2	3	5	0	8	20	38(95)		
Housing											14.9	0.384
Cage system	80	1	0	0	6	6	1	11	55	79(98.7)		

DL, fully	304	6	4	14	12	11	23	53	181	298(98)	
Ventilation										30.5	0.040
Poor	20	1	0	1	1	2	2	7	6	19(95)	
Good	160	4	1	4	11	7	10	19	104	156(97.5)	
V/good	40	1	0	1	0	3	1	9	25	39(97.5)	
Excellent	164	1	3	8	6	5	11	29	101	163(99.3)	
Hygiene										31.7	0.038
Poor	60	2	0	2	1	2	4	11	38	58(96.6)	
Good	100	3	0	3	9	7	7	10	61	97(97)	
V/good	104	1	1	2	3	5	7	24	61	103(99)	
Excellent	120	1	3	7	5	3	6	19	76	119(99.1)	
Flock size										33.5	0.032
1-500	20	1	0	1	1	2	1	5	9	19(95)	
500-1500	148	3	3	4	11	7	3	23	94	145(97.9)	
1500-10000	152	3	1	7	6	8	16	25	86	149(98)	
>=10000	64	0	0	2	0	0	4	11	47	64(100)	
Egg production										28.901	0.4176
> 85%	60	0	0	4	1	2	3	10	40	60 (100)	
75-84%	80	1	0	0	6	3	2	7	61	79 (98.7)	
50-74%	120	2	3	5	6	7	9	21	67	118 (98.3)	
1-50%	80	2	1	3	4	3	5	19	43	78 (97.5)	
Not yet Started	44	2	0	2	1	2	5	14	18	42 (95.5)	

4.6. Association of Risk Factors with the development of antibody levels against Infectious bursal disease virus

The minimum standard protective level of IBDV vaccine as per the the manufacturer recommendation was 875 antibody titers. Interestingly, results of the current study showed that out of the total of 384 sera samples analyzed 363 (94.5%) of vaccinated layer chickens with infectious bursal disease vaccine achieved protective antibody levels against infectious bursal disease (IBD) suggesting the development of good protective immunity in chickens. Regarding the location of study area, all vaccinated chickens demonstrated above the minimum protection level in Aa'ea 101 (93.5%) district followed by Bishoftu city 170 (96%), and Lume district 92 (92.9%) with statistically significantly ($p < 0.05$) differences in protection level of chickens among the different study areas.

The comparative detection of seo-conversion among different breeds of chickens including Bovans Brown, Classic Lohman Brown, Cockek and Lohman brown showed lowest (80%) seroconversion and protection level in Cockek breed whereas all the other breeds of Chickens had demonstrated above the protection level for IBDV without the presence of statistically significant ($p > 0.05$) variation among the different breeds. The results of the present study indicated absence of statistically significant ($p > 0.05$). Difference among the different age groups of chickens (≤3 months, 4–6 months, 7–9 months, and >10 months) in protection levels of antibody titers following vaccination against Infectious Bursal Disease Virus (IBDV).

On the other hand, statistically significantly $p < 0.05$) highest proportion of birds with protective antibody levels was observed one month post-vaccination (96.2%) followed by two months (91.2%) and three months (90%) among a total of 384 sera samples collected from chickens. The impact of housing systems on seroconversion was also assessed with birds raised in deep litter systems showed a protection level of 94.4%, while those in cage systems had a slightly higher level at 95%. However, this difference was not statistically significant ($p >0.05$).

Ventilation quality had a significant effect on antibody protection ($p < 0.05$). Farms with excellent ventilation had the highest seroprotection levels (98.1%), followed by very good

(95%), good (91.2%), and poor ventilation (90%). The present study demonstrated that higher protection levels was detected in birds kept under farms with excellent hygiene (98.3%), followed by very good (95.1%), good (92%), and poor hygiene conditions (90%) with the presence of statistically significant ($p < 0.05$), differences among different hygiene levels.

Likewise, birds kept under farms with more than 10,000 birds showed statistically significant ($p < 0.05$) highest protection level (96.8%), followed by those with 1,500–10,000 birds (94.7%), 500–1,500 birds (93.9%), and fewer than 500 birds (92%) suggesting that larger flocks tend to achieve better protective antibody titers against Infectious Bursal Disease.

The conversion level in different egg production performance was compared among >85%, 75-84%, 50-74%, 1-50% and not yet started egg production of chickens. The seroconversion and protection level of 1-50% egg production was relatively lower (87.5%), whereas all the other performance of chickens had demonstrated. Above the protection level against IBDV however, the variation among the different egg production performance showed statistically significant ($p <0.05$) variations.

Table 6: Association of risk factors with development of antibody titer levels against Infectious bursal disease virus

