2023-03

Sero-Epidemiology of Bovine Herpes Virus-1 in Dairy Cattle In Bahir Dar City And Bahir Dar Zuria Disrict, Northwest Ethiopia

Zelalem Getahun

`

BAHIR DAR UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICINE

DEPARTMENT OF VETERINARY SCIENCE

MASTERS OF SCIENCE IN VETERINARY EPIDEMIOLOGY AND ECONOMICS

SERO-EPIDEMIOLOGY OF BOVINE HERPES VIRUS-1 IN DAIRY CATTLE IN BAHIR DAR CITY AND BAHIR DAR ZURIA DISRICT, NORTHWEST ETHIOPIA

MSc Thesis

By

Zelalem Getahun

MARCH 2023

BAHIR DAR, ETHIOPIA

BAHIR DAR UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICINE

DEPARTMENT OF VETERINARY SCIENCE

MASTERS OF SCIENCE IN VETERINARY EPIDEMIOLOGY AND ECONOMICS

SERO-EPIDEMIOLOGY OF BOVINE HERPES VIRUS-1 IN DAIRY CATTLE IN BAHIR DAR CITY AND BAHIR DAR ZURIA DISTRICT, NORTHWEST ETHIOPIA

MSc Thesis

BY

Zelalem Getahun

A THESIS SUBMITTED TO THE COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCE OF BAHIR DAR UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIRMENTS OF THE DEGREE OF MASTER SCIENCE (MSc.) ''IN VETERINARY EPIDEMIOLOGY AND ECONOMICS"

March 2023

Bahir Dar, Ethiopia

THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by **Zelalem Getahun** entitled **Sero-Epidemiology of Bovine Herpesvirus-1 in Dairy Cattle in Bahir Dar city and Bahir Dar Zuria district, Northwest Ethiopia".** We hereby certify that, the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (M.Sc.) in **"Veterinary Epidemiology and Economics".**

Board of Examiners

Prof. Wassie Molla Signature for Date 16/03/2023

Name of External Examiner

Mussie H/Meret (PhD) Signature Date 16/03/2023

Name of Internal Examiner

Habtamu Tassew (DVM) Signature Date 16/03/2023

Name of Chair Person

DECLARATION

This is to certify that this thesis entitled "**Sero-Epidemiology of Bovine Herpesvirus-1 in Dairy Cattle in Bahir Dar City and Bahir Dar Zuria district, Northwest Ethiopia**" submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in the Graduate Program of **"Veterinary Epidemiology and Economics"** to the College of Agriculture and Environmental Sciences, Bahir Dar University is a record of original work carried out by me and has never been submitted to this or any other institution to get any other degree or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

Name of the student

Zelalem Getahun

Signature & Date 16/03/2023

1) Major Advisor

Dr.Yeshwas Ferede (DVM, MSc, assistant professor)

Signature & date 16/03/2023

2) Co-Supervisors

Dr. Yechale Teshome (DVM, MSc, assistant professor)

Signature & date 16/03/2023

Dr. Yoseph Kerie (DVM, MSc, Veterinary Microbiologist)

Signature & date 16/03/2023

ACKNOWLEDGEMENTS

First and for most, my heartiest gratitude shall belong to my almighty GOD and his Mother Virgin Marry, for their compassionate protection and help throughout my personal and professional career development.

I would like to express my profound appreciation and sincere thanks to my major advisor Dr. Yeshwas Ferede for his willingness and tireless cooperation in correcting and giving constructive comments guidance, ideal and moral support from inception to completion of this MSc thesis.

I would like to thank my co-advisors Dr.Yechale Teshome from Bahir Dar University and Dr. Yoseph Kerie from Bahir Dar Regional Animal Health Diagnostic and Investigation Laboratory for their guidance and technical support and encouragement during the production of this MSc thesis.

I wish to express my thanks to staff of Bahir Dar Regional Animal Health Diagnostic and Investigation laboratory for their kind cooperation in providing the required laboratory materials, sample collection and laboratory processing. My special thanks go to Dr. Endeshaw Demil for his technical support during the laboratory investigation process.

My profound gratitude goes to Dr. Semahegn Yilkal and Mr. Zemen Birhan, and to all livestock owners, district animal health personnel, and the assistant veterinarians for their cooperation during field data collection.

Finally, I would like to acknowledge Bahir Dar University, College of Agriculture and Environmental Sciences which offered me to persue this MSc study opportunity.

DEDICATION

I dedicate this work to my family members for nursing me with affection and love and for their dedicated partnership in the success of my life.

TABLE OF CONTENTS

CONTENTS **page**

(Continued)

(Continued)

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF APPENDICES

Appendix **Page**

LIST OF APPENDIX TABLES

Appendix Table **page**

LIST OF APPENDIX FIGURES

Appendix Figure **Page**

LIST OF ABBREVIATIONS

AI	Artificial insemination
BoHV-1	Bovine Herpes Virus-1
BRDC	Bovine Respiratory Disease Complex
CNS	Central Nervous System
CPE	Cyto Pathic Effect
CVE	Coital Vesicular Exanthema
DIVA	Differentiating Infected from Vaccinated Animals
DNA	Deoxyribonucleic Acid
EU	European Union
HveC	Human vascular endothelial Cell
IBR	Infectious Bovine Rhinotracheitis
ICTV	International Committee for Taxonomy of Virus
IPB	Infectious Pustular Bbalanoposthitis
IPV	Infectious Pustular Vulvovaginitis
MDBK	Madin Darby Bovine Kidney
MLVV	Modified Live Virus Vaccines
OD	Optic Density
OIE	Office International des Epizooties
TMB	Tetra Methyl Benzidine
TNLC	Test Negative Latent Carrier
VNT	Virus Neutralization Test

SERO-EPIDEMIOLOGY OF BOVINE HERPES VIRUS-1 IN DAIRY CATTLE IN BAHIR DAR CITY AND BAHIR DAR ZURIA DISTRICT, NORTHWEST ETHIOPIA

Zelalem Getahun[1], Yeshwas Ferede[2], Yechale Teshome[2] and Yoseph Kerie[3]

[1]Bure Town Administration Agriculture Office

[2]School of Animal Science and Veterinary Medicne, Bahir Dar University

[3]Bahir Dar Bahir Dar Regional Animal Health Diagnostic and Investigation Laboratory

ABSTRACT

Bovine herpesvirus-1 (BoHV-1) causes infectious bovine rhinotracheitis (IBR), and infectious pustular vulvovaginitis (PV) in cows and infectious balanoposthitis (IBP) in bulls. Bovine herpesvirus-1is highly contagious cattle disease, which causes significant reproductive losses in the dairy industry worldwide. A cross-sectional study was conducted using a multistage stratified sampling method between April 2022 to December 2022 to estimate the sero-prevalence of BoHV-1 in dairy cattle and its associated risk factors in Bahir Dar city and Bahir Dar Zuria district, northwest Ethiopia. A total of 384 serum samples from 121 herds of cattle aged above 6 months reared in rural, peri-urban, and urban dairy production systems were collected. Competitive ELISA assay was performed to detect antibodies directed against the gB glycoprotein of BoHV-1. Besides, a semi-structured questionnaire was administered to dairy cattle producers to obtain data on household demography, dairy cattle production system, animal level and herd level management practices. The present study revealed that the overall animal level and herd level seroprevalences of BoHV-1 were 63.54% (95% CI: 58.8–68.2) and 81.82 % (95% CI: 74.8–88.7) respectively and varied between production systems. The overall animal level prevalence of BoHV-1 was significantly higher in rural dairy production systems (AOR=4.2, 95% CI: 1.78–9.85; p<0.001) than in peri-urban (AOR=2.2, 95% CI: 1.13-4.42; p=0.019) and urban dairy production systems. Age was found one of the significant risk factors affecting the prevalence of BoHV-1 infection (P<0.001). Animals aged above five years (>5 years) were 3.2 times (AOR=3.2, 95%CI: 1.76–5.86; P<0.001) more likely to be affected by BoHV-1 compared to cattle below two years (< 2 years). Regarding the origin, Purchased cattle were more likely at risk of acquiring BoHV-1 (AOR=2.9, 95% CI: 1.57–5.52; p=0.001) as compared to homebred cattle. The prevalence of BoHV-1 was also significantly influenced by parity (OR=2.9; p=0.020) in which multiparous

