

WALLAGA UNIVERSITY
SCHOOL OF GRADUATE STUDY
DEPARTMENT OF VETERINARY MICROBIOLOGY IMMUNOLOGY AND
PUBLIC HEALTH.



ISOLATION, MOLECULAR DETECTION AND DETERMINING THE CASE OF
VACCINE FAILURE OF NEWCASTLE DISEASE VIRUS OUTBREAKS IN
CHICKENS IN WESTERN OROMIA, ETHIOPIA.

By
ABEBE TEMESGEN

A PROPOSAL SUBMITTED TO THESIS ADVISOR IN PARTIAL FULFILLMENT
OF A REQUIREMENT FOR THE VETERINARY MICROBIOLOGY MSc THESIS.

ADVISOR: DR. DEREJE A (DVM, MSc, ASSOCIATE PROF.)

OCTOBER, 2025
NEKEMTE, ETHIOPIA

APPROVAL SHEET

This thesis proposal paper titled: **Isolation, Molecular Detection and Determine Case of vaccine Failure of Newcastle Disease Virus in Chickens in Western Oromia, Ethiopia** has been submitted for presentation with my approval as school advisor.

Submitted by:

ABEBE TEMESGEN ALEMAYEHU	_____	_____
	Signature	Date

Approved by

DR. Dereje Abera	_____	_____
	Signature	Date

Evaluated by

Dr. _____	_____	_____
Name of main evaluator	signature	Date

Dr. _____	_____	_____
Name of co-evaluator	signature	Date

Dr. _____	_____	_____
Department chair person	Signature	Date

Dr. _____	_____	_____
Name of Dean SGS	Signature	Date

ACKNOWLEDGEMENT

First and for most let me praise and honor the God for the opportunity and capacity given to me to realize my aspiration.

My special and sincere gratitude goes to my Advisor Dr. Dereje Abera for his encouragement, support and continual guidance and understandings throughout my works. I wish to record my sincere appreciation to my parents and my friends for their encouragement, advice and moral appreciation.

At the end, I would like to acknowledge wallaga University specially School of Veterinary Medicine for facility support and internet access.

TABLE OF CONTENTS

APPROVAL SHEET	I
ACKNOWLEDGEMENT	II
TABLE OF CONTENTS	III
LIST OF ABBREVIATIONS	V
LIST OF TABLES	VI
LIST OF FIGURE	VII
SUMMARY	VIII
1. INTRODUCTION.....	1
1.1 Statement of the Problem:	3
1.2 General Objective:	3
1.3 Specific Objectives:	3
1.4 Hypothesis testing.....	4
1.5 Research Questions	4
2. LITERATURE REVIEW	5
2.1 Definition and Etiology	5
2.2. Distribution, host and transmission.....	5
2.3. Pathogenicity, clinical forms and lesions	6
2.4. Diagnosis, Control and Prevention	6
2.5. Current status of NCD disease in Ethiopia.....	7
<i>2.5.1. History, prevalence and significance</i>	<i>7</i>
<i>2.5.2. Risk factors associated with Newcastle outbreaks</i>	<i>9</i>
<i>2.5.3. Control of Newcastle disease in Ethiopia.....</i>	<i>11</i>
<i>2.5.4. Status of the Disease in Ethiopia</i>	<i>12</i>
2.6. Determining Cases of Vaccine Failure of Newcastle Disease Virus.....	13
<i>2.6.1 Overview of Vaccine Failure.....</i>	<i>13</i>
<i>2.6.2 Causes of Vaccine Failure.....</i>	<i>13</i>
<i>2.6.2.1 Antigenic Variation Between Field and Vaccine Strains.....</i>	<i>13</i>
<i>2.6.2.2 Inadequate Vaccination Practices</i>	<i>14</i>
<i>2.6.2.3 Maternal Antibody Interference.....</i>	<i>14</i>
<i>2.6.2.4 Host and Environmental Factors.....</i>	<i>14</i>
<i>2.6.3 Molecular Evidence of Vaccine Failure</i>	<i>14</i>
<i>2.6.4 Diagnostic Approaches to Identify Vaccine Failure</i>	<i>15</i>

2.6.5 Strategies to Mitigate Vaccine Failure.....	15
3. MATERIALS AND METHODOLOGY.....	16
3.1. Description of the Study Area	16
3.2. Study Population	17
3.3. Study Design	18
3.4. Sample Size Determination	18
3.5. Sample Collection.....	18
3.6. Virus Isolation	18
3.7. Molecular Detection	19
3.7.1 RNA Extraction.....	19
3.8 Steps to Determine Cases of Vaccine Failure of Newcastle Disease Virus.....	19
3.8. Data Management and Analysis	20
3.9. Quality Control.....	21
4. EXPECTED OUTCOMES	22
TABLE: 1 WORK PLAN.....	23
TABLE 2: BUDGET BREAK DOWN.....	24
5. REFERENCES.....	25

LIST OF ABBREVIATIONS

BSL-2	Biosafety Level 2
cDNA	Complementary Deoxyribonucleic Acid
CSA	Central Statistical Agency
DNA.	Deoxyribonucleic Acid
F	Fusion (gene)
HA.	Haemagglutination
HI	Haemagglutination Inhibition
ICPI	Intracerebral Pathogenicity Index
NAHDIC	National Animal Health Diagnostic and Investigation Center
ND	Newcastle Disease
NDV	Newcastle Disease Virus
OIE	World Organisation for Animal Health (formerly Office International des Epizooties)
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction (Real-Time PCR)
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SPF	Specific Pathogen-Free
VTM.	Viral Transport Medium
WOAH.	World Organisation for Animal Health
µL	Microliter
°C	Degree Celsius

LIST OF TABLES

Table 1: WORK PLAN	23
Table 2: BUDGET BREAK DOWN.....	24

LIST OF FIGURE

Figure 1: Map of study area	17
--	----

SUMMARY

Poultry production is an important agricultural activity that provides food, income, and employment opportunities for millions of people in Ethiopia and across the world. Despite its potential, poultry productivity is constrained by infectious diseases, among which Newcastle disease (ND) is one of the most devastating and economically important. ND, caused by Newcastle disease virus (NDV) of the Paramyxoviridae family, leads to high mortality, reduced egg production, and trade restrictions in both backyard and commercial flocks. Although vaccination is widely practiced, ND remains endemic in many parts of Ethiopia, indicating the circulation of virulent NDV strains and potential vaccine failure. . This study will be conducted to isolate, molecularly detect, and determine case of vaccine failure in chickens. A purposive sampling design will be used, and samples will be collected from both live and dead birds for virus isolation, molecular detection using RT-PCR, and determine case of vaccine failure following WOAHA guidelines. The study is expected to provide isolate, molecularly detect and determine case of vaccine failure and supporting improved vaccination, surveillance, and control programs in Western Oromia, Ethiopia.