Variables	Total sample	Antibody titer ranges (%)								No of protected birds (%)	Chi-square	p-value
		<=875	876-1999	2000-3999	4000-5999	6000-8999	9000-11999	12000-14999	>=15000			
Location											29.3662	0.048
Adea	108	7(8.3)	3(2.8)	9(8.3)	21(19.4)	32(29.6)	22(20.4)	6(5.6)	8(6.6)	101(93.5)		
Bishoftu	177	7(4.6)	6(3.4)	20(11.3)	28(15.8)	49(27.7)	30(16.9)	16(10.3)	21(11.9)	170(96)		
Lume	99	7(6)	2(2)	13(13.1)	22(222)	33(33.3)	16(15.2)	4(4)	2(2)	92(92.9)		
Breed											26.1895	0.199
Bovans	80	0	2	6	17	26	14	5	10	80(100)		
Classic LB	20	1	2	3	1	5	5	1	2	19(95)		
Cockek	20	4	1	2	3	7	2	1	0	16(80)		
Lohman	264	16	6	31	50	76	49	16	20	248(93.9)		
Age											24.2281	0.282
<=3months	64	4	3	8	11	15	13	4	6	60(93.7)		
4-6months	120	4	2	12	23	42	21	11	5	116(96.6)		
7-9month	140	7	4	18	25	33	20	5	28	133(95)		
>10months	60	6	2	4	12	24	7	3	2	54(90)		
Last vacc/n											31.1966	0.010
1month	264	10	5	32	52	68	53	16	28	254(96.2)		
2month	80	7	4	7	12	26	12	4	8	73(91.2)		
3month	40	4	2	3	7	16	5	3	0	36(90)		
Housing											8.8614	0.840
Cage syste	80	4	1	8	15	25	13	9	5	76(95)		
DL, fully	304	17	10	34	56	88	57	14	28	287(94.4)		
Ventilation											30.7749	0.040
Poor	20	2	0	3	5	5	5	0	0	18(90)		
Good	160	14	3	18	33	45	29	7	11	146(91.2)		
V/good	40	2	0	2	5	15	7	5	4	38(95)		
Excellent	164	3	8	19	28	45	29	11	21	161(98.1)		

Hygiene										30.6370	0.041
Poor	60	6	0	7	12	18	10	2	5	54(90)	
Good	100	8	2	12	23	30	15	7	3	92(92)	
V/good	104	5	4	11	12	27	23	6	16	99(95.1)	
Excellent	120	2	5	12	24	35	22	8	12	118(98.3)	
Flock size										59.2940	0.000
1-500	20	2(5)	0	4(20)	6(30)	6(35)	2(10)	0	0	18(92)	
500-1500	148	9(4.7)	3(2)	12(8.1)	33(22.3)	45(30.4)	25(16.9)	13(8.8)	8(5.4)	139(93.9)	
1500-10000	152	8(10.6)	3(1.9)	16(10)	27(16.9)	50(31.3)	32(20)	5(3.)1	11(6.3)	144(94.7)	
>=10000	64	2(3.1(	5(7.8)	10(15.6)	5(7.8)	12(18.8)	11(17.2)	5(7.8)	14(6.3)	62(96.8)	
Egg production										45.008	0.02
>85 %	60	1	4	10	9	15	7	3	11	59(98.3)	
75-84%	80	2	1	10	17	18	16	6	10	78(97.5)	
50-74%	120	3	4	10	26	42	22	9	4	117(97.5)	
1-50%	80	10	2	6	15	24	18	2	3	70(87.5)	
Not yet started	44	5	4	6	4	8	6	3	8	39 (88.6)	

5. DISCUSSION

Ethiopian poultry industry is continuously suffered by the impact of infectious viral diseases, particularly, NCD, IB and IBD. In this study, a total of 384 blood samples were collected from chickens found in 19 different flocks of layer breeders in Bishoftu town, Ada'a and Lume district, Ethiopia to assess the seroconversions and antibody titter status in birds vaccinated using commonly available vaccines against viral diseases circulating in the study areas.

The results of the present study indicate that almost all flocks showed antibody titers above the minimum protective level (99%) in the tested layer chickens. This was verified by the positive NCD antibody protection level observed across all 19 farms, achieving the critical protective titer threshold of ≥ 85% as recommended by the World Organisation for Animal Health (OIE, 2013). This finding highlights the importance of periodic serological monitoring of commercial poultry farms to ensure the efficacy of the vaccines and the status of the chicken's antibody protection level for specific pathogens on regular basis. Post vaccination antibody titer measurement in serum is the standard method used to evaluate the level of immune responses and vaccine efficacy monitoring (Sarba *et al.*, 2021).

In the present study seroconversion and protective immunity were assessed based on the detectable antibody levels reaching a minimum threshold level. Various factors have been associated to affect the outbreaks of NCD globally including insufficient biosecurity measures, poor vaccination, and immunization practices, antigenic variation, the interference of maternal antibodies with live vaccines, and immunosuppression (Lebdah *et al.*, 2024; Sultan *et al*., 2020).

Out of the total of 384 samples analyzed, 99% of vaccinated layers had antibody levels above the minimum protective threshold, indicating the development of strong immune protection. This finding of the current study is in agreement with the previous findings reported by Numan *et al*. (2005) in Pakistan, where 98.07% of sera samples showed protective of NCDV antibody titer, and Demeke *et al*. (2024) from Ethiopia also reported 90.5% of vaccinated birds exceeding the minimum protective level.

Statistically significant ($P < 0.05$) variation in antibody titer was detected among birds taken vaccines during different months after the last vaccination. Sera samples collected one month of post-vaccination demonstrated the highest protective level (99.6 %), while the seroconversion rate at three months post-vaccination was slightly lower (95%). This observation coincides with previous finding reported by Parvin *et al.* (2015), who detected that the highest antibody titer was recorded at day 21 post-vaccination (100% protective) and decreased to 90% at 35 days of post-vaccination.

Comparable results were also repeoted by Okwor *et al.* (2013) in which chickens vaccinated with the LaSota vaccine developed a maximum antibody titer at 4 weeks post-vaccination and then gradually declined. The present study also assessed the impact of types ventilation used by poultry farms on the minimum antibody titer in vaccinated chickens reared in excellent and very good ventilation status had (100%) significantly ($p < 0.05$) higher protective antibody titer, than those raised in poor ventilation environment had 95%.