cow were more affected by BoHV-1 compared to primiparous cows. Furthermore, a significantly higher prevalence of BoHV-1infection was recorded in cows with a history of reproductive disorders. Likewise, the prevalence of BoHV-1infection was significantly higher (OR=4.1, 95% CI: 2.17–7.7; p<0.001) in animals with a history of respiratory disorders when compared to animal with no history of respiratory disorder. In conclusion, this study revealed that the prevalence of bovine herpes virus-1 (BoHV-1) was found to be higher in the present study area. Besides, a multitude of host and management risk factors affecting the prevalence of BoHV-1 infection were investigated. Such higher prevalence could significantly affect the productivity and overall production of dairy herds in the study areas. This necessitates evidence based integrated BoHV-1 control and prevention strategy in the study area.

***Keywords**: Bahir Dar city; Bahir Dar Zuria district; BoHV-1; Competitive ELISA; Cross-sectional; Dairy Cattle; Sero-epidemiology*

Chapter 1. INTRODUCTION

1.1 Background of the Study

Bovine herpesvirus type 1 (BoHV-1) is an enveloped DNA virus which belongs to the order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae* and the genus *Varicellovirus* (Albayrak *et al*., 2020). BoHV-1 is a major pathogen of cattle that is known to be highly contagious and responsible for severe economic loss to the cattle industry throughout the world (Kuotsu *et al*., 2019b). The clinical spectrum of the disease is complex and the severity of the infection and pathogenesis depends upon its virulence (Biswas *et al*., 2013). The virus generates losses in (sub)clinically diseased cattle and may result in trading restrictions both within and between countries (Muylkens *et al*., 2007).

BoHV-1 causes important cattle diseases such as infectious bovine rhinotrachietis (IBR), infectious pustular balanoposthitis (IPB), infectious pustular vulvovaginitis (IPV) (Newcomer, 2021). The virus has also been associated with other clinical disease manifestations including infertility, abortion, encephalitis, conjunctivitis, mastitis, and enteritis (Adeli *et al*., 2017). BoHV-1 infection increase susceptibility to secondary bacterial infection of the lower respiratory tract through injury and induction of other changes in the tract this initiates and contributes to shipping fever or bovine respiratory complex (Levings and Roth, 2013).

The major characteristics feature of BoHV-1 is the establishment of lifelong latency (Farooq *et al*., 2019a). After initial infection and disease, cattle become carriers of the virus for life, as it remains latent in the trigeminal and sciatic ganglia (Ackermann and Engels, 2006) and lymph nodes and nasal mucosa are also considered to be sites of latency (Bilge-Dagalp *et al*., 2008). Cattle infected with BoHV-1 could excrete the virus for a prolonged period of time and develop latency following recovery from the infection (Nandi *et al*., 2009). Increased corticosteroid levels, due to food and water deprivation during shipping of cattle, weaning, and/or dramatic weather changes increase the incidence of BoHV-1 reactivation from latency (Jones, 2014).

The main sources of infection are nasal exudates and cough droplets, genital secretions, semen, fetal fluids and tissues (Nandi *et al*., 2009). The BoHV-1 infection is mainly transmitted by respiratory, ocular or genital secretions through direct contact between animals. Direct contact with non-nasal mucosal discharges, contaminated semen, fetal tissues, and genital fluid can also lead to transmission (Muylkens *et al*., 2007). The viruses is also shed and transmit following stimuli, for example, transport, parturition and glucocorticoid therapy (Jones and Chowdhury, 2007). However, the infectivity duration of these aerosols is dependent on environmental factors such as humidity and temperature (Kipyego *et al*., 2020).

The symptoms of the BoHV-1 are mainly mild and non-life-threatening but have a rather wide host range that limits animal trade (Mahmoud and Salem, 2012). Generally, BoHV-1 is characterized by clinical signs of serous nasal discharge, salivation, fever, in appetence, milk drop, abortion, stillbirth, vulvovaginitis, and even death (Muylkens et al., 2007; Woodbine et al., 2009b). Where natural mating is practiced, genital infection can lead to pustular vulvovaginitis or balanoposthitis (Raaperi *et al*., 2012). Secondary bacterial or viral agents may contribute to a multifactor disease complex resulting in severe respiratory disease of young animals (shipping or crowding fever) (Biswas *et al*., 2013).

The disease can be diagnosed by using conventional procedures (like cell culture, immune-histopathology, and enzyme-linked immunosorbent assay (ELISA)) as well as highly sensitive modern techniques (like nested PCR and southern hybridization) with the virus neutralization test regarded as gold standard (Kathiriya *et al*., 2018). However, the ELISA is a specific, sensitive, and more practical test for the detection of BoHV-1 antibodies (Varela *et al*., 2010) From the currently available diagnostic tests, it is not possible to identify animals which have a latent BoHV-1 infection (Malla *et al*., 2018).

Conventional vaccines are widely used to prevent the development of clinical signs and markedly reduce the shedding of virus after infection. The use of conventional vaccines, however, do not appear to have resulted in a reduction in the prevalence of infection (Adeli *et al*., 2017). Several eradication campaigns have been carried out or are currently running in different countries including test-and-removal programmes or vaccination campaigns (Biswas *et al*., 2013). BoHV-1 eradication campaigns have been implemented in some European

Union countries. These tend to employ vaccination (using marker vaccines), serological testing and movement control (Brock, 2021).

BoHV-1 is a ubiquitous pathogen affecting cattle populations worldwide with the exception of the BoHV-1-free countries, paralleling the distribution of domestic cattle (Biswas *et al*., 2013). Cattle of all breeds and ages are equally susceptible, and the disease is common in cattle above 6 months of age due to waning of maternal antibody protection and increased mixing of cattle populations (Adeli *et al*., 2017). Approximately 50% of the adult cattle population have had experience of this disease (Seal *et al*., 2007). Various intrinsic and extrinsic factors influence the occurrence of infection among cattle population (Kuotsu *et al*., 2019b). Seroprevalences reported during the past 15 years varied between 35.9- 77.5% in Europe and 37–67% in Latin America (Raaperi *et al*., 2014). Only a few countries are known to be free of the disease (Iscaro *et al*., 2021).

Studies undertaken on seroprevalence BoHV-1 so far in Ethiopia revealed the existence of the disease in different parts of the country with prevalence ranging from 25.6%–77.6% (Berhanu Sibhat *et al*., 2018; Tadeg Wedajo *et al*., 2021). These studies showed the widespread nature of BoHV-1 with possible variation of the prevalence status. As there is no animal movement restriction in place, it is highly likely that the disease could have been spread to the country.

The financial and animal welfare consequences of the virus are significant, epidemiological data of the virus and the risk factors thus greatly important to design suitable prevention and control program (Kaddour *et al*., 2019). The ability to study this virus not only aids in prevention and control program but it also lays the foundation for a better understanding of BoHV-1 infection in general. Since the documentation of the epidemiological data of BoHV-1 in cattle requires the assessment and estimation of the sero-epidemiology and evaluation of associated risk factors in the country, the present study was conducted to investigate the sero-epidemiology of BoHV-1 in dairy cattle in Bahir Dar city and Bahir Dar Zuria district, Northwest Ethiopia.