Keywords: *Newcastle disease, NDV, virulence, Western Oromia, poultry, Ethiopia*

1. INTRODUCTION

Poultry production is an essential agricultural activity worldwide, especially in developing countries where it significantly contributes to food security, income generation, and poverty alleviation. In sub-Saharan Africa, chickens are the most commonly kept domestic animals, primarily managed by smallholder and rural households under traditional backyard systems (Alders & Pym, 2009). In Ethiopia, poultry farming plays a crucial socio-economic role, providing animal protein, employment opportunities, and a source of income for millions of rural families (CSA, 2021). The sector predominantly consists of indigenous chickens raised under low-input systems, accounting for over 90% of the total poultry population (Girma *et al.*, 2020). Despite its importance, poultry production in Ethiopia is challenged by several factors, including feed scarcity, poor management, and diseases. Among these, infectious diseases—particularly Newcastle Disease (ND)—are recognized as one of the most severe constraints affecting productivity and causing high mortality rates in chickens. Newcastle Disease is a highly contagious viral disease affecting domestic and wild birds worldwide, causing significant economic losses in the poultry industry (Alexander & Senne, 2008).

The disease is caused by the Newcastle Disease Virus (NDV), a member of the genus *Avulavirus* in the family *Paramyxoviridae*. NDV strains vary widely in their pathogenicity, classified into lentogenic (low virulence), mesogenic (moderate virulence), and velogenic (high virulence) pathotypes (Miller & Koch, 2013). The clinical presentation of ND depends on the strain, host species, immune status, and environmental conditions, with common symptoms including respiratory distress, nervous system disorders, decreased egg production, and sudden death (Alexander, 2001).

Control and prevention of ND primarily rely on vaccination programs, which are the most effective means to reduce disease incidence and economic losses (Spradbrow, 2014). Vaccines such as live attenuated strains (Lasota, HB1, and I2) and inactivated vaccines are commonly used in Ethiopia's poultry sector (Girma *et al.*, 2020). However, despite routine vaccination efforts, ND outbreaks remain frequent, particularly in smallholder and village poultry systems (Chaka *et al.*, 2013). These recurrent outbreaks in vaccinated flocks raise concerns about the efficacy of vaccines and possible vaccine failure, which can be attributed to multiple factors, including improper vaccine handling, inadequate cold chain maintenance,

improper administration, interference of maternal antibodies, and the circulation of antigenically distinct or more virulent NDV strains (Diel *et al.*, 2012; Bello *et al.*, 2020).

Recent molecular epidemiological studies have revealed considerable genetic diversity among NDV strains circulating globally and within Ethiopia (Bello *et al.*, 2020; Awol *et al.*, 2021). In particular, strains from genotypes differing from those used in vaccines have been associated with field outbreaks and vaccine failures. For example, vaccines based on genotype II strains (such as Lasota) may provide suboptimal protection against outbreaks caused by genotype VII or other virulent strains. Such mismatches highlight the need for continuous monitoring and molecular characterization of circulating NDV strains to inform vaccine selection and development (Diel *et al.*, 2012).

Western Oromia, a major poultry-producing region in Ethiopia, includes zones such as East Wollega, West Wollega, Horo Guduru Wollega, Kellem Wollega and Illubabor. Poultry farming in this region is dominated by rural households practicing traditional scavenging and semi-intensive production systems. Despite regular vaccination campaigns, frequent ND outbreaks have been reported, causing substantial losses and threatening the livelihoods of smallholder farmers (Local veterinary reports, 2022). However, there is a scarcity of comprehensive data on the reasons behind apparent vaccine failures.

Therefore, this study aims to address this gap by isolating and molecularly detecting NDV from outbreak cases in chickens in Western Oromia and investigating possible causes of vaccine failure. The study seeks to:

- Identify and characterize the circulating NDV strains during outbreaks
- Compare field strains with vaccine strains to assess antigenic and genetic similarities or differences
- Evaluate vaccination histories and management practices that may contribute to vaccine failure
- Provide evidence-based recommendations to improve ND control strategies in the region
- The outcomes of this research will enhance understanding of the epidemiology of Newcastle Disease in Western Oromia and guide policymakers and others.

1.1 Statement of the Problem:

Newcastle Disease (ND) remains one of the most significant infectious diseases affecting poultry worldwide, particularly in developing countries such as Ethiopia, where poultry farming is a vital source of income and nutrition for smallholder farmers. Despite widespread use of vaccines, frequent ND outbreaks continue to occur, causing high mortality and severe economic losses (Chaka *et al.*, 2013; Girma *et al.*, 2020).

In Western Oromia, Ethiopia, recurrent ND outbreaks have been reported even in flocks that have been vaccinated, indicating possible vaccine failure. This situation threatens the sustainability of poultry production and the livelihoods of rural communities dependent on it. Vaccine failure may arise from several factors including improper vaccine handling, suboptimal vaccination protocols, or the circulation of genetically distinct NDV strains that are not effectively covered by the existing vaccines (Diel *et al.*, 2012; Bello *et al.*, 2020).

However, there is a lack of comprehensive data on the reasons behind vaccination failure under field conditions. Without such information, control strategies remain inadequate and ineffective, leading to persistent outbreaks and continued losses. Therefore, understanding the epidemiology, viral genetics, and causes of vaccine failure is critical for improving Newcastle Disease control and enhancing poultry production in the region.

1.2 General Objective:

- To isolate, molecularly detect, and determine the causes of vaccine failure of Newcastle Disease Virus (NDV) during outbreaks in chickens in Western Oromia, Ethiopia.

1.3 Specific Objectives:

- To isolate and identify Newcastle Disease Virus from outbreak cases in vaccinated and unvaccinated chicken flocks in Western Oromia.
- To perform molecular characterization of the isolated NDV strains to determine their genetic relationship with commonly used vaccine strains.
- To assess the vaccination history, management practices, and other risk factors that may contribute to vaccine failure in affected flocks.
- To evaluate the effectiveness of current ND vaccines against circulating NDV strains in Western Oromia.

1.4 Hypothesis testing

Null Hypothesis (H_0):

There is no significant association between Newcastle Disease outbreaks in vaccinated chickens and vaccine failure in Western Oromia, Ethiopia.

Alternative Hypothesis (H_1):

Newcastle Disease outbreaks in vaccinated chickens in Western Oromia are significantly associated with vaccine failure due to factors such as improper vaccine handling, administration errors, immunosuppressive conditions, or mismatch between field and vaccine virus strains.

1.5 Research Questions

- What Newcastle Disease Virus (NDV) strains are circulating during outbreaks in vaccinated chicken flocks in Western Oromia?
- Can NDV be isolated and molecularly detected from vaccinated chickens showing clinical signs of Newcastle Disease?
- Is there a genetic or antigenic difference between the circulating NDV strains and the vaccine strains used in Western Oromia?
- What are the possible factors contributing to vaccine failure in the affected chicken flocks (e.g., vaccine handling, administration, flock management, or host immunity)?
- How effective are the currently used vaccines in providing protection against the circulating NDV strains in the region

2. LITERATURE REVIEW

2.1 Definition and Etiology

Newcastle disease (NCD) is one of the most contagious and devastating viral diseases of poultry. Synonymously, it is also recognized as avian pnemoencephalitis, psedo-fowl pest, pseudo-poultry plague, avian pest an avian distemper. NCD is caused by a Paramyxovirus belonging to the Paramyxoviridae family together with morbilivirus and pneumovirus. On the basis of antegenic relatedness in haemagglutination inhibition(HI) tests, Paramyxovirus 1(PMV1) is divided in nine serotypes(PMV1,2,3...9). On the basis of intra-cerebral pathogenicity indices (ICPI), three strains of NCDvirus have been recognized; namely the velogenic (very pathogenic), mesogenic (mildly pathogenic),and lentogenic(less pat hogenic)strains(OIE,2000). The velogenic strain causes the most devastating disease of poultry with mortality reaching up to 100% in unvaccinated flocks (Nasser,1998).