This finding is in agreement with the previous report of Tucker et al. (2020) who demonstrated that exposure to high atmospheric ammonia concentrations negatively impacts both growth performance and immunological responses of layer and broiler chickens. This is most probably attributed to the fact that inadequate ventilation potentially leads to elevated ammonia levels. This ammonia build up in poultry farms compromises the immune response in poultry.

Antibody titers against NCD can vary in different breeds, because of differences in the speed of metabolism or the stress induced by the onset of laying (Eze, 2013; Ershad, 2005). However, in this study, significant variation was not detected among four different commercial layer breeds. This finding is in line with the earlier finding of Oberländer *et al.* (2020), who reported absence of breed-based variation in post-vaccination antibody protection level. However, on the opposite Sarba *et al.*, (2021) reported differences in serological responses among seven chicken ecotypes, including local and commercial breeds. This difference might be probably due to the fat that the present study compared only four commercial layer breeds.

The observation that flock maintained under excellent poultry farm hygiene conditions showed a (100%) protective titer, while those in a farm with poor farm hygiene had slightly reduced level (96.6%) in the present study contrasts the previous finding by Messaï *et al*. (2019), who reported that the antibody titers were significantly ($p < 0.05$) elevated in the flocks with bad hygiene compared with those with good hygiene in Algeria. This is most probably attributed to factors related to farm hygiene and husbandry, poor vaccine quality, improper storage, the use of expired batches, and inadequate application techniques are common challenges in developing countries (Vui *et al*., 2002; Mohamed *et al.*, 2019).

Additionally, heat stress and water deprivation also lead to the production of steroids and, thus, resultantly immunosuppression (Hossain *et al*., 2010; Sarker *et al.*, 2021). The quality of water which is offered to the birds was also found questionable, which might hinder the development of specific immunity, possibly due to acid-base imbalance. Unsuitable vaccination schedule also leads to the neutralization of maternally derived antibodies and making the birds more susceptible to the infection (Numan *et al.*, 2005; Alexander *et al.*, 2004; Ghaniei *et al.*, 2012).

The observation of significantly higher antibody protection level in large flock size (100%) than small flock size (95%) in the present study is in agreement with previous finding he reported by de Bruyn *et al*. (2017), who found that higher chicken flock size was significantly ($p < 0.05$) higher in protective antibody production amongst households participating consistently in vaccination campaigns. This is most probably attributed to the presence of better vaccine handling, cold chain management, and professional health supervision in larger commercial operations great as compared to that small-scale farming system.

In addition large poultry farms have the potential storing vaccines in a stable cold chain and can use more effective vaccines. Moreover, large poultry farms have permanently employed professionals for vaccination and overall health management. But the small folk or small-scale poultry farm have financial and human resource limitations to properly vaccinate their chickens and properly manage farm health.

The results of the present study indicate that almost all flocks showed antibody titers above the minimum protective level against IBV and IBDV (98% and 94.5%) in the layer chicken tested respectively which was verified by the positive IBV and IBDV antibody protection level observed across all 19 farms, achieving the critical protective titer threshold of ≥ 80% (Alam *et al*,. 2021).

The current application of hatchery vaccination strategies, application techniques, and vaccine schedule of administrating IBV and IBDV live vaccine, as recommended by the vaccine Production Company and breeder farms are highly effective, as evidenced by the protective titers observed in most of the farms in this study area. The control of IBV and IBDV in poultry farms significantly contributes to enhanced farm productivity, food security and poverty reduction in Ethiopia (Mihret *et al.,* 2023; Anaraki *et al.,* 2022).

Various factors have been associated with outbreaks of IBV and IBDV globally, such as insufficient biosecurity measures, poor vaccination, and immunization practices, antigenic variation, the interference of maternal antibodies with live vaccines, and immunosuppression (Alam *et al*,. 2021). Vaccination is a crucial method of preventing infectious diseases in poultry, including Infectious Bronchitis Virus and Infectious Bursal Disease Virus. Vaccination works by introducing a weakened or inactive form of the virus, stimulating the immune system to produce antibodies. These antibodies provide a specific defence against the virus, protecting the chickens from infection (Ike *et al.,* 2021).

Of 384 samples analysed, IBV and IBDV (98% and 94.5%) of vaccinated layers had antibody levels above the minimum protective threshold, indicating strong immune protection. In a study of chicken serum samples, a protective level of antibodies against both Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease Virus (IBDV) was detected. This finding indicates that the chickens had developed adequate immunity to protect against these viruses, likely due to vaccination. The current research finding is similar to the findings of reports published in 2001 on the Vet Immunology Journal (Vet Immunol, 2001). In Chicks hatched with high levels of maternal antibody had excellent protection (> 95%) against infectious bronchitis virus (IBV) challenge at 1 day of age, but not at 7 days (< 30%). This protection significantly ($P < 0.05$) correlated with levels of local respiratory antibody.

Significant variation in antibody titer was observed among the different lengths since last vaccination ($P < 0.05$). A sample taken from one month of post-vaccination demonstrate the highest protective level for IBV and IBDV (98.8% and 96.2), while the seroconversion rate at three months post-vaccination was slightly lower (95% and 90%) respectively. A similar finding was reported by (De Wit *et al.,* 2022), which is found in the highest antibody titer was post-vaccination (95% protective) and decreased to 80% at 40 days of post-vaccination.

The study also assessed the impact of poultry farm ventilation status on the minimum antibody titer. Vaccinated chickens reared in excellent ventilation status had (99.3 and 98.1%) protective antibody titer respectively, whereas those raised in poor ventilation environment had 95% and 90% respectively. This suggests that inadequate ventilation potentially leads to elevated ammonia levels, this ammonia build up in poultry farms compromises the immune response in poultry that is why the ventilation status of the farm significantly ($p < 0.05$) affects the protection level of the antibody produced by the Infectious bronchitis and Infectious bursal disease in layer farms respectively. This finding align with the report of (Dominguez *et al.* (2022). Who demonstrated that exposure to high atmospheric ammonia concentrations negatively impacts both growth performance and immunological responses of layer chickens.