1.2 Statement of the Problem

Bovine herpesvirus-1 (BoHV-1) is widespread, and is an important pathogen of cattle that causes considerable economic losses due to reproductive failure and increased calf mortality, as well as respiratory disease (Kampa *et al*., 2004). It is an enzootic disease on the list of the World Animal Health Organization. The disease is common in many countries, although there are differences in prevalence (Ackermann and Engels, 2006). It is distributed worldwide although only a few countries are known to be free of the virus (Raaperi *et al*., 2014). Some countries are working toward controlling or eradicating the infection but in developing countries it is continuing as one of the economically important diseases due to weak implementation of preventive measures (Raaperi, 2012).

BoHV-1 first reported in Ethiopia in mid-1970 and late 1980s. Thereafter, few recent studies showed that BoHV-1 is widely spreading in some parts of the country (Berhanu Sibhat *et al*., 2018). Although BoHV-1 infections have been registered in Ethiopia cattle herds for years no systematic control programs exist against the disease. Nevertheless, nationwide epidemiological data is needed to device evidence-based effective disease control and prevention measures.

Despite its high economic importance in cattle production, no previous study was found on the sero-epidemiology of BoHV-1 virus in cattle in Bahir Dar city and Bahir Dar Zuria district, Northwestern Ethiopia. Bahir Dar city is considered as one of Ethiopia's potential milk shed (a potential growth corridor for dairy), where several market-oriented small holders and commercial dairy farms have been emerging.

As there is no animal movement restriction in place, it is highly likely that the disease could have been spread to the country since contact with infected cattle is the main source of BoHV-1 infection to susceptible animals and herds (Kipyego *et al.*, 2020). On the other hand, Movement and mixing in new herds is stressful for cattle, resulting in recrudescence of the virus in infected cattle that then infect susceptible cattle (Woodbine *et al*., 2009b).

Animal marketing is carried out between rural, peri-urban, and urban production settings without the application of any health security measures in the area, which might facilitate the

transmission of BoHV-1 among cattle populations (Tadeg Wedajo *et al*., 2021). Furthermore, animals not successfully sold will return and introduced to their original herd without quarantine anymore. Communal grazing mixed with sheep and goats is a common practice particularly in rural cattle production system; this might increase the chance of exposure and transmission of BoHV-1 among cattle and livestock populations. Sheep and goats are known to be carriers of BoHV-1 (Biswas *et al*., 2013). Indeed, breeding of the bull is no longer under the annual inspection of all animals in the herd for BoHV-1; thus Virus may be transmitted by semen and, the disease may persist and circulate across the cattle population.

Understanding this virus and its effect on host animals will aid in making sound management decisions. In addition, knowing which risk factors are objectively relevant in cattle herds is indispensably important for designing most beneficial and economical control programmes for BoHV-1 in Ethiopia in general and in Bahir Dar city and Bahir Dar Zuria district in particular.

Based on the above background, this study was initiated to meet the following general and specific objectives;

1.3 Study Objectives

1.3.1 General Objective

To investigate the Sero-epidemiology of Bovine herpesvirus-1 in Bahir Dar City and Bahir Dar Zuria district, Northwest Ethiopia

1.3.2 Specific Objectives

- ✓ To estimate the seroprevalence of Bovine herpesvirus-1 in dairy cattle
- ✓ To identify associated risk factors affecting the occurrence of Bovine herpes virus-1 in the study area
- ✓ To examine the association between Bovine herpesvirus-1 infection with reproductive and respiratory disorders in dairy cattle herds

1.4 Research Questions

- What is the prevalence of BoHV-1 infection in dairy cattle kept under different dairy production systems in Bahir Dar city and Bahir Dar Zuria district, Northwest Ethiopia?
- What are the potential risks factors influencing the occurrence of BoHV-1 in dairy cattle in the study area?
- Is there any association between Bovine herpesvirus-1 infection with reproductive and respiratory disorders in dairy cattle herds in Bahir Dar City and Bahir Dar Zuria district

Chapter 2. LITRATURE REVIEW

2.1 Historical Perspective and General Aspects of BoHV-1

Various viral diseases including Bovine herpesvirus type 1 (BoHV-1) are hindering the development of dairy industry due to heavy economic loss (Graham, 2013a). Bovine herpesvirus 1 has gained a lot of attention worldwide during the last half of a century. The first report of a disease probably caused by bovine herpesvirus-1 (BoHV-1) was recorded in Germany in 1841 where a condition referred to as Bläschenausschlag (in German literature), or coital vesicular exanthema (CVE) was described (Adeli *et al*., 2017). However, the viral etiology was demonstrated and proved in 1928 by Reisinger and Reimann, who transmitted this venereal disease by a filterable agent (Biswas *et al*., 2013). Thereafter, CVE, more commonly referred to as infectious pustular vulvo-vaginitis in cows and heifers (IPV) and infectious pustular balanoposthitis in bulls (IPB) remained the primary recognized manifestation of infection with BoHV-1 until the 1950s (Graham, 2013b). Meanwhile, BoHV-1 was detected and named in the 1950s in feedlots in the western United States of America and was named BoHV-1 (Graham, 2013a).

The first published report on respiratory IBR came from Schroeder and Moys (1954) where they described an apparently new upper respiratory disease of dairy cattle that occurred in California since 1953 (Chen *et al*., 2018). It appeared suddenly and was characterized by high fever and reduced milk production in addition to respiratory signs (Muylkens *et al*., 2009). By 1954, it was occurring in dairy cattle and all ages of beef cattle both in feedlots and occasionally in cattle on pasture (Raaperi *et al*., 2014). In the year 1955, at a meeting of the USA livestock sanitary association, the conventional name for the disease became Infectious bovine rhinotracheitis (Majumder *et al*., 2015).

In 1958, the virus was isolated successfully for the first time and its antigenic identity was revealed and this viral agent was classified under the family of *Herpesviridae* (Biswas *et al*., 2013). In 1961, Armstrong suggested that the IBR virus (BoHV-1) belongs to the *Herpesvirus* group (Yadav *et al*., 2018).

Following the apparent emergence of IBR in the USA, it was diagnosed in many countries and is now reported worldwide. Through cattle trade (including semen and embryos), the virus was introduced in Europe in 1960 (Woodbine *et al*., 2009b). In Europe the virus was first isolated from cattle with respiratory disease in the United Kingdom in the early 1960 (Majumder *et al*., 2015). Within a decade, the virus had become endemic in most countries; and subsequently, IBR emerged as a clinical condition in Europe, from the 1970s onward (Graham, 2013b). Soon research about the epidemiology of the disease started to clarify the extent of the virus spread (Ackermann and Engels, 2006). In 1986, almost 35 per cent of cattle and 48 per cent of herds in Europe evidenced the antibody to BoHV-1 (Ganguly *et al*., 2008).

The initial description of infectious pustular vulvovaginitis (IPV) was made by Kendrick in 1958, while Huck described its association with Balanoposthitis in 1971 (Fulton *et al*., 2016) . BoHV-1 is currently widespread and reported all over the world and the virus is recognized to cause a range of other clinical conditions in cattle, including conjunctivitis; reproductive tract causing vulvovaginitis and balanoposthitis, skin lesions as well as neonatal infection causing red nose, necrotic rhinitis, epididymitis, abortion, infertility, dermatitis and mastitis (Newcomer, 2021). However, BoHV-1 is over the years successfully eradicated in several European countries or regions through different mechanisms (Valas *et al*., 2019).

Now a day it is a problem of domestic cattle populations, both in beef, cattle and dairy. The disease occurs usually after recent addition to a herd, and the virus is transmitted to susceptible cattle (Wathes *et al.*, 2020). The disease is most common in cattle over 6 months of age (Tuncer-Göktuna *et al*., 2016). Although the infection is infrequently life threatening, the introduction of BoHV-1 into a cattle farm can cause severe economic losses due to production losses and restrictions in the international trade of livestock (Nandi *et al*., 2009)

It is difficult to make an accurate estimation of the real economic impact of BoHV-1 because of the absence of clinical signs in latently infected animals (Segura-Correa *et al*., (2016)). The BoHV-1 associated risk factors possibly vary from farm to farm, place to place, and region to region because of the number of animals, uneven husbandry, microclimatic differences, and other circumstances (Kaddour *et al*., 2019).