2.2. Distribution, host and transmission

NCD is reported from all poultry raising countries. The disease usually occurs in chicken although all bird species are probably susceptible to the infection and it may vary considerably from one avian species to another with any given viruses strain. All age groups are susceptible. Those people who came in close contact with NCD virus frequently develop temporarily localized eye infection (conjunctivitis) (Kaleta and Baldauf, 1988). Transmission and spread of NCD virus occurs through virus containing excretions from infected birds. Aerosol transmission is the main mode of NCD infection. Furthermore, contaminated feed and water, footwear, clothing, equipment and the poultry environment itself are also incriminated. Consequently, the main modes of virus spread among different flocks are movements of live birds, movement of poultry products, contaminated poultry food or water and through non-avian hosts. Natural routes of infection (nasal, oral and ocular) appear to emphasis the respiratory nature of the disease (Alexander, 1988; Alexander, 2000).

2.3. Pathogenicity, clinical forms and lesions

The incubation period of NCD virus after natural exposure is on average 5-6 days. The pathogenicity of NCD virus strains varies greatly with the type of the avian species affected. Chicken are highly susceptible but turkeys ducks and geese may be infected and show few or no clinical signs even with strains that are lethal for chicken. In chicken the pathogenicity of NCD virus is determined chiefly by the strain of the virus, although the ages of the chicken and environmental conditions all have an effect. Breed or genetic stock appears to have very little effect on the susceptibility of chicken to the disease (Beard and Hanson, 1984).

Based on the strains and clinical signs observed, NCD has five forms (Beard and Hanson, 1984). Visotropic velogenic: Newcastle Disease is characterized by an acute lethal infection of chicken of all ages. Hemorrhagic lesions and signs of digestive tract disturbances are frequently present. It causes high mortality (50-100%) among adult birds. Neurotropic velogenic: Newcastle Disease is characterized by an acute lethal infection of chicken of all ages. It produces respiratory and neurogenic signs and lesions. Less pathogenic form: causes death only in young birds. The viruses causing this type of infection are the mesogenic pathotype and moderate respiratory disease has been registered. Mild or inapparent respiratory infection: caused by viruses of lentogenic pathotype. It is also called Hitchner's form. Asymptomatic enteric form: caused by lentogenic viruses, which produce chiefly gut infection with no obvious lesions or diseases (Beard and Hanson, 1984).

2.4. Diagnosis, Control and Prevention

Serological tests such as haemagglutination inhibition (HI) and enzyme linked immunosorbent assay (ELISA) tests can be used to diagnose NCD in non-vaccinated flocks (OIE, 1996). NCD virus can most easily be isolated from tissue samples or faecal or tracheal swabs from infected birds by inoculation of chicken egg embryos via allantoic cavity (Alexander, 1997). The strains of NCD virus were differentiated according to the mean death time of chicken embryos after infection by determining the intra-cerebral pathogenicity index on day old chicks after intra cerebral infection and by determining the intravenous pathogenicity index on six weeks old chickens after intravenous infection (OIE, 2000).

Generally, vaccination is the only means to reduce the occurrence of NCD in commercial poultry farms (Allan *et al.*, 1978.) Routine vaccinations were carried out with low virulent live vaccines and/or inactivated (killed) oil emulsion vaccines (administered parentally as a final vaccine). The effectiveness of NCD vaccines in the control of the disease depends on the virulence of the field strains, the types and states of the vaccine, the immunological status of the birds and the methods of the vaccine application. NCD vaccine is usually given at 10 and 35 days of age and repeated every three months. The commonly used vaccines in Ethiopia are Hitchener's B1 (HB1) and La Sota, which are produced at the National Veterinary Institute (NVI), Debre Zeit. These vaccines are applied as mass application using spray/aerosol or via drinking water. Application of good biosecurity measures also help in the control of the disease (NVI, 1974). Biosecurity measures, such as cleaning and disinfection of bird housing facilities and equipment are very important. New introductions or birds returning to the farm should be isolated for several weeks before being placed into the flock. A vaccine is available for birds and is routinely used in poultry flocks (NVI, 2005).

2.5. Current status of NCD disease in Ethiopia

2.5.1. History, prevalence and significance

NCD is the major constraint of poultry production in Ethiopia. One of the local names of the disease is 'Fengle'. The first documented evidence of NCD in Ethiopia dates back to 1971 (NVI, 1974). The virus strains involved in those early outbreaks were the velogenic ones and caused up to 80% mortality. It is not known how the virus was introduced but it might have been introduced through the port. Since then, in subsequent years the disease was dissimilated and reported from different agro-climatic areas. Those early outbreaks had occurred in Addis Ababa, Alemaya and Debre Zeit. Despite the routine vaccinations with HB1 and Lasota strains at least nine outbreaks of NCD have been reported in three big poultry farms (Dembi, Shola and Lemlem) between 1983-1995 (Nasser, 1998).

The major outbreaks of NCD recorded in the year between 1983-1995 in three major poultry development enterprises in Ethiopia accounted mortality rates of 14.9% in vaccinated flocks. In the same year's concurrent disease and nutritional problems aggravated mortality rates due to NCD infection. In 1995, NCD outbreaks killed almost 50% of the local or village chicken in the surrounding areas of Debre Zeit, Nazareth and Addis Ababa (Nasser, 1998). Strain

characterization performed on field isolates of NCD virus from outbreaks showed the widely distributed nature of the velogenic strain in Ethiopia. Moreover, Almargot (1987) has indicated that NCD was the first and most prevalent disease accounting prevalence up to 80% in industrial poultry farms. Similarly, the study of Ashenafi (2000) has indicated a seroprevalence rate of 43.7% in local chicken from the highlands of Ethiopia. Dessie (1996) has shown the seasonal patterns of NCD where serious losses have been observed at the beginning of the rainy seasons. No seasonality of NCD has been documented with the rainy seasons (Nasser, 1998; Ashenafi, 2000). According to Minda *et al.*, 2016 seroprevalence of NCD in Sinana district (33.04%) is higher when compared to Agarfa (20.13%) district. The prevalence in each kebele ranges from 15.63% to 40%; the highest prevalence of 40% was found at Horaboka, but insignificantly associated with Newcastle disease (ND) seropositivity.