As described in different literature, antibody titers against IBV and IBDV can vary in different breeds, because of differences in the speed of metabolism or the stress induced by the onset of laying (Rama *et al,*. 2023, Eze, 2013; Ershad, 2005). However, in this study, no significant variation was detected among four different commercial layer breeds. This finding aligns with the finding of (Arango *et al.*, 2024), who found no breed-based variation in post-vaccination antibody protection level.

The research also found that poultry farm hygiene significantly affects post-vaccination antibody titer in commercial layers. Flock maintained under excellent poultry farm hygiene conditions IB and IBD showed a (99.1% and 98.3%) protective titer, while those in a farm with poor farm hygiene hade slightly reduced level (96.6% and 90%) respectively. This finding contrasts with Messaï *et al*. (2019), who reported that the antibody titers were

significantly (p=0.04) elevated in the flocks with bad hygiene compared with those with good hygiene in Algeria.

Factors related to farm hygiene and husbandry, poor vaccine quality, improper storage, the use of expired batches, and inadequate application techniques are common challenges in developing countries (Bejo, 2010, Allam *et al*., 2012). Additionally, Heat stress and water deprivation also lead to the production of steroids and, thus, resultantly immunosuppression (Numan *et al.,* 2005). The quality of water which is offered to the birds was also found questionable, which might hinder the development of specific immunity, possibly due to acid-base imbalance. Unsuitable vaccination schedule also leads to the neutralization of maternally derived antibodies and resultantly making the birds more susceptible to the infection (Alexander *et al* 2004; Ghaniei *et al.,* 2012).

Moreover, the study indicated that flock size influenced Infectious bronchitis and Infectious bursal disease post-vaccine antibody titer level, with a large flock size (100% and 96.8%) achieving a minimum antibody protection level, while a small flock size (95% and 92%) respectively. The variation across different flock sizes in this study has shown significant variation. This may reflect better vaccine handling, cold chain management, and professional health supervision in larger commercial operations great as compared to that small-scale farming system.

Additionally, large poultry farms have the potential storing vaccines in a stable cold chain and can use more effective vaccines. Moreover, large poultry farms have permanently employed professionals for vaccination and overall health management. But the small flock or small-scale poultry farm have financial and human resource limitations to properly vaccinate their chickens and properly manage farm health. This finding aligns with the report of (Van *et al.,* 2017, de Bruyn *et al.,* (2017), who found that higher chicken flock size was significantly ($p < 0.05$) higher in protective antibody production amongst households participating consistently in vaccination campaigns.

Furthermore, several factors including route of vaccination, vaccine storage, the hygiene of the administering vessel, and the number of vaccine doses per bird is all important aspects to be considered. All of these factors result in a reduction in vaccine dose per chicken or even the injection of entirely damaged virus, rendering immunization ineffective (Gary *et*

al., 2022). In general, results of the present study clearly showed that the type of vaccine used for the flocks was an excellent inducer of immunity and able to protect birds from the infection (Bhuiyan *et al.*, 2021).

6. CONCLUSION AND RECOMMENDATIONS

Newcastle disease, Infectious bronchitis and Infectious bursal diseases are costly poultry diseases that affects commercial farmers and poultry production sectors around the world. NCD is a leading cause of mortality, morbidity and economic losses in Ethiopia. The result of seroconversion and protection level for NCD was found to be higher than recommended by WOAH limits. In addition, the result recorded in this study showed IB and IBD has maximum protection levels. Observations of the current study revealed that NCD, IB and IBD live vaccine types were effective in protecting chickens from the aforementioned diseases although uniformity of antibody production is variably interrupted among the flocks. The present study also demonstrated the management systems and its impact on seroconversion and antibody protection levels for boosting chicken immunities, where good management system have positive responses on immune developments. Furthermore, farm location, post-vaccine length of time, ventilation, hygiene and flock size were found to be associated with significant difference in seroconversion and protection level of vaccines in chicken of the present study.

Limitations

- Failure to take samples repeatedly which help for seromonitoring is considered as one limitation.
- Failure to use DIVA kit (which I conducted using conventional kit) is considered as a limitation because the induced immunity is weather from the vaccine or infection is not differentiated.

Therefore, based on this conclusion, the following recommendations are forwarded

- Implement routine serological surveillance of post-vaccination in all poultry farms at national level for seromonitoring.
- Maintain good biosecurity and management practices including correct vaccines storage and administration and adjustment of vaccination schedules based on seroconversion data.

- Community awareness on vaccine handling and management practices to sustain production of high antibody protection level.
- Further in detail research on the seroconversion and antibody protection level of vaccines used against for common viral diseases of poultry in different parts of Ethiopia.
- Regulation and policy action regarding vaccine and vaccination should be developed and forwarded to stockholders.

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8. ANNEXES

Annex 1: Structured questioner

This questionnaire is intended to obtain primary data to assess Managements of vaccine and major viral diseases in commercial layer chickens. We thank you for your valuable time.

Section 1: General Information

Demographics:

Name of the farm/owner _____:

Location of the farm Region _____ District_____
Kebele_____ GPS location Latitude_____
Longitude_____

Year of establishment_____: Ownership type -Individual ☐ Group ☐ Company ☐

Education level of farm manager/owner_____

Farming experience

Which poultry farming experience do you have? Mention the number of chickens you manage and years of experience.