2.2 Taxonomic Classification BoHV-1

The international committee for taxonomy of virus (ICTV) named the virus as bovid herpes virus type-1 on the basis of the family of virus and the host it infects (Kaur and Chandra, 2016). BoHV-1 is a member of the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae,* which belongs to the *Herpesviridae* family, order *Herpesvirales* and class *Herviviricetes* (Yazici *et al*., 2015). According to ICTV the herpesviridae family includes three subfamilies. The three subfamilies are *Alphaherpesvirinae*, *Betaherpesvirinae* and *Gammaherpesvirinae* each having distinctive biological properties (McGeoch *et al*., 2008). The classification of *Herpesvirus* based on biological properties, DNA sequence homology, similarities in genome sequence arrangements, and similarities in the functions of important viral proteins (Muylkens *et al*., 2007).

Alphaherpesviruses replicate and spread in the host, rapidly and destroying the host cells (Biswas *et al*., 2013). It has a wide host range, efficient and short reproductive cycles and establish latency in sensory neuronal cells (Muylkens *et al*., 2007).

Betaherpesviruses replicate and spread in the host slowly. They cause infected cells to enlarge hence they are commonly named as cytomegaloviruses. They become latent in the secretary gland and lymphoreticular cells of the hosts (Muylkens *et al*., 2007).

Gammaherpesvirus are distinguished by reproducing at a more variable rate than other subfamilies of herpesviridae. The virus infect the T and B lymphocytes of the host and produce latent infection in these cells (Ackermann, 2006) Table 1).

Table 1. Subfamily *alphaherpesvirinae* and respective diseases (Ackermann, 2006)

Subfamily	Genus	Name	Common Name
Alphaherpesvirinae	*Varicellovirus*	*Bovine herpesvirus 1*	*Infectious bovine rhinotracheitis virus*
Alphaherpesvirinae	*Varicellovirus*	*Bovine herpesvirus 5*	*Bovine encephalitis herpesvirus*
Alphaherpesvirinae	*Varicellovirus*	*Canid herpesvirus 1*	*Canine herpesvirus*
Alphaherpesvirinae	*Varicellovirus*	*Equine herpesvirus 1*	*Equine abortion virus*
Alphaherpesvirinae	*Varicellovirus*	*Felid herpesvirus 1*	*Feline herpesvirus*
Alphaherpesvirinae	*Varicellovirus*	*Suid herpesvirus 1*	*Pseudorabies virus*
Alphaherpesvirinae	*Simplexvirus*	*Bovineherpesvirus 2*	*Bovine mammillitis virus*
Alphaherpesvirinae	*Iltovirus*	*Gallid herpesvirus 1*	*Infectious laryngotracheitis virus*
Alphaherpesvirinae	*Iltovirus*	*Psittacid herpesvirus 1*	*Pacheco's disease virus*
Alphaherpesvirinae	*Mardivirus*	*Gallid*	*Marek's disease virus*
Betaherpesvirinae	*Probiscivirus*	*Elephanti herpesvirus1*	*Elephant endotheliotrphic herpesvirus*
Betaherpesvirinae	*Muromegalovir*	*Muridherpesvirus 1*	*Murine cytomegalovirus*
Gammaherpesvirinae	*Macavirus*	*Ovineherpesvirus 2*	*Sheep-associated malignant catarrhal fever virus*

2.3 Etiology

BoHV-1 is highly contagious and infectious virus responsible for great economic loss to the livestock industry (Muylkens *et al*., 2007). There are four *Bovine herpesvirus* including BoHV-1, BoHV-2, BoHV-4, and BoHV-5 in the family *herpesviridae* (Yang, 2019). There is

only one antigenic type of BoHV-1, irrespective of whether the isolate is derived from cases of IBR or IPV (Nandi *et al*., 2009).

BoHV-1 isolates are classified into 3 subtypes by the use of restriction endonuclease analysis: subtypes BoHV-1.1, BoHV-1.2a, and BoHV-1.2b (Yang, 2019). The subtypes are differentiated based on antigenic and genomic analysis (Owen *et al*., 2015). BoHV-1.1 is usually associated with respiratory syndrome and abortions, while BoHV-1.2a is linked with genital tract infections such as IPV and IPB. Subtype 2b is mainly manifested as IPV/IBP but not abortifacient and have slow pattern of spread (Muylkens *et al*., 2007). Isolates of BoHV-1.1 are more virulent than are isolates of BoHV-1.2b and have a rapid pattern of spread across the herd (Nandi *et al.*, 2009).

BoHV-1.3, which is a neuropathogenic agent, has been reclassified as BoHV-5 (Glazov *et al*., 2010). All of the subtypes are antigenically similar but may be differentiated by restriction enzyme fragment polymorphisms (Zhou *et al*., 2020). Different prototypes of *herpesviridae* are listed Table 2 below.

Table 2. prototypes of *herpesviridae* host range and their associated disease in animals (Biswas *et al*., 2013)

Virus	Host Species	Disease
Alphaherpesviridae subfamily		
Bovine herpesvirus-1	Cattle (goat, sheep, red deer, rein deer)	Infectious bovine rhinotracheitis
Bovine herpesvirus-2	Cattle and African ruminants	Bovine herpes mammillitis Pseudo lumpy skin disease
Bovine herpesvirus 5	Cattle (goat, sheep)	Bovine encephalitis
Suid herpesvirus-1	Swine (ruminants, carnivores)	Fatal encephalitis
Cervid herpesvirus-1	Red deer	Ocular disease
Cervid herpesvirus-2	Rein deer (cattle)	Subclinical genital infection
Caprine herpesvirus-1	Goat (cattle, sheep)	Genital disease, fatal neonatal
Bubaline herpesvirus-1	Buffalo	Subclinical genital infection
Elk herpesvirus-1	Elk (cattle)	Subclinical genital infection
Ovine herpesvirus-1	Sheep	Isolated in ovine pulmonary adenomatosis

2.4 Herpes Virus Structure and Genome

Like all herpesviruses, BoHV-1 has a relatively long, double-stranded DNA genome. Herpesviruses share characteristic, highly conserved structural components. All herpesviruses have a lipid envelope covered with viral glycoprotein spikes, a proteinaceous tegument, an icosahedral viral capsid, and a linear, double-stranded DNA core (Paulus *et al*., 2010). The capsid is a 100 nanometer diameter structure composed of 162 capsomers (Biswas *et al*., 2013). Surrounding the capsid is a layer of globular material, known as the tegument, which is enclosed by a typical lipoprotein envelope with numerous small glycoprotein spikes (Zaher *et al*., 2014). The tegument provides presynthesized proteins to a newly infected cell, which facilitates suppression of host protein synthesis, inhibition of cellular defense mechanisms,

and induction of viral gene expression (Muylkens *et al*., 2007). The viral glycoproteins are located in the envelope on the surface of the virion and play an important role in pathogenesis and immunity (Nandi *et al*., 2009). Herpesviruses acquire a primary envelope through budding at the inner nuclear membrane (Russell *et al*., 2018). Herpes virus genomic structure depicted figure 1.