The prevalence of households with at least one seropositive chicken was higher during the dry season (27.4%) than during the wet season (17.4%). Viral genome was detected in 14.2% of households during the wet season using a fusion (F) gene assay and in 23.5 % of households during the dry season using a polymerase (L) gene assay that targets both class I and class II viruses. At the markets sampled overall bird level prevalence was 4.9% for period 1 (F gene assay), and 38.2% and 27.6% for periods 2 and 3, respectively (L gene assay) (Hassen *et al.*, 2012). Serkalem *et al.*, (2005) reported that sero-prevalence rates of 28.57%, 29.69%, and 38.33% NCD in the high, mid-range, and low altitudes, respectively.

This study has shown that NCD is one of the major infectious diseases threatening the survival and productivity of traditionally managed local chickens in Western Oromia, Ethiopia (Serkalem *et al.*, 2005). Desalegn (2015) reported a sero-prevalence of 28.6% (149/521) NCD in East Showa zone of Oromia regional State. Furthermost, four years (2012-2015) retrospective data analysis had figured out the occurrence of 86 outbreaks of NCD in seven national regional states of the country (Amhara, South nation and nationalities (SNN), Oromiya, Tigray, Addis Ababa, Gambella and Benshangul-Gumz).

Table 1: Four years 2012 to 2015) NCD outbreak reports from seven national regional states of Ethiopia

Region	Number out breaks	Number cases	of Number of deaths	Population at risk
Amhara	54	3461	1540	178038
South Nation and Nationalities	32	2258	1263	465876
Oromiya	286	14602	5084	1885704
Tigray	17	7267	6179	218733
Addis Ababa	1	20	19	400
Gambella	1	18	8	506
Benshangul- Gumz	14	359	3324	54173

Source: Ministry of Agriculture and Livestock Resources (2018)

2.5.2. Risk factors associated with Newcastle outbreaks

Although, comprehensive data are lacking concerning the risk factors that predispose birds to NCD ranges of factors have been mentioned. Villagers by recognizing early signs of NCD or by notifying outbreaks in the neighboring household they can depopulated their stocks either by random sales of infected or clinically sick birds. Therefore, local open markets can be a potential common resource of NCD infection. Similarly the correlation of NCD outbreaks may be related with the selling /buying of chickens to celebrate socially and ritually functional cultural and social events in Ethiopia. On top of the villagers also carry chickens as gifts to heir kinfolk. These practices are frequently practiced in many regions of Ethiopia (Personal observation). In previous outbreaks, villagization had contributed to the highest prevalence of the disease probably because of close contact among flocks of different households. In rural Ethiopia, where many flocks have communal scavenging environment different age groups and chickens of different health status can intermingle with each other. Although, chickens are the most important host for NCD other avian species may be a

potential source of infection as well. Therefore, this maintains the cycle of velogenic virus to remain endemic among the flocks (Dessie, 1996; Dessie and Jobre, 2004).

In commercial poultry farms the main source and dissemination of infectious virions might be due to lack of proper sanitary or quarantine measures. On top of this wild birds could also remain a potential source of infection. The risk factors incriminated for the outbreaks in the three major commercial farms in the year between 1983-1995 were related with poor bio security measures or insufficient sanitary and quarantine measures nutritional problems and concurrent infections. All of these factors might have contributed to the introduction spread and persistence of the diseases in the farms. The presence of multiple age groups in the farms minimal distances and lack of physical separation between different units could be suggested as the factors responsible for the outbreaks. Poor disposal of dead bird's absence of all in-all-out system on the farms and maintaining different types of chickens in the same farm also created favorable conditions for the outbreaks (Nasser, 1998).

Lack of strict control of movement of people and use of the same objects in and between infected farms favored the dissemination of the disease among the farms. Visits by attendants and farm managers without changing clothes or boots between houses disseminated the disease to all functional houses. Another factor of equally important in the spread was free movement of pets and wild bird between infected and non-infected premises. Infections with aflatoxicosis, salmonellosis, coccidiosis, mycoplasmosis and nutritional deficiencies that are prevalent in the farms could inhibit vaccine response and render the birds more susceptible to NCD infections (Nasser, 1998). With the considerations of low genetic potential of indigenous local breeds, there is trend of importing high producing exotic day old chicks and fertilized eggs from Africa and Europe. In the last four years (2012-2015) 430,735 fertilized eggs and 1,151,219 day old chickens were imported from 2012 to 2015 by private sector to improved breeding. This might be considered as risk factors of introducing diseases of various natures and their subsequent dissemination in poultry flocks. Parallel to this, there is a practice of distributing improved chickens to the farmers in rural and peri-urban areas through the extension and package systems. These chickens could transmit the virus to those birds managed by the farmers and to other avian species (Ministry of Agriculture and Livestock Resources, 2018).

2.5.3. Control of Newcastle disease in Ethiopia

Of the many intervention strategies to control poultry diseases, vaccination is the most and highly practical and cost effective method that ensures successful poultry production via maintaining poultry health at high level (Al-Garib *et al.*, 2003). With this notion, the National Veterinary Institute (NVI) since its inception has been well organized in the production of many for livestock diseases, including vaccines for NCD using specified pathogens free chicken eggs (NVI, 1974). Strategies towards an efficient control of NCD were practiced by vaccination and hence attenuated and inactivated oil emulsion vaccines have been produced from the lentogenic strain of NCD virus (HB1 and Lasota) (NVI, 2000). However, these conventional NCD vaccines that were effective in commercial poultry farms were found to have little use in local/villages chickens. The probable explanation may be due to small flock sizes, scattered presence of chickens mixing up of multi-aged groups and poor management in the village system. In addition to these the vaccines were heat labile (requires cold chain from their production up to their administration to individual chickens) relatively expensive and produced in large dose units suitable for large commercial flocks. Therefore, this remains an obstacle to design nationwide preventive strategies against the disease (Spradbrow *et al.*, 1997).

To control NCD in village chickens whereby each household owns chickens in scattered fashions, thermostable vaccines applied as feed baits seemed the most appropriate methods for introducing heat stable NCD vaccine to village chickens and are currently under production and its effect under field circumstance is similarly under experimental situation. This vaccine was prepared from NDV-V4 strain and was in practice in Australia and other countries and results were found to be encouraging (Spradbrow *et al.*, 1997). It was demonstrated that vaccination with NDV-V4 induces mucosal immunity constituting of specific IgA antibodies. The same vaccine had conferred substantial protection in broilers. This experience was adopted by the NVI and thermostable vaccines were prepared from both NDV-V4 and NDV I2 strains and is currently under experimental conditions and provided affluent results (NVI, 1995; Nasser *et al.*, 1998). Nasser (1998) did various experiments on the efficacy vaccines and came up with the observation that the chickens vaccinated with the thermostable NDVI2 vaccine via the ocular and drinking water routes or orally with parboiled barley as vaccine carrier were substantially protected against challenge with virulent NCD. The results were found to be comparable with conventional HB1 and Lasota

when applied via the drinking water or ocular route. Although some proportion of birds vaccinated with thermostable vaccine had low HI titers, however, they were found to be resistant to challenge with the velogenic strains. These may be due to the effect of local or cell mediated immunity they could play roles in the protection of vaccinated chickens against velogenic NCD virus challenges. However, the efficacy is not tested at field levels. According to the information obtained from National Veterinary Institute (NVI), currently the institute produces four types of NCD vaccines (HB1, Lasota, NDV-I2 thermostable and inactivated oil emulsion Newcastle. The first two vaccines (HB1 and lasota) are produced in vials of 500 doses. The costs of the vaccines are 0.5, 0.5 and 0.30 birr per dose, respectively. Moreover, the thermostable vaccine may be popular in the future to control NCD in village chickens as the preparation and handling is convenient than the conventional live vaccines (NVI, 2004).