☐ Egg Hub farm: No of Chickens _____ No Years _____

☐ Small-scale: No of Chickens _____ No Years _____

☐ Medium-scale farm: No of Chickens _____ No Years _____

☐ Large scale farm: No of Chickens _____ No Years _____

How long have you been managing your current poultry farm?

☐ Less than 1 year: ☐ 1–3 years : ☐ More than 3 years

Farm Characteristics:

Total number of layers _____

Age of the chicken's _____

Breed(s) of layers used_____

Average egg production per farm/day_____ per layer/day _____

Type of housing system used - cage ☐ deep litter ☐ other _____

Section 2: Vaccinations

2.1. Source of vaccine_____

2.2. Type of vaccine_____

2.3. Handling of Vaccine_____

2.4. Transportations of vaccine_____

2.4. Storage of vaccine_____

Section 3: Husbandry Practices

Housing Management:

Total area allocated for poultry production (Compound dedicated for poultry farming) _____

What is the area of the poultry house _____ stocking density (number of birds per square meter) _____?

How is temperature regulated in the poultry house_____?

How is the ventilation condition: Excellent ☐ Very Good ☐ Good ☐ Bad ☐

How is the hygienic condition of the house: Excellent ☐ Very Good ☐ Good ☐ Bad ☐

How often do you clean poultry houses?

☐ Daily ☐ Weekly ☐ Monthly ☐ Rarely

How is poultry manure managed or disposed of?

Are there any measures to control odours or pests related to waste?

Health and Disease Management:

Was there a disease outbreak in your farm_____ How Frequent _____

What was the common Disease in you farm _____

List out the Common Poultry Diseases in the area in priority order_____

_____ _____ _____

What are the most frequent health issues or diseases you encounter in your poultry flock and the surrounding areas, _____

and how do they impact your production? _____

What specific disease symptoms or patterns did you observe in your bird during disease outbreaks? Please describe them. _____

Which times of the year do you typically notice an increase in poultry disease outbreaks_____

How do these align with environmental or seasonal changes?

_____

How do you monitor bird health and respond to disease outbreaks?

_____

Are there regular veterinary check-ups? If yes, how frequently?

_____

Do you vaccinate your chickens? ☐ Yes ☐ No

If yes, what diseases are they vaccinated against? (Newcastle disease, IB, IBD, others)

_____

How often do you vaccinate your chickens? (Schedule-specific options)

_____

Do you follow a regular vaccination schedule provided by a veterinarian?

☐ Yes ☐ No

Disease Impact

Have you observed any poultry diseases on your farm in this production season?

Yes □ No □; if yes,

If yes, which diseases have affected your chickens? (Newcastle disease, Infectious bronchitis, Infectious bursal disease, Coccidiosis, Salmonellosis, etc.- please relate with the symptom they describe)

What was the mortality rate (%) during the most recent disease outbreak?

_____

How many chickens did you lose in the last disease outbreak? (Specify number)

_____

How has disease impacted egg production? (Reduced eggs per day by __%, no impact, others) _____

How long did the production loss last during the disease outbreak? (Days/Weeks)

Did you observe reduced feed intake during the disease outbreak?

☐ Yes ☐ No

If yes, by how much (%) did feed intake decreases? _____

What do you do when a bird shows signs of illness? (Isolate, seek veterinary care, cull, others)

Do you separate sick chickens from healthy ones?

☐ Always ☐ Sometimes ☐ Never

Do you quarantine new or sick birds before introducing them to the flock?

☐ Yes ☐ No

How do you dispose of dead chickens?

☐ Burial ☐ Burning ☐ Throwing away ☐ other (Please specify)

_____

Do you have access to veterinary services for your poultry farm?

☐ Yes, regularly ☐ Occasionally ☐ No

What challenges do you face in accessing veterinary services? (Select all that apply)

☐ Distance to veterinary service

☐ Cost of service

☐ Lack of skilled personnel

☐ Other (Please specify)

Biosecurity Practices

What type of housing do you provide for your chickens? (Open yard, semi-enclosed shelter, fully enclosed housing)

Do you separate chickens by age or production type (e.g., layers, broilers)? ☐ Yes ☐ No

Do you mix birds from different sources in the same flock? ☐ Yes ☐ No

Are visitors allowed to enter your poultry farm? ☐ Yes ☐ No

If yes, are there hygiene protocols in place for visitors (e.g., use of foot baths, protective clothing)? ☐ Yes ☐ No

How do you ensure farm workers maintain hygiene before handling chickens? (Handwashing, use of boots, protective gear, etc.)

Are workers restricted from visiting other poultry farms while employed at your farm? ☐ Yes ☐ No

Do you have measures in place to prevent wild birds, rodents, and other animals from entering the poultry house? ☐ Yes ☐ No

If yes, what measures do you use? (Netting, traps, repellents, other)

Have you observed wild birds or rodents inside or near your poultry house?

☐ Yes ☐ No

How do you manage feed storage to prevent contamination by rodents and pests? (Covered storage, daily cleaning, others)

How often do you clean and disinfect the poultry house? (Daily, Weekly, Monthly, Occasionally, Never)

What disinfectants do you use to clean the poultry house and equipment?

Are tools and equipment shared between different poultry houses or farms? ☐ Yes ☐ No

Annex 2: Indirect ELISA

1, a) Pre-dilute the samples as follows:

- Mix 80 µl of **Wash Solution 1X** with 20 µl of egg yolk.
- Mix well before testing by inversion or vortexing.

Note: These diluted egg yolk samples may be stored at -20°C for future testing. Samples may not undergo more than 3 freeze-thaw cycles.