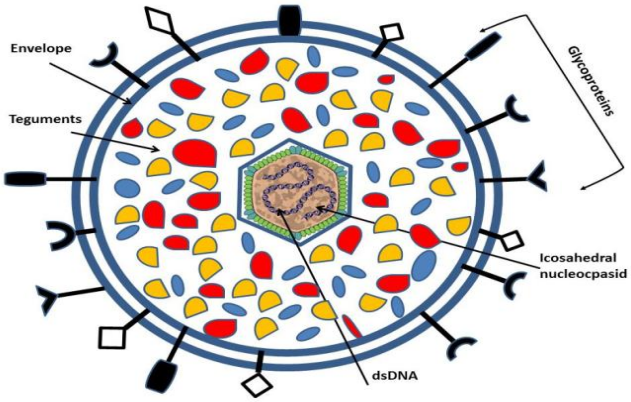


Figure 1. Schematic representation of BoHV-1 structure (Givens, 2018)

BoHV-1 genome belongs to a D group in the classification, the genome consists of 11 enveloped glycoproteins, among the enveloped glycoprotein gB, gC, and gD proteins are considered as major proteins and highly essential for entry, pathogenesis and immunity development in the hosts (Kuotsu *et al*., 2019a). the genome of the virus is 135-140 kilobase pairs and can be divided into unique long (UL) and unique short (US) segments (Verbruggen, 2021). Terminal repeat (T_R) and Internal repeat (I_R) sequences may bracket unique sequences (U_L, U_S) of both L and S or only S. Herpesvirus virions contain over 30 structural proteins, of which 6 are present in the nucleocapsid and 2 are DNA associated (Muylkens *et al*., 2007). In addition, about 11 glycoproteins are located in the envelope (Biswas et al., 2013) from which most project as peplomers (figure 2).

Both of these unique regions can be inverted, meaning that there are actually four isomeric forms of the herpesvirus genome that can be found in a BoHV1 virion. These inverted sequences are likely attributable to recombination during genome replication (McGeoch *et al*., 2006). The genomic structure of herpes virus showed figure 2.

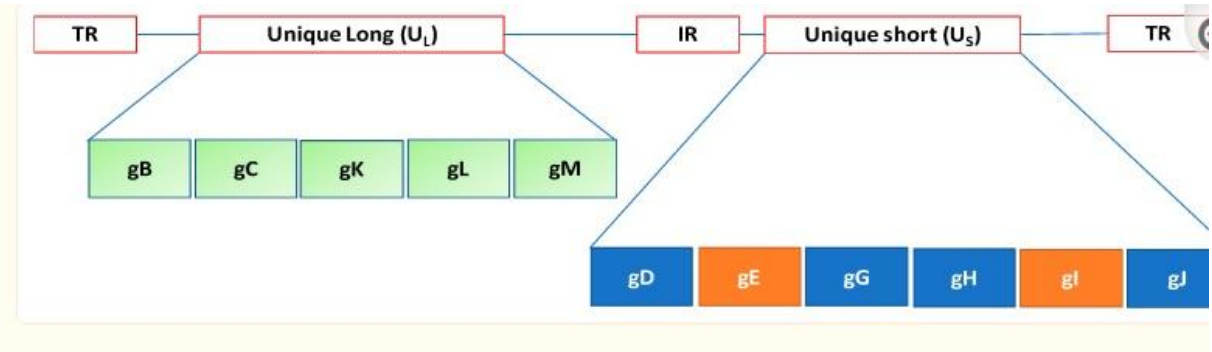


Figure 2. The genome structure of BoHV-1 (Petrini *et al*., 2019)

2.5 Physico-Chemical Properties of the Virus

The virus is fairly resistant to environmental influences (Biswas *et al*., 2013). Inactivation of the virus in the environment depends on factors such as temperature, pH, light, humidity, and the kind of medium harboring the virus (Tibary, 2014). The virus labile at pH 4.5 to 5.0 but stable at pH 6 to 9; relatively at higher temperatures the virus is inactivated more rapidly, i.e, when the temperature increase the survivality of the virus life decrease in such a way that it is inactivated at 56 ºC within 21 min, at 37°C within 10 days and at 22ºC within 50 days (Craig *et al*., 2022). BoHV-1 survives better in aerosols at 90% relative humidity than in aerosols of 35 and 10% relative humidity. In favorable conditions, e.g., in feed stuffs, the virus may survive for more than 30 days (Dangas *et al*., 2016).

Since the virus is enveloped, it is sensitive to organic solvents such as chloroform, ether, Alcohol, acetone and bile salts (Nandi *et al*., 2009). It is extremely sensitive to 0.5% NaOH, 0.01 % $HgCl_2$, 1% CaCl2 (chlorinated lime), 1% phenolic derivatives, 1% quaternary ammonium bases and 10% Lugol's iodine and inactivate the virus within seconds (Farooq *et al*., 2019a). Formalin (5%) inactivates BoHV-1 within 1 minute. Thus, the virus is fragile and sensitive to detergents and lipid solvents. It is unstable in the environment but can survive in the frozen semen indefinitely (Segura-Correa *et al*., (2016)) .

2.6 Molecular and Antigenic Characterization of BoHV-1

Antigenically, BoHV-1 is closely related to cervine herpesvirus-1 (CvHV-1), buffalo herpesvirus-1, and elk herpesvirus and related to equine rhinopneumonitis virus (BoHV1) in

complement fixation test (Keuser *et al*., 2004). It shares one-way relationship with pseudorabies virus, goat herpesvirus (Takacs, 2013). The virus also shares a common antigen with Marek's disease virus and Burkitts lymphoma virus. The virus can be isolated with the help of bovine cell cultures as well as cell lines of other species (Kaur and Chandra, 2016).

There is only a single serotype of BoHV-1 recognized as antigenicity is concerned. There are three strains of BoHV-1, namely, I, II, and III on the basis of endonuclease pattern of viral DNA (Petrini *et al*., 2019). They reclassified as BoHV-1.1 (respiratory type), BoHV-1.2 (genital type), and BoHV-1.3 (encephalitis subtype); BoHV-1.3 reclassified as distinct herpesvirus designated as BoHV-5 (Levings and Roth, 2013). BoHV-1 consists of 25-33 polypeptides of which 11 are glycosylated and associated with the virus envelope. The three sets of enveloped glycoproteins gI, gIII, and gIV were involved in the immune response (Zhang *et al*., 2010).

2.7 Epidemiology of Bovine Herpes Virus-1

2.7.1 Global Distribution of BoHV-1

BoHV-1 occurs in all continents, although there are differences in prevalence and incidence (Muylkens *et al*., 2007). Cattle populations of many countries are endemically infected with BoHV-1 (Boelaert *et al*., 2000; Rajkhowa *et al*., 2004; Yesilbag *et al*., 2003). Studies conducted in different countries across the world over the last 15 years reported varying sero-prevalence of BoHV-1 ranging from 14 to 60% in Africa, 35.9–77.5% in Europe, 37–60.8% in Latin America and 36 to 48% in Central and South America (Raaperi *et al*., 2014).

In Europe, although some countries have controlled BoHV-1 since long ago (Ackermann and Engels, 2006), the virus is still present in some other countries, with the infection tending to be endemic in most populations, but with national and regional variations (Gonzalez-Garcia *et al*., 2009; Kampa *et al*., 2009).

There is a paucity of reports on the prevalence of BoHV1 in Sub-Sahara Africa, a region where millions of ruminant livestock species abound (Fry *et al*., 2019). In these countries, no strategy is put in place by governments to control the disease. The prevalence of BoHV-1 in some country scenario is listed below Table 3.