Two large scales and one small scale private poultry farms located in Debre Zeit were interviewed pertaining to the use of vaccines to control NCD in their respective farms. Accordingly, it was noted that in the two large scale commercial poultry farms, vaccination was done by NCD vaccine imported from the Netherland whereas the small scales poultry farm vaccinated their chickens with vaccines from National Veterinary Institute (NVI).The two large scale farms use both the live (HB1) and inactivated forms of the NCD vaccines for vaccination of breeder flocks and commercial layers whereas all the three farms use the live (HB1) vaccine for broilers. In addition, the small scale farm very recently started to use the thermostable vaccine (NDV-I2) produced by the NVI.

2.5.4. Status of the Disease in Ethiopia

Newcastle disease is considered as endemic in the village chicken population in Ethiopia. A number of studies have been conducted to determine the prevalence of NCD in different agro ecology and season of Ethiopia. It is mentioned as one of the most important disease problems in backyard chickens in most parts of Ethiopia. Mortality may be very high, often reaching 50 to 100 %. The prevalence of NCD varies among years in Ethiopia. Literature review shows that, starting from 2005 the prevalence of NCD is endemic as reported by different researchers. Some previous research work reported from some parts of the country include: Zeleke *et al.* (2005) reported 0%, 0%, 12.9%, 16.7%, 35.9% and 47.6% from Arbegona, Shebedino, Hawassa, Butajira, Alage, Hossana, respectively; Serkalem *et al.* (2005)

reported 28.6%, 29.7% and 38.% at Debreberhan, Sebeta and Adama, respectively; Mazengia *et al.*(2010) reported 21.7% from Bahir Dar; Chaka *et al.* (2012) reported 5.14% and 7.12% from Adamitulu gido kombolcha (ATGK) and Adea, respectively; Nega *et al.* (2012) reported seroprevalence of 55.8% ; Chaka *et al.* (2013) reported 16.73% and 32.2% from Adamitulu gidokombolcha and Adea, respectively; Belayheh *et al.* (2014) reported 5.6% at Kersna Kondality; Desalegn *et al.*(2016) and Miressa *et al.*(2016) reported 28.6% and 26.7% from east Shewa respectively .In the same year Minda *et al.*(2016) reported prevalence of 27.86% from Bale zone.

2.6. Determining Cases of Vaccine Failure of Newcastle Disease Virus

2.6.1 Overview of Vaccine Failure

Vaccine failure refers to the inability of a vaccinated flock to develop adequate protection against infection or disease following immunization. In the case of Newcastle disease virus (NDV), vaccine failure is a multifactorial issue involving viral, host, environmental, and management-related factors (Miller *et al.*, 2009). Despite extensive vaccination programs using live and inactivated ND vaccines in many countries, outbreaks continue to occur even among vaccinated flocks, indicating gaps in vaccine efficacy, application, or virus evolution (Cattoli *et al.*, 2011; Dimitrov *et al.*, 2017).

2.6.2 Causes of Vaccine Failure

2.6.2.1 Antigenic Variation Between Field and Vaccine Strains

One of the major reasons for NDV vaccine failure is the antigenic and genetic divergence between vaccine strains (such as LaSota or Hitchner B1) and circulating field strains. These traditional vaccines are derived mainly from genotype II viruses, whereas most currently circulating virulent field strains belong to genotypes VII and XIII (Miller & Koch, 2013). Studies have demonstrated that these antigenic mismatches can reduce cross-protective immunity, allowing vaccinated birds to become infected and shed virulent virus (Mase *et al.*, 2009; Diel *et al.*, 2012). In Ethiopia, similar findings were reported, with vaccine genotype II strains showing suboptimal protection against field genotype VII viruses (Getachew *et al.*, 2021).

2.6.2.2 *Inadequate Vaccination Practices*

Improper vaccine handling and administration significantly contribute to vaccine failure, especially under rural conditions. ND vaccines are thermo-labile and lose potency if exposed to high temperatures or direct sunlight (Alders & Spradbrow, 2001). Additionally, incorrect dosage, poor mixing of vaccine with diluent, and use of contaminated equipment can compromise vaccine efficacy. In many village systems, cold chain maintenance is unreliable, leading to the use of ineffective vaccines (Habte *et al.*, 2015).

2.6.2.3 *Maternal Antibody Interference*

Chicks that receive high levels of maternal-derived antibodies (MDA) may experience interference with early vaccination. These antibodies can neutralize live vaccine viruses before they stimulate an active immune response, leaving the birds unprotected as maternal antibodies wane (Degefa *et al.*, 2004). Proper timing of vaccination, typically at 10–14 days of age for the first dose, is crucial to avoid this interference.

2.6.2.4 *Host and Environmental Factors*

Nutritional deficiencies, concurrent infections (such as infectious bursal disease or coccidiosis), and environmental stressors like overcrowding or temperature extremes can suppress the immune response to vaccination (Allan *et al.*, 1978; Musa *et al.*, 2012). Moreover, improper biosecurity and mixing of vaccinated with unvaccinated flocks promote virus circulation and challenge that may overwhelm vaccine-induced immunity.

2.6.3 *Molecular Evidence of Vaccine Failure*

Recent molecular epidemiological studies have confirmed post-vaccination outbreaks of NDV associated with genotype mismatch. In several African and Asian countries, ND outbreaks have occurred in vaccinated flocks where the field virus differed significantly from the vaccine strain (Cattoli *et al.*, 2011; Miller *et al.*, 2009).

Molecular sequencing of the F gene cleavage site has revealed that virulent field isolates often possess multiple basic amino acids (112R-R-Q-K-R-F117), distinct from those of vaccine strains (112G-R-Q-G-R-L117) (Diel *et al.*, 2012). Such differences are responsible

for the reduced neutralization capacity of antibodies induced by genotype II vaccines against genotype VII viruses.

In Ethiopia, Getachew *et al.* (2021) reported NDV isolates from vaccinated flocks showing velogenic characteristics and genotypic divergence from vaccine strains used by the National Veterinary Institute (NVI). This indicates that vaccine failure is not only operational but also genetic, underscoring the need for local vaccine strain development based on circulating genotypes.

2.6.4 Diagnostic Approaches to Identify Vaccine Failure

Determining vaccine failure involves integrating field observations with laboratory analyses:

1. Virus Isolation and Molecular Typing: Detection of virulent NDV from vaccinated birds through RT-PCR and sequencing confirms infection despite vaccination, supporting molecular evidence of failure (Diel *et al.*, 2012).

2. Epidemiological Assessment: Reviewing vaccination records, cold chain integrity, and flock management practices provides insight into operational failures (Habte *et al.*, 2015).