1, b) 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.
   - **50 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).

3. Cover the plate and incubate **30 min ± 3 min** at 21°C (± 5°C).

For all sample types:

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 at least 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 at least 300 µL of the **Wash Solution 1X.** Avoid drying of the wells between washes.
9. Add 100 µL 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 the same order as in step No. 9 to stop the reaction.
12. Read and record the O.D. at 450 nm.

Annex 3: Indirect ELISA result interpretation of NCDV, IBV and IBDV

Results are interpreted as follows:

S/P value	ELISA Antibody titre	ND Immune status
S/P ≤ 0.3	Titre ≤ 993	Negative
S/P > 0.3	Titre > 993	Positive

Results are interpreted as follows:

S/P value	ELISA Antibody titer	IBV Immune status
S/P ≤ 0.2	Titer ≤ 1625	Negative
S/P > 0.2	Titer > 1625	Positive

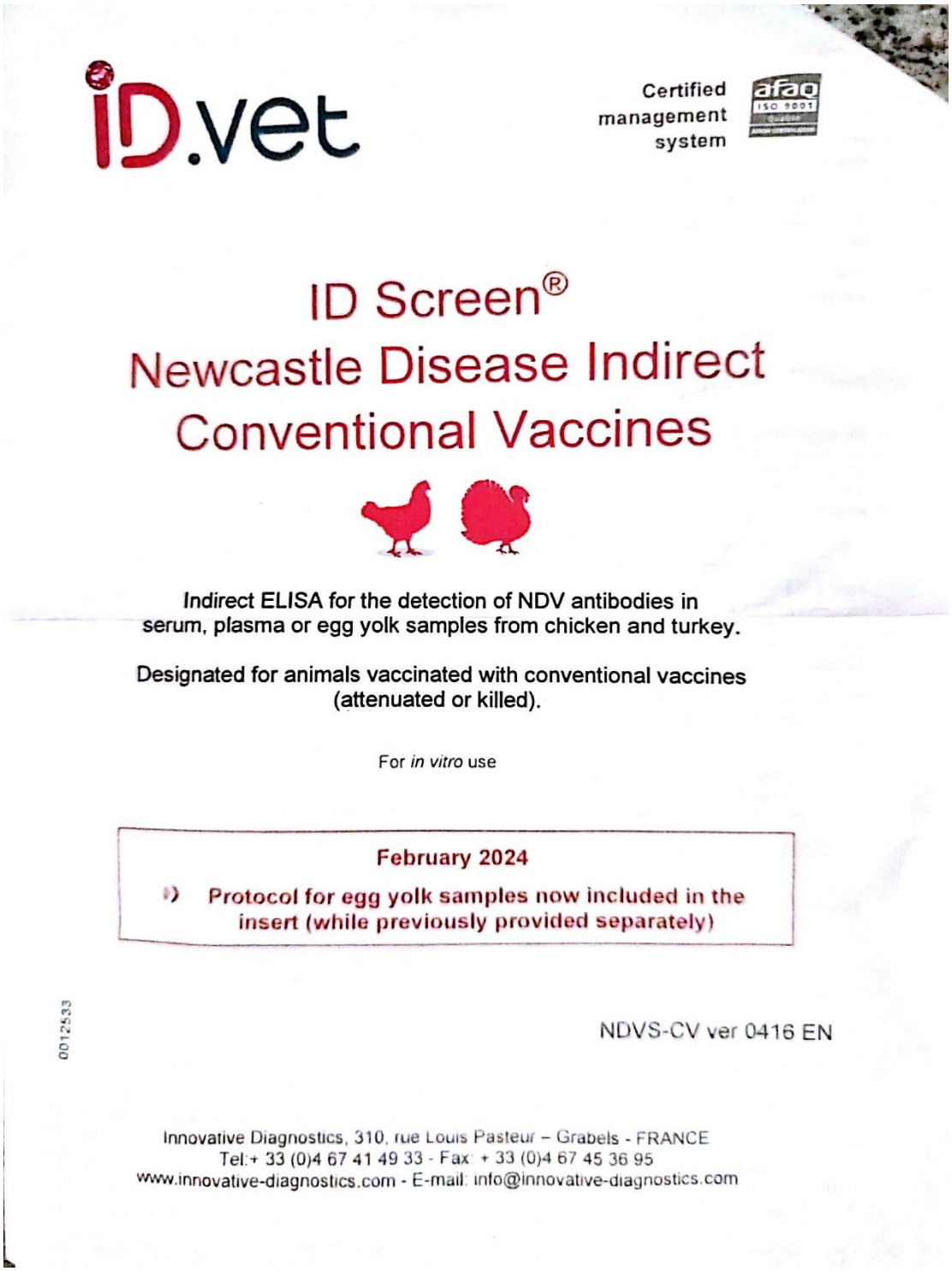
Results are interpreted as follows:

S/P value	ELISA Antibody titer	IBD Immune status
S/P ≤ 0.3	Titer≤ 875	Negative
S/P > 0.3	Titer > 875	Positive

Kit Components

Reagents*
Microplates coated with purified IBV recombinant protein
Positive Control
Negative Control
Concentrated Conjugate (10X)
Dilution Buffer 3
Dilution Buffer 14
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0,5 M)

Annex 4: Pictures taken during research work



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ID Screen®
Newcastle Disease Indirect Conventional Vaccines

Indirect ELISA for the detection of NDV antibodies in serum, plasma or egg yolk samples from chicken and turkey.

Designated for animals vaccinated with conventional vaccines (attenuated or killed).