Table 3. Prevalence of BoHV-1 in different countries

Country	Test type	Prevalence (%)	References
Sudan	Direct ELISA	51.7	(Elhassan *et al*., 2011)
Egypt	Indirect ELISA	17.4	(Mahmoud and Allam, 2013)
Kenya	Indirect ELISA	17.4	(Kipyego *et al*., 2020)
Cameroon	Indirect ELISA	16.7	(Athingo, 2018)
Algeria	Indirect ELISA	14.16	(Kaddour *et al*., 2019)
Mexico	competitive-ELISA	64.5	(Romero-Salas *et al*., 2013)
Ecuador	Indirect ELISA	43.2	(Carbonero *et al*., 2011)
Brazil	Indirect ELISA	59.0	(Dias *et al*., 2013a)
Colombia	Indirect ELISA	57.5	(Ortiz-González *et al*., 2022)
Spain	Blocking ELISA	45.7	(Gonzalez-Garcia *et al*., 2009)
Italy	Serum neutralization test	34.99	(Castrucci *et al*., 1997)
England	Ab-ELISA	42.5	(Woodbine et al., 2009b)
Turkey	Indirect ELISA	39.5	(İnce and Şevik, 2022)
Pakistan	Competitive ELISA	49.5	(Batool *et al*., 2022)
Iran	Indirect ELISA	58.74	(Bahari *et al*., 2013)
China	Competitive ELISA	35.8	(Yan *et al*., 2008)

2.7.2 Seroprevalence and Distribution of BoHV-1 in Ethiopia

Ethiopia was also affected by this disease as the virus was detected in some parts of the country. The first time, BoHV-1 has been clinically documented in the mid-1970s and late 1980s though the information on how BoHV-1 was introduced and established in Ethiopia is not documented yet (Berhanu Sibhat *et al*., 2018). Few serological studies done in different localities of Ethiopia indicated that the virus is a widespread and endemic disease of cattle in different production systems of the country. According to the study the seroprevalence in central, southern and western part of Ethiopia was 30.8%, 45.5% and 55.9% respectively (Berhanu Sibhat *et al*., 2018).

Apart from this evidences seroprevalence of BoHV-1 was conducted and recorded in a dairy cattle at Dessie and Kombolcha Twon, North Central Ethiopia, and the reported overall prevalence of the disease was 25.6% (Tadeg Wedajo *et al*., 2021). Similar study was also conducted in local zebus of extensively and semi-intensively reared cattle in Northwestern parts of the country such as West Gojjam, East Gojjam, and Awi zone (shown Table 4) and this study showed the wide spread nature of the virus with animal level overall prevalence recorded 77.6% (Demeke Zewde *et al.*, 2021). Since that time BoHV-1 has been diagnosed in many cattle herds, and is related to clinical respiratory disease outbreaks or reproduction problems. All of the recorded signifies that virus naturally and latently exists in the country and needs farther investigation. The Seroprevalence of BoHV-1 collected from different studies in Ethiopia were listed below Table 4

Table 4. Seroprevalence of BoHV-1 compiled from different studies in Ethiopia

Study site	No. Sample	Test type	Seroprevalence (%)	References
Central Ethiopia	555	Blocking ELISA	30.8	(Berhanu Sibhat *et al*., 2018)
Southern Ethiopia	629	Blocking ELISA	45.5	Berhanu Sibhat *et al*., 2018)
Western Ethiopia	195	Blocking ELISA	55.9	Berhanu Sibhat *et al*., 2018)
Dessie and Kombolcha Town	332	Competitive ELISA	25.6	(Tadeg Wedajo *et al*., 2021)
West Gojjam zone	160	Indirect ELISA	69.4	(Demeke Zewde *et al*., 2021)
East Gojjam zone	137	Indirect ELISA	93.4	(Demeke Zewde *et al*., 2021)
Awi zone	145	Indirect ELISA	71.7	(Demeke Zewde *et al*., 2021)

2.7.3 Risk Factors for BoHV-1

Various intrinsic and extrinsic factors influence the occurrence of infection among cattle population (Kuotsu *et al*., 2019b). Based on serological surveys, a number of studies have been identifying the risk factors for BoHV-1 seropositivity (Brock *et al*., 2020). Some of them are well characterized below.

Age

The age of an animal is a risk factor for BoHV-1 seropositivity (Carbonero *et al*., 2011; Woodbine *et al*., 2009a). Age is an alternative measure of the amount of exposure-time (Raaperi, 2012), and this occurs with diseases that induce life-long seropositivity. Calves have a lower prevalence probably because of reduced contact with cattle in other herds, and shorter duration of exposure (Solis-Calderon *et al*., 2003). However, animals younger than 24 months

of age had a higher incidence of seroconversion for BoHV-1 than those >24 months of age (Segura-Correa *et al*., 2010b). Seroconversions were often restricted to cattle aged between 24–28 months, which is the period in which the young stock generally entered the milking cow population. Possibly the seronegative young stock became infected with BoHV-1 when they were added to the milking cows (Mars *et al*., 2001).

Sex

The sex of an animal (males are more frequently positive than females) has been shown to be a risk factor for BoHV-1 seropositivity (Boelaert *et al*., 2005). Bulls have more risky contacts compared to cows, for example, more frequent participation in cattle shows and other risky behaviour, such as escaping and mingling with other cattle (Waldeck *et al*., 2021). However, contrary results on the prevalence among cows and bulls have been found in different serological investigation (Guarino *et al*., 2008).

Farm Type

Farm type is a common risk factor for BoHV-1. Mixed herds (herds with dairy as well as beef) have an increased risk of being BoHV-1 positive than herds with only dairy cattle (García *et al*., 2009). Studies in Northern Ireland found that no significant difference was found between dairy and beef herds in BoHV-1 prevalence (Cowley *et al*., 2011). In a study, if the herd was a dairy herd rather than a suckler herd, the rate of seroconversion in adults was greater, possibly due to winter housing or other stressors related to dairy cow management (Woodbine *et al*., 2009b).

Origin of cattle

Purchase of cattle (Raaperi, 2012) as well as cattle that escape and associate with other cattle were a significant risk factors for the introduction of BoHV-1 (Van Schaik *et al*., 2002. Direct contact with other cattle (i.e., allowing cattle to return to the farm when not successfully sold, and grazing cattle at other farms) was found to be a risk factor for BoHV-1 introduction in a study conducted by (Van Schaik *et al*., 2002). Movement and mixing in new herds is stressful for cattle, resulting in recrudescence of the virus in infected cattle that then infect susceptible cattle (Woodbine *et al*., 2009b).

Herd Size

A positive association between herd size and BoHV-1 seropositivity has frequently been found and this indicated that this is the very important risk factor for BoHV-1(Boelaert *et al*., 2005; Segura-Correa *et al*., 2010a). According to some study that large herds or herds with high stock density are associated with high odds for BoHV-1 (Ortiz-González *et al*., 2022; Raaperi *et al*., 2014). Although no association between herd size and herd´s BoHV-1 status was found by in rural regions of Peru (Ståhl *et al*., 2002). In smaller herds, there are fewer susceptible animals throughout the year, so that the infection may not be sustained because it is below the epidemic threshold (Boelaert *et al*., 2005). Herd size must be considered as a representation for other risk factors (e.g., purchase of stock and professional visitors, recrudescence of infection through stress, or exposure to more viral types) (Nardelli *et al*., 2008; Woodbine *et al*., 2009b).

Management system

According to different studies conducted in different countries, extensive and intensive management system of dairy cattle affects the occurrence and frequency of BoHV-1. In extensive management system, there is obvious practice of natural bull mating with bulls of unknown health status that causes the spread of the disease (Ackermann and Engels, 2006). While in intensive management system there is there is a confinement and cattle density which may facilitate the transmission of the virus around the cattle population and the animal would suffered an increased risk of reactivation of BoHV-1 at the farm (Van Schaik *et al*., 2001). The reactivation was facilitated when the barn was overcrowded (i.e., more cows than compartments in the barn). Mostly the intensive management system there was overcrowded barn which leads to higher stress levels of the animal, which might in turn lead to an increased reactivation rate of BoHV-1 and more contacts between the cows (Van Schaik *et al*., 2001).

2.7.4 Host Range

Although cattle are the natural host, antibody to BoHV-1 also have been revealed in goats, swine, eastern cottontail, rabbits, water buffalos, and East African wildlife (Biswas et al., 2013). Cattle are the principal reservoir and usual source of infection. However, the virus has been shown to infect and cause disease in sheep and goats, also it has been isolated from

apparently healthy pronghorn, antelope, wildebeest, mink, and ferrets as well as from the soft-shelled tick (Thiry *et al.*, 2006). The virus also affects endangered bovine species like mithun and Yak but it does not infect mice, rats, guinea pigs, or chick although immunocompromised mice that lack interferon receptors can be infected if the virus is inoculated into the peritoneal cavity (Stults *et al*., 2022). Rabbits can be experimentally infected if the virus is injected into the conjunctiva sac of their eyes (Piacenti *et al*., 2006).