2.6.5 Strategies to Mitigate Vaccine Failure

To reduce the incidence of vaccine failure, several strategies have been recommended:

Use of thermostable vaccines (e.g., I-2 strain) suitable for rural settings where cold chains are unreliable (Alders & Spradbrow, 2001).

Genotype-matched vaccines, which offer better protection against circulating virulent strains (Mase *et al.*, 2009; Dimitrov *et al.*, 2017). Improved vaccination management, including training farmers on proper handling, dosing, and timing. Regular surveillance and molecular monitoring to detect emerging genotypes and guide vaccine updates.

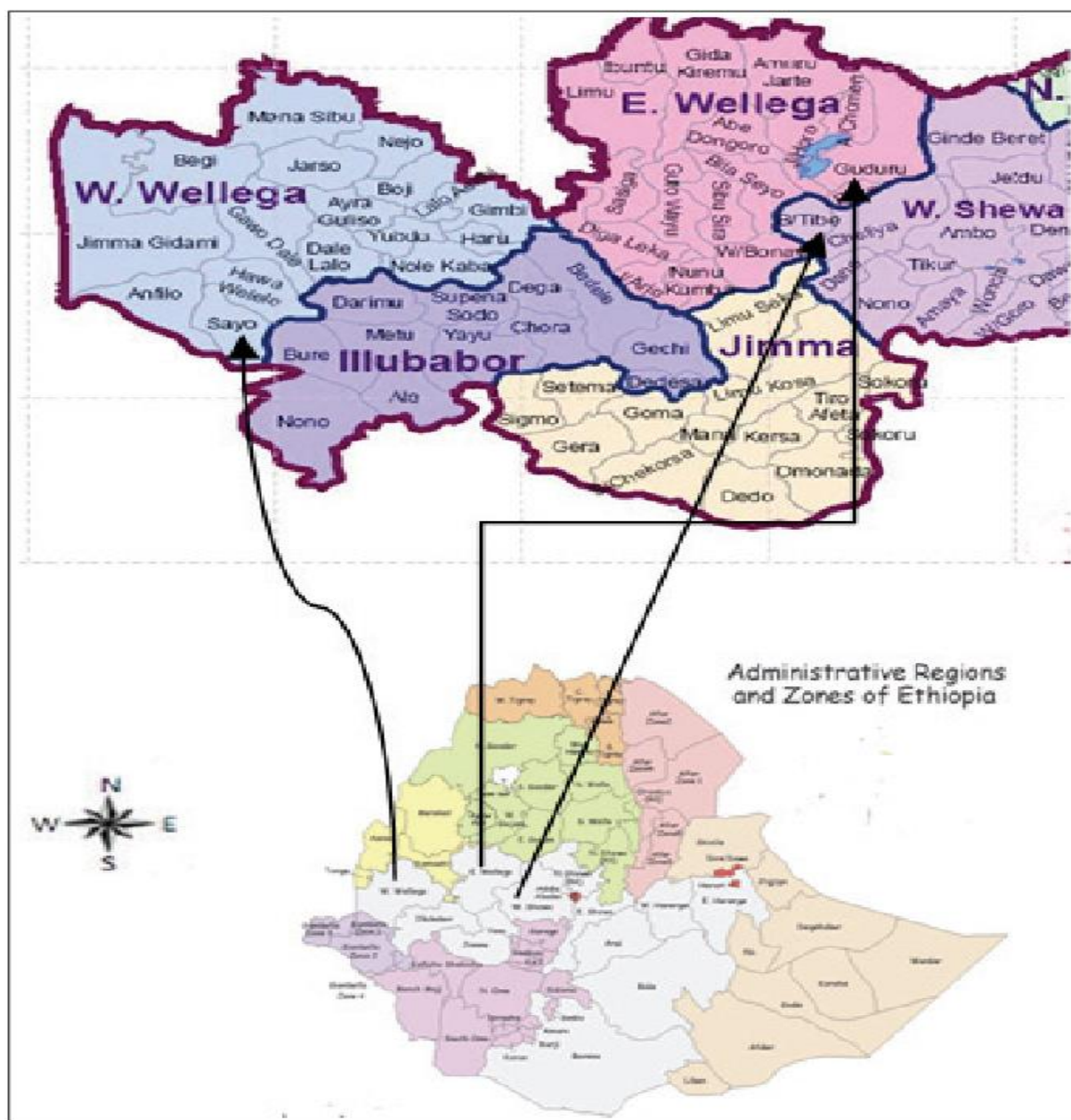
3. MATERIALS AND METHODOLOGY

3.1. Description of the Study Area

The study was conducted in selected locations across Western Oromia, Ethiopia, focusing on areas with significant poultry populations and frequent reports of Newcastle Disease (ND) outbreaks. Western Oromia encompasses several administrative zones, including East Wollega, West Wollega, Kellem Wollega, Horo Guduru Wollega and Illubabor Zones. These areas were purposively selected based on poultry population density, history of ND outbreaks, and accessibility for sample collection and field investigation.

Western Oromia is located in the western part of the Oromia Regional State and is characterized by a predominantly mixed crop-livestock farming system, where poultry, especially chickens, play a critical role in the livelihoods of smallholder farmers. The region includes both highland and midland agroecological zones, with elevations ranging from 1,300 to over 2,600 meters above sea level. The climate of Western Oromia is sub-humid to humid tropical, with an average annual rainfall ranging from 1,200 mm to over 2,000 mm, supporting diverse agricultural production. The area experiences a bimodal rainfall pattern, with the main rainy season (Kiremt) occurring between June and September, and a shorter rainy season (Belg) from March to May. Average annual temperatures vary between 15°C and 25°C, depending on altitude and season.

Administratively, the zones in Western Oromia are divided into several rural woredas and kebeles, where poultry farming is practiced under traditional, semi-intensive, and limited intensive systems. Chickens are mostly managed under free-range or backyard production systems, with minimal biosecurity measures and inconsistent vaccination practices, making them highly vulnerable to infectious diseases, especially Newcastle Disease Virus (NDV). Due to the repeated occurrence of Newcastle Disease outbreaks in vaccinated flocks across the region, Western Oromia provides a relevant context to investigate the isolation and molecular detection of circulating NDV strains and to assess the possible causes of vaccine failure. The findings from this study are expected to inform vaccination strategies and improve poultry health management in the region.



Source: Adopted from Administrative Region, Zone and Woreda Map of Oromia, 2005, viewed n.d., from <http://www.dppc.gov.et/downloadable/map/administrative/2005/Oromia.pdf>

FIGURE 1: Map showing the location of the study area.

3.2. Study Population

The study population consisted of domesticated chickens of various ages and breeds, selected from vaccinated and unvaccinated flocks in different districts of Western Oromia, Ethiopia. Birds were sampled from traditional backyard, semi-intensive, and intensive poultry production systems. Chickens showing clinical signs of Newcastle Disease, those from recent outbreak areas, and apparently healthy birds from the same flocks were included. Both live

symptomatic birds and recently deceased chickens were sampled. Flocks were chosen based on vaccination history, reported vaccine failure, and outbreak reports, aiming to identify circulating NDV strains and investigate potential causes of vaccine failure.