For *in vitro* use

February 2024

- **Protocol for egg yolk samples now included in the insert (while previously provided separately)**

0012533

NDVS-CV ver 0416 EN

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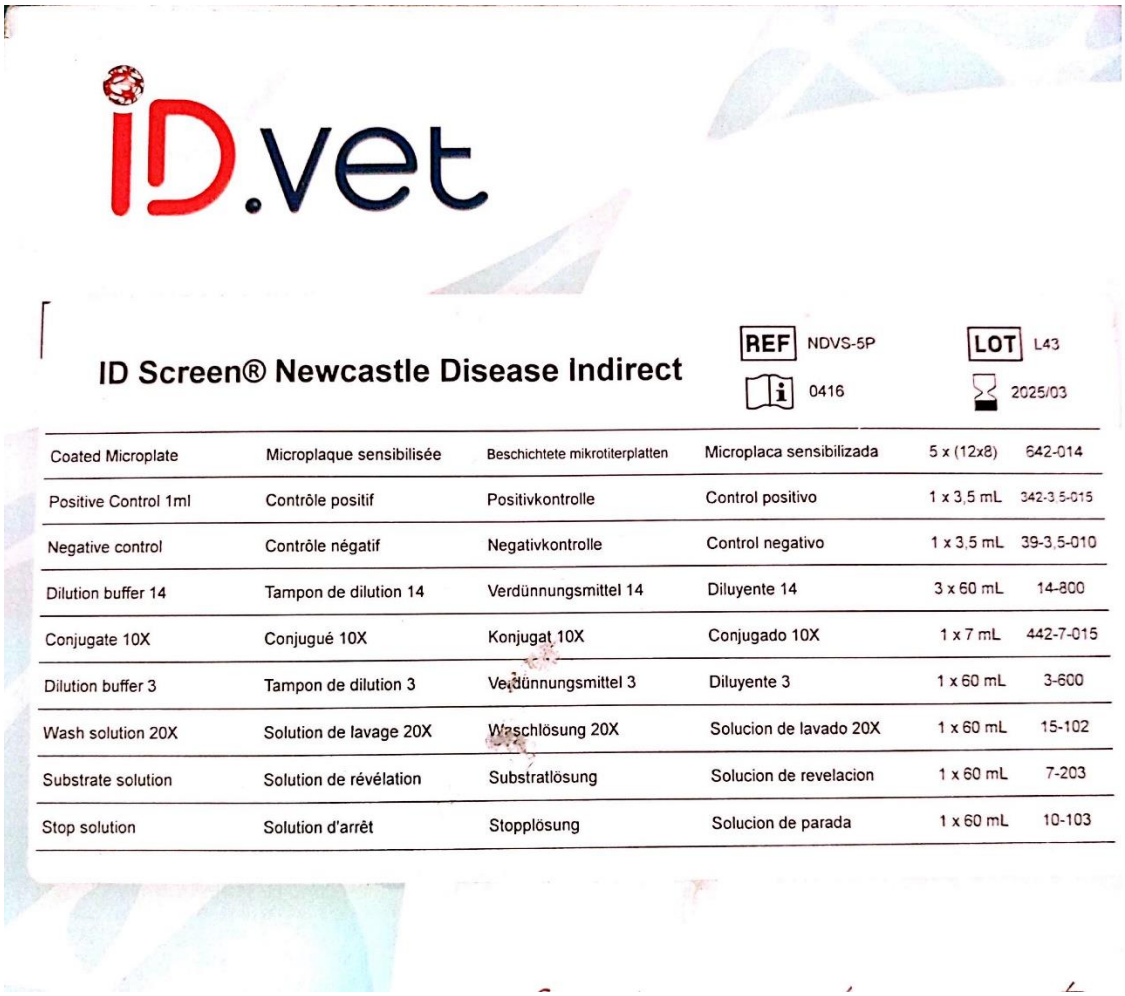

ID Screen® Newcastle Disease Indirect

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0416

LOT L43

2025/03

Coated Microplate	Microplaque sensibilisée	Beschichtete mikrotiterplatten	Microplaca sensibilizada	5 x (12x8)	642-014
Positive Control 1ml	Contrôle positif	Positivkontrolle	Control positivo	1 x 3,5 mL	342-3,5-015
Negative control	Contrôle négatif	Negativkontrolle	Control negativo	1 x 3,5 mL	39-3,5-010
Dilution buffer 14	Tampon de dilution 14	Verdünnungsmittel 14	Diluyente 14	3 x 60 mL	14-800
Conjugate 10X	Conjugué 10X	Konjugat 10X	Conjugado 10X	1 x 7 mL	442-7-015
Dilution buffer 3	Tampon de dilution 3	Verdünnungsmittel 3	Diluyente 3	1 x 60 mL	3-600
Wash solution 20X	Solution de lavage 20X	Waschlösung 20X	Solucion de lavado 20X	1 x 60 mL	15-102
Substrate solution	Solution de révélation	Substratlösung	Solucion de revelacion	1 x 60 mL	7-203
Stop solution	Solution d'arrêt	Stopplösung	Solucion de parada	1 x 60 mL	10-103

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ID Screen®
Infectious Bronchitis Indirect 2.0

Indirect ELISA for the detection of antibodies against IBV *in chicken* serum, plasma or egg yolk samples.