In vitro studies in cell culture showed that BoHV1 can bind weakly to human vascular endothelial cells (HveC) (nectin-1) or to the human poliovirus receptor (Minh, 2011) without detectable viral replication. Cattle of all breeds and ages are equally susceptible, and the disease is common in cattle above 6 months of age due to due to waning of maternal antibody protection and increased mixing of cattle populations (Ortiz-González *et al*., 2022). There are no records of human infection with BoHV-1 (Biswas *et al*., 2013).

2.7.5 Latency and Reactivation of BoHV-1

Following acute infection, BoHV-1 establishes latency in the sensory neurons (Jones, 2009). Bovine herpes virus-1 persists in the peripheral sensory ganglia such as trigeminal, sacral, lumbar or thoracic and excretes the virus in response to various tensions and such cattle were found spreading the infection to immunocompromised cattle (Kampa *et al*., 2004). Viral DNA persists in in sensory ganglionic neurons for the lifetime of infected cattle but can periodically reactivate and spread (Gonzalez-Garcia *et al*., 2009). The reactivated virus is transported intra-axonally back to the periphery, to the original portal of entry, where it is available for transmission to other susceptible hosts (Jones, 2016). This ability makes the virus highly effective pathogens with seropositivity rates approaching extremely very high in some populations (Biswas *et al*., 2013).

The reactivation stimulus may also occur on several occasions related to stress, such as at parturition, from transport (Jones and Chowdhury, 2007), subsequent to the introduction of heifers into another herd, moving cattle from one site to another, climatic changes, introduction into a new herd, associated viral or bacterial infections, poor management conditions, scarcity of diet, overcrowding of animals and vaccination (Muylkens *et al*., 2007).

During latency, latency related transcript (LR) region is expressed in BoHV-1 leading to the inhibition of the lytic cycle and the induction of an anti-apoptotic state of the infected cells. Inhibition of apoptosis, inhibit entry to S phase, and bICP0 expression are attributed to the function of LR (Muylkens *et al*., 2007). The Abundant transcription of latency related (LR) gene and gE gene coding for glycoprotein E was responsible for the latency of BoHV-1 (Jones and Chowdhury, 2010). Viral replication in the course of reactivation may cause recurrence of the disease (Farooq *et al*., 2019b). The latency reactivation cycle can be operationally divided distinct phase: (1), establishment, (2), maintenance and (3), reactivation from latency (Jones, 2014). Figure 3 Putative steps of BoHV-1 latency-Reactivation cycle in cattle (Jones and Chowdhury, 2008). Latency reactivation cycle of BoHV-1 is illustrated in figure 3.

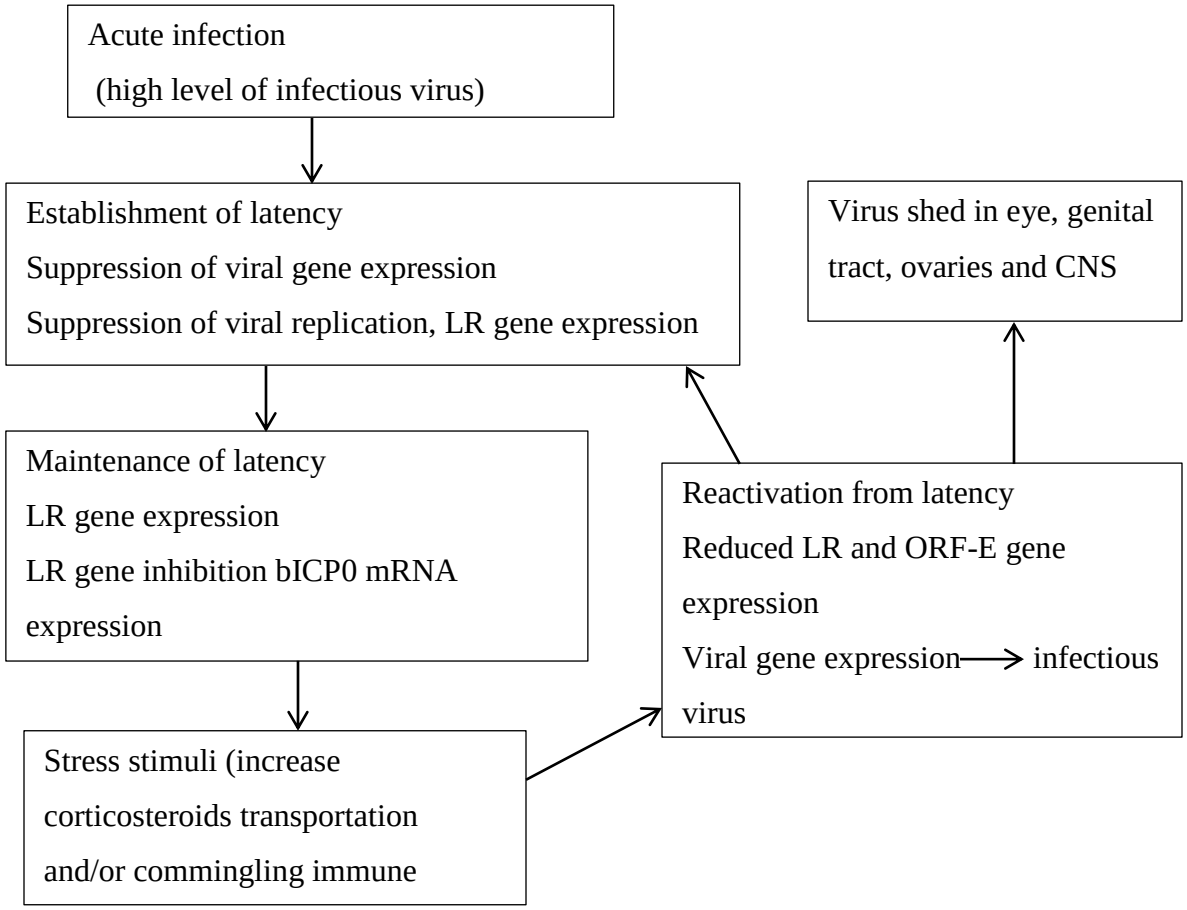


Figure 3. Latency reactivation cycle of BoHV-1 virus (Jones and Chowdhury, 2008)

2.8 Source of Infection and Mode of Transmission

The main sources of infection for the disease are nasal exudates and cough droplets, genital secretions, semen, fetal fluids, and tissues (Nandi *et al.*, 2009). Periodically, BoHV-1 reactivates from latency, the virus is shed, and consequent virus transmission occurs (Jones and Chowdhury, 2007). Therefore, the BoHV-1 antibody carrier should always be considered as a potential source of infection to other animals (Jones and Chowdhury, 2010; Nandi *et al*., 2009). The virus may alive and survive up to 1 year in semen frozen at -196 ^{0}C for artificial insemination and can survive for 5 to 13 days in warmer environments for airborne transmission to occur (Biswas *et al*., 2013). Latently infected cows or heifers can re-excrete virus during delivery (Thiry *et al*., 2006). Their calves can become infected and, after transport, transmit virus to other groups on the farm or to other herds. The virus is existing in all fetuses aborted as a result of BoHV-1 infection, and these fetuses can serve as a source for transmission of disease (Crook *et al*., 2012). In this case, the virus can be transmitted to large numbers of cows and may cause miscarriages, infertility, endometritis, and embryonic death (Biswas *et al*., 2013).