3.3. Study Design

A purposive sampling design will be conducted according to OIE guidelines to investigate outbreaks of Newcastle disease virus in study area to the isolate, molecularly detect, and potential causes of vaccine failure during Newcastle Disease Virus (NDV) outbreaks in chickens across selected areas of Western Oromia, Ethiopia.

3.4. Sample Size Determination

The sample will be collected from outbreaks of Newcastle disease virus in chicken in Western Oromia, Ethiopia. Sample will be 40 samples by considering of

- ❖ Availability of the instrument
- ❖ Interest of laboratory worker
- ❖ Period of the research
- ❖ Outbreak of the disease and
- ❖ Budget available

3.5. Sample Collection

From live birds, sterile cotton-tipped swabs will be inserted 2–3 cm into the oropharynx and cloaca to collect secretions (WOAH, 2021). Each swab will be placed into a 2 mL viral transport medium (VTM) composed of phosphate-buffered saline (PBS) (pH 7.2) supplemented with 10,000 IU/mL penicillin, 10 mg/mL streptomycin, and 25 µg/mL gentamicin (Worku *et al.*, 2022). From freshly dead chickens, 1–2 g of trachea, spleen, and brain tissue will be aseptically collected using sterile scissors and forceps and pooled in a 5 mL sterile tube containing 2 mL VTM (Yadeta *et al.*, 2024). Samples will be labeled (bird ID, location, date) and transported in an ice-box at 4 °C to the Animal health institute (Alemu *et al.*, 2024).

3.6. Virus Isolation

Each pooled sample (approximately 0.1 mL) will be inoculated into the allantoic cavity 9–11-day-old specific-pathogen-free (SPF) embryonated chicken eggs using sterile 1 mL syringes. Eggs will be sealed with paraffin wax and incubated at 37 °C for 5 days, candled twice

daily(WOAH, 2021).Embryos dying within 24 h will be discarded as nonspecific deaths. After incubation, allantoic fluid will be harvested from each egg using sterile pipettes and stored at -20°C until tested(WOAH, 2021)

The harvested allantoic fluids will be screened for hemagglutination (HA) activity by mixing 25 μL of fluid with 25 μL of 1% chicken red-blood-cell (RBC) suspension in V-bottom microtiter plates and incubating at room temperature for 30 minutes (Worku *et al.*, 2022). Positive samples showing complete HA will be confirmed by hemagglutination inhibition (HI) using known NDV antiserum at four hemagglutinating units (4 HAU) per test (WOAH, 2021).

3.7. Molecular Detection

3.7.1 RNA Extraction

RNA will be extracted from 200 μL of HA-positive allantoic fluid using a Viral RNA Mini Kit according to the manufacturer's protocol, eluting in 60 μL RNase-free water (Alemu *et al.*, 2024).

3.8 Steps to Determine Cases of Vaccine Failure of Newcastle Disease Virus

In this study, the following steps will be undertaken to determine cases of vaccine failure of Newcastle Disease Virus (NDV) in chickens:

❖ Collection of Background Information:

Detailed vaccination histories, including vaccine types, batch numbers, storage conditions, routes of administration, and vaccination schedules, will be collected from affected farms. Information on flock management, biosecurity, and environmental conditions will also be documented.

❖ Clinical Assessment:

Clinical signs and mortality rates among vaccinated flocks will be observed and recorded to identify symptoms consistent with Newcastle Disease.

❖ Sample Collection:

Tracheal and cloacal swabs, as well as tissue samples from sick and dead birds, will be collected following standard procedures. All samples will be transported under appropriate cold chain conditions to the laboratory.

❖ **Laboratory Diagnosis:**

Virus isolation will be performed using embryonated chicken eggs or cell culture methods to confirm the presence of NDV. Molecular detection through RT-PCR will be conducted to detect NDV RNA and confirm infection.

❖ **Genetic Characterization:**

The fusion (F) and hemagglutinin-neuraminidase (HN) genes of isolated viruses will be sequenced. The sequences will be compared with vaccine strains to identify potential genetic variations that may contribute to vaccine failure.

❖ **Evaluation of Vaccine Handling and Administration:**

The cold chain maintenance, vaccine dosage, administration route, and vaccination schedules will be evaluated through farm visits and interviews with farmers and vaccinators.

❖ **Analysis of Management and Host Factors:**

Potential immunosuppressive factors such as stress, nutrition, and concurrent infections will be investigated. The presence of maternal antibodies interfering with vaccination in young birds will also be assessed.

❖ **Interpretation of Findings:**

Based on clinical, molecular, and management data, it will be determined whether vaccine failure has occurred and the likely causes, including vaccine mismatch or procedural issues.

❖ **Reporting and Recommendations:**

Findings will be compiled into a report, and recommendations for improving vaccination practices, biosecurity measures, and vaccine selection will be provided to stakeholders.

3.8. Data Management and Analysis

All laboratory and field data (sample ID, location, HA, RT-PCR, ICPI results) will be recorded in Microsoft Excel 2019 spreadsheets. Statistical analysis will be conducted using SPSS version 26. Descriptive statistics (frequency, percentage, mean $\pm$ SD) will summarize laboratory results, while 95% confidence intervals will be calculated for prevalence estimates (Worku *et al.*, 2022). Results will be presented in tables and figures.

3.9. Quality Control

Positive and negative controls will be included in all HA, HI, and RT-PCR runs to ensure validity (WOAH, 2021). All reagents will be stored according to manufacturer recommendations (2–8 °C for buffers, –20 °C for enzymes), and all instruments will be calibrated before use (Alemu *et al.*, 2024).

4. EXPECTED OUTCOMES

➤ **Virus Isolation and Identification**

Successful isolation of NDV (Newcastle Disease Virus) from field samples (e.g. tissues from sick or dead chickens, swabs from trachea, cloaca, etc.).

Confirmation of NDV by standard virological methods (e.g. embryonated chicken eggs inoculation, cell culture with cytopathic effect [CPE], syncytia formation).

➤ **Molecular Detection and Characterization**

Detection of NDV in field samples using molecular methods (e.g., RT-PCR or real-time RT-PCR) targeting conserved genes such as **M (matrix) gene**, and optionally **F (fusion) gene**, etc.

➤ **Vaccine Failure Investigation**

Assessment of vaccine history: which vaccine strains were used, the vaccination schedule, booster doses, routes, interval, cold-chain / storage conditions, handling.

➤ **Comparative Analysis: Field Strains vs Vaccine Strains**

Evidence that field NDV isolates are genetically distinct from vaccine strains (e.g., vaccine might be genotype II, field strains genotype VII or others).

➤ **Risk Factors and Epidemiology**

Identification of management, biosecurity, vaccine delivery / handling, bird age, flock type, and other risk factors associated with vaccine failure or with infection despite vaccination.

Association of vaccine failure with factors such as cold chain breakdowns (storage temperature, duration), misuse or inappropriate vaccination schedules, mismatched genotype, poor immune responses, improper vaccine administration, etc.