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- **New antibody titer formula and associated cut-off titer value** (titer >1625 instead of 853)

0014110

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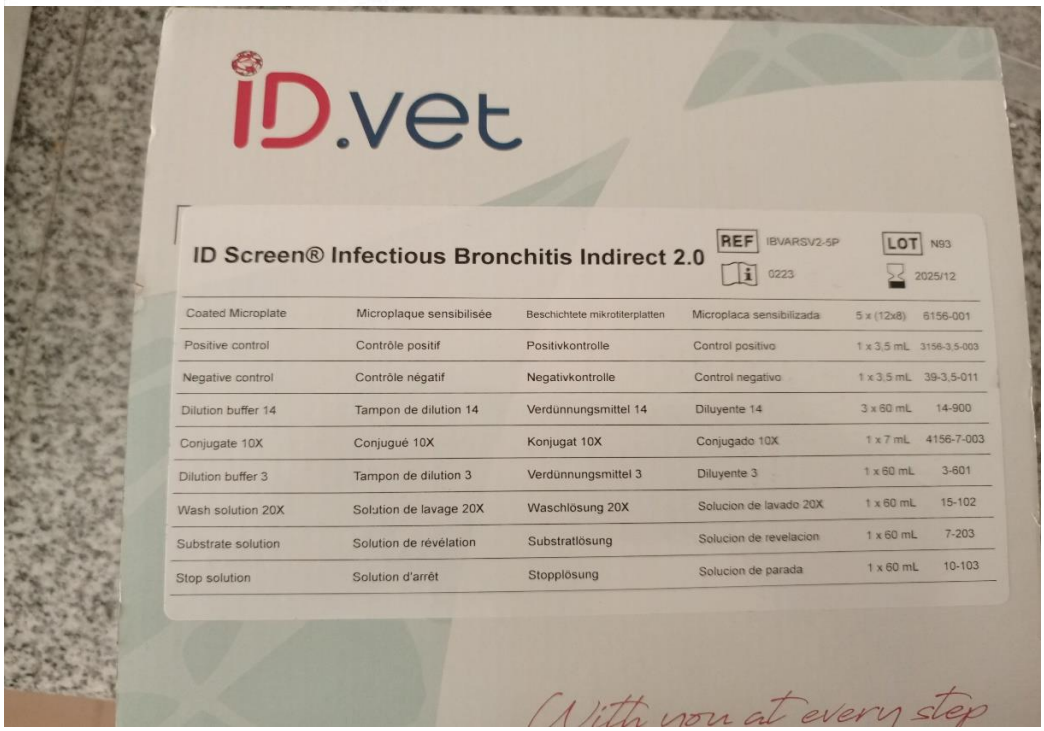

Certified management system

ID Screen®
IBD Indirect

Indirect ELISA for the detection of antibodies against IBDV in chicken serum, plasma or egg yolk samples

For *in vitro* use

September 2021

- **Protocol for egg yolk samples now included in the insert (while previously provided separately)**

0012530

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ID Screen® IBD Indirect
REF IBDS-5P
LOT 007
0416
0715
2025/12
Coated Microplate | Microplaque sensibilisée | Beschichtete mikrotiterplatten | Microplaca sensibilizada | 5 x (12x8) | 680-020
Positive control | Contrôle positif | Positivkontrolle | Control positivo | 1 x 3,5 mL | 380-3,5-024
Negative control | Contrôle négatif | Negativkontrolle | Control negativo | 1 x 3,5 mL | 39-3,5-011
Dilution buffer 14 | Tampon de dilution 14 | Verdünnungsmittel 14 | Diluyente 14 | 3 x 60 mL | 14-900
Conjugate 10X | Conjugué 10X | Konjugat 10X | Conjugado 10X | 1 x 7 mL | 480-7-023
Dilution buffer 3 | Tampon de dilution 3 | Verdünnungsmittel 3 | Diluyente 3 | 1 x 60 mL | 3-601
Wash solution 20X | Solution de lavage 20X | Waschlösung 20X | Solucion de lavado 20X | 1 x 60 mL | 15-102
Substrate solution | Solution de révélation | Substratlösung | Solucion de revelacion | 1 x 60 mL | 7-203
Stop solution | Solution d'arrêt | Stopplösung | Solucion de parada | 1 x 60 mL | 10-103

Loading serum on indirect ELISA Kit

Annex 5: Thesis document originality report

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ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH DEPARTMENT

ASSESSMENT OF SEROCONVERSION AND PROTECTION LEVEL AGAINST VIRAL DISEASES IN COMMERCIAL LAYER CHICKENS IN BISHOFTU TOWN, ADA'A AND LUME DISTRICTS, CENTRAL ETHIOPIA

A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science Poultry health and management

BY
NABON DEBELA

MAIN ADVISOR: DR. HIKA WAKTOLE (ASSOCIATE PROFESSOR, PHD)
CO-ADVISORS: PROFESSOR BERSISSA KUMSA (DVM, MSC, PHD)
DR. SAMUEL MULAT (DVM, MSc)

JUNE, 2025
BISHOFTU, ETHIOPIA

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ASSESSMENT OF SEROCONVERSION AND PROTECTION LEVEL AGAINST VIRAL DISEASES IN COMMERCIAL LAYER CHICKENS IN BISHOFTU TOWN, ADA'A AND LUME DISTRICTS, CENTRAL ETHIOPIA BY NABON DEBELA

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and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/04/58/17/2025

Name of Applicant: Nabon Debela Wayima (MSc student)

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *assessment of sero-conversion and protection level against viral diseases in commercial layer chickens in Bishoftu town, Ada'a and Lume districts, Ethiopia*

Date of application:	**December, 2024**
Nature of the study:	**Farm investigation**
Target animal species:	**Chicken**
Number of animals involved:	**384**
Study area:	**Central Oromia, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/17/025, 25/02/2025**

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 Nabon Debela.

Professor Getachew Terefe (DVM, PhD)
Chairperson

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine & Agriculture

Signature

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