TABLE: 1 WORK PLAN

N O-	Activity	Sept.	Oct	Nove	Dece	Jan	Feb	March	April	May
1	Site selection									
2	Proposal writing									
3	Soumission of Proposal									
4	Acquisition of materials									
5	Sample collection									
6	Sample transportation									
7	Sample processing									
8	Data entery and evaluation									
	Thesis submission and defense									

TABLE 2: BUDGET BREAK DOWN

No.	items	quantity	Unity price	unities	Total price
1	Glove	500	50	piece	25000
2	Macks	200	30	piece	6000
3	Transportation			km	15000
4	Paper printing	200	5	piece	1000
	Reagents			piece	25000
5	Marker	2	50	piece	100
6	Paper for questionaries'	1000		piece	3000
10	Total				75,100

5. REFERENCES

- Abdeta, D., et al. (2022). Seroprevalence and associated risk factors of infectious bursal disease in chickens managed under intensive and backyard production systems in Western Oromia, Ethiopia. *Veterinary Medicine: Research and Reports*, **13**, 67–75.
- Alders, R. G., & Spradbrow, P. B. (2001). Controlling Newcastle disease in village chickens: A field manual. Canberra: Australian Centre for International Agricultural Research (ACIAR).
- Aldous, E. W., & Alexander, D. J. (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *Avian Pathology*, **30**(2), 117–128.
- Alemu, F., Belayneh, R., & Taye, M. (2012). Seroprevalence of Newcastle disease virus in village chickens in the central part of Ethiopia. *Tropical Animal Health and Production*, **44**(3), 573–578.
- Alemu, E. E., Senbata, B., Sombo, M., Guyassa, C., Alemayehu, D. H., Kidane, E., Mihret, A., Mulu, A., & Dinka, H. (2024). Molecular detection and characterization of Newcastle disease virus from chickens in Mid-Rift Valley and Central Ethiopia. *Veterinary Medicine: Research and Reports*, **15**, 149–157.
- Alexander, D. J. (2001). Newcastle disease. *British Poultry Science*, **42**(1), 5–22.
- Alexander, D. J. (2009). Ecology and epidemiology of Newcastle disease. *Avian Diseases*, **53**(2), 202–210.
- Alexander, D. J. (2011). Newcastle disease in the European Union 2000 to 2009. *Avian Pathology*, **40**(6), 547–558.
- Allan, W. H., & Gough, R. E. (1974). A standard haemagglutination inhibition test for Newcastle disease: A comparison of macro and micro methods. *Veterinary Record*, **95**(6), 120–123.
- Bari, F. D., Gelaye, E., Tekola, B. G., Harder, T., Beer, M., & Grund, C. (2021). Antigenic and molecular characterization of virulent Newcastle disease viruses circulating in Ethiopia between 1976 and 2008. *Veterinary Medicine: Research and Reports*, **12**, 129–140.
- Central Statistical Agency (CSA). (2023). Agricultural sample survey: *Livestock and poultry population report, Oromia Region. Addis Ababa, Ethiopia*.

- Chaka, H., Goutard, F., Bisschop, S., & Thompson, P. (2013). Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa Zone, Ethiopia. *Preventive Veterinary Medicine*, **110**(3–4), 562–567.
- Dohoo, I., Martin, W., & Stryhn, H. (2010). Veterinary epidemiologic research (2nd ed.). Charlottetown, Canada: VER Inc.
- Ezeokoli, C. D., Umoh, J. U., Adesiyun, A. A., & Abdu, P. A. (1984). Newcastle disease in Nigeria: A review. *Nigerian Veterinary Journal*, **13**(2), 39–45.
- FAO (Food and Agriculture Organization). (2010). Guidelines for animal welfare and ethics in veterinary research. Rome, Italy: FAO.
- Getachew, F., Teshome, W., & Molla, B. (2014). Serological and molecular detection of Newcastle disease virus in village chickens in Central Ethiopia. *Tropical Animal Health and Production*, **46**(3), 529–534.
- Haile, B., et al. (2020). The role of live chicken markets as a source of replication and dissemination of Newcastle disease in Ethiopia. *Poultry Science*, **99**(12), 6651–6658.
- Kariuki, L., Mbugua, H., & Njenga, M. (2013). Newcastle disease in backyard chickens in Africa: Epidemiology and control. *Avian Pathology*, **42**(5), 437–444.
- Kassa, S. A., Sahle, M., & Yami, M. (2016). Seroprevalence and risk factors of Newcastle disease in local chickens in central Ethiopia. *Journal of Veterinary Science and Technology*, **7**(2), 321–327.
- Mazengia, H. (2012). Review on major viral diseases of chickens reported in Ethiopia. *Journal of Infectious Diseases and Immunity*, **4**(1), 1–9.
- Miller, P. J., & Koch, G. (2013). Newcastle disease. In D. E. Swayne (Ed.), *Diseases of poultry* (13th ed., pp. 89–138). Wiley-Blackwell.
- Molla, G., et al. (2025). Draft genome sequences of four Newcastle disease virus isolates from vaccinated chickens in Central Ethiopia. *Microbiology Resource Announcements*, **14**(1).
- Nigussie, D., Tadelle, D., Alemu, Y., & Sambo, E. (2012). Village chicken production and the impact of Newcastle disease vaccination in central Ethiopia. *Addis Ababa: Ethiopian Institute of Agricultural Research*.
- Sahle, M., Kassa, S. A., & Wondmeneh, E. (2019). Molecular characterization of Newcastle disease virus in backyard and commercial chickens in Ethiopia. *African Journal of Microbiology Research*, **13**(6), 112–120.

- Sonaiya, E. B., & Swan, S. E. J. (2004). Small-scale poultry production: Technical guide. Rome: *FAO Animal Production and Health Manual*.
- Tadesse, G., Ashenafi, H., & Zeleke, A. (2005). Village chicken production system and constraints in western Ethiopia. *Livestock Research for Rural Development*, **17**(11).
- Thrusfield, M. (2018). Veterinary epidemiology (4th ed.). Oxford, UK: *Wiley-Blackwell*.
- Tibebu, A. (2024). Prevalence of Newcastle disease in chickens in Ethiopia: A regional synthesis. *CGIAR/CGSpace Repository*.
- Worku, T., Dandecha, M., Shegu, D., Aliy, A., & Negessu, D. (2022). Isolation and molecular detection of Newcastle disease virus from field outbreaks in chickens in central Ethiopia. *Veterinary Medicine: Research and Reports*, **13**, 65–73.
- World Organisation for Animal Health (WOAH). (2021). Manual of diagnostic tests and vaccines for terrestrial animals: Newcastle disease (Avian paramyxovirus type 1). Paris, France: *WOAH*.
- Yadeta, W., Amosun, E., Mohammed, H., Woldemedhin, W., Sherefa, K., Legesse, A., Deresse, G., Birhanu, K., Abayneh, T., Getachew, B., Farnós, O., Kamen, A. A., & Gelaye, E. (2024). Isolation and genetic characterization of genotype VII velogenic pathotype Newcastle disease virus from commercial chicken farms in Central Ethiopia. *Viruses*, **16**(8), 1249.
- Yonas, A., Berhanu, A., & Sisay, T. (2018). Molecular detection and characterization of Newcastle disease virus isolates from chicken in central Ethiopia. *African Journal of Microbiology Research*, **12**(3), 56–63.