Nose -to-nose contact is the main mode of transmission occurring between the infected to susceptible cattle (Ellis, 2009). This is made possible because of the virus sloughing off into the mucus (Muylkens *et al*., 2007). However, airborne transmission by the aerosol route has been demonstrated over short distances (Farooq *et al*., 2019a). An indirect route of spread is also possible, by means of contaminated instruments (Jones and Chowdhury, 2007). Transmission can also occur in artificial insemination with semen from sub-clinically infected bulls (Jones and Chowdhury, 2007; Jones *et al*., 2011b; Wrathall *et al*., 2006).

Ticks are mechanical transmitters of the virus, although multiplication of the virus in the tick might also occur (Biswas *et al*., 2013). Transmission by ticks occurs when they feed on animals during the first stage of the disease when the virus is present in the macrophages and monocytes (Nandi *et al*., 2009).

2.9 Immune Response to BoHV-1 Infection

Following primary infection, non-specific inflammatory and cellular reactions are the first response to BoHV-1 infection (Muylkens *et al*., 2007). Infected animal with BoHV-1 build up an innate immune response; however, efficient virus copying and spread occurs (Chase *et al*., 2017). The immune response to BoHV-1 infection is triggered when the virus begins to replicate (Jones and Chowdhury, 2010). Cytotoxic T cells response to viral glycoproteins occurs in cattle following infection (Muylkens *et al*., 2007).

2.9.1 Humeral Response

Envelope glycoproteins gB, gC, gD and gH are the most potent inducers of virus-neutralizing antibodies (Jones and Chowdhury, 2007). The antibody response includes mostly neutralizing antibodies and contributes to antiviral antibody-dependent cellular cytotoxicity, which is often complement mediated (Nardelli *et al*., 2008). Maternal antibody to BoHV-1 persists for 123 days after weaning at 2 months of age. Newborn calves are protected from BoHV-1 infection after being fed colostrum collected from vaccinated cows (Petrini *et al*., 2019) although maternal antibody is not completely protective since calves have latent BoHV-1 infections early in life in the presence of maternal antibody too (Gupta, 2019).

2.9.2 Cell-Mediated Immune Response

Although antibodies have been correlated with protection and recovery from BoHV-1 infection, the cell-mediated immune response is a critical defense mechanism because cell-to-cell spread occurs before haematogenous spread (Petrini *et al*., 2022). It is also important in determining the duration and severity of recurrent infection. Cell-mediated immune responses play an important role in killing virus-infected cells that express viral antigens on the cell surface (Jones and Chowdhury, 2007). The response is first noticed about two days post infection and takes place at nearly 8 to 10 days post infection (Jones and Chowdhury, 2007).

The cell-mediated immune response to BoHV-1 infection is under the control of macrophages, interleukin-2, and interferon-à production, natural killer cells, proliferation of and stimulation of cytotoxic T lymphocyte activity. Particularly, macrophage is influential in focusing the specific immune response by producing various cytokines (Raaperi, 2012)and

subsequently responding to cytokines produced by T-cells to kill to virus infected cells. This activity is detectable within 2 days after infection in lung parenchymal cells and 5–7 days in peripheral blood leukocytes. Cytotoxic T cell responses to viral glycoproteins occur in cattle following infection (Tikoo *et al*., 1995).

2.10 Diagnostic Approaches of BoHV-1

2.10.1 Isolation of the Virus in Cell culture

BoHV-1 can be readily isolated in cell culture of primary or secondary bovine kidney, lungs, testis, turbinate, or trachea and established cell lines such as Madin–Darby Bovine Kidney (MDBK) cells (Nandi *et al*., 2009). The virus can be isolated from nasal swabs, conjunctival swabs, vaginal swabs, preputial washing, placental cotyledons of aborted foetus, fetal liver, lung, spleen, kidney, lymph node, mucous membrane of the respiratory tract, tonsils, and lungs collected in virus transport medium (Biswas *et al*., 2013). Raw or frozen semen with preservatives may also be collected for virus isolation. The presence of virus in specimens is detected by a cytopathic effect. The cytopathic effect of BoHV-1 is characteristic and usually appears within 3 days after inoculation. There are grape-like clusters of rounded cells present around a microplaque in cell culture (Turin and Russo, 2003). The cell culture is passaged at least three times before the sample is considered negative (Nandi *et al*., 2009).

2.10.2 Immune Histopathology

Intranuclear viral inclusions of cow dry type A can occasionally be identified in the epithelial cells of vaginal biopsy tissues collected in the early stage of IPV but not in cells collected in nasal discharge of cattle with BoHV-1 (Nandi *et al*., 2009). These inclusions are also present in the brain in cases of encephalitis, and in tissues of aborted fetuses. As these inclusions are transitory, the use of histological examination for diagnosis is of limited value (Turin and Russo, 2003). Perivascular cuffing with neuronophagia, satellitosis, haemorrhage, and neuronal degeneration are seen in encephalitic form of BoHV infection (Biswas *et al*., 2013)

2.10.3 Serological Test

The virus can be diagnosed by detection of virus or virus components and antibodies by serological tests such as Enzyme Linked Immunosorbet Assay (ELISA), Virus Neutralization

Test (VNT), Indirect Fluorescent Antibody Test (IFAT) (Woodbine et al., 2009b)or by detection of genomic DNA by polymerase chain reaction (PCR) (Biswas *et al*., 2013). ELISA is a specific, sensitive, and more practical test for the detection of BoHV-1 antibodies (Nandi *et al*., 2009). A variety of ELISAs, namely, indirect ELISA, competitive ELISA, and AB-ELISA, have been employed to screen serum samples of cattle in different countries of the world (Romero-Salas *et al*., 2018). Unfortunately, with currently available diagnostic tests, it is not possible to identify animals which have a latent BoHV-1 infection (Biswas *et al*., 2013).

2.10.4 Electron Microscopy

The use of electron microscopy to identify virus particles in clinical material has been a rapid method for the diagnosis of BoHV-1 (Elashmawy *et al*., 2019). It can be used to identify the virus in the early stage of the disease, but it must be considered provisional as all herpesviruses are vague by electron microscopy. It cannot be used regularly due to non-availability of an electron microscope in the small laboratory (Nandi *et al*., 2009).

2.11 Differential Diagnosis of BoHV-1

BoHV-1 was defined by anorexia, acute rhinotracheitis, excessive nasal discharge, bilateral conjunctivitis, coughing, nasal lesions, fever, and gradual recovery within a few days (Nettleton and Russell, 2017). However, a secondary forms of pneumonia and bacterial tracheitis may occur (Nettleton and Russell, 2017). Thus, BoHV-1 should differentiate from malignant catarrhal fever, pneumonic pasteurollosis, bovine viral diarrhea, calf diphtheria, viral pneumonia and allergic rhinitis (Serem, 2019).

2.12 Impact over Livestock Industry

BoHV-1 causes important economic losses to the cattle industry due to fertility disorders, abortions, neurological and respiratory problems, reduced milk production, and restrictions in the international livestock trade (Nandi *et al*., 2009). Cattle recovering from BoHV-1 infection are very sensitive to become a silent carrier. These animals remain carriers for the virus for the rest of their lives and immunosuppressive treatments or conditions might reactivate virus replication, leading to spread of the infection to the rest of the cattle once more (Raaperi *et al*., 2014). BoHV-1 infection causes a quick drop in milk production with

loss of body weight, abortion, and decreased value of animals due to bilateral conjunctivitis, eye irritation, and extreme restlessness (Biswas *et al*., 2013).

Economic losses due to decreased milk production are associated with the virus infection evaluated in different previous studies. Losses of 2.6 kg of milk per day in cows with subclinical BoHV-1 compared to seronegative cows have been reported in England (Sayers, 2017). A study conducted in Turkey reported milk production reductions of up to 10 % for animals that had detectable BoHV-1 (seropositive) compared with animals seronegative (Can *et al*., 2016).

2.13 Control and Prevention of the Disease

Due to the significant losses in the cattle industry, there is an increased interest in the control and eradication of BoHV-1 across the world (Raaperi *et al*., 2014). Several countries have started to implement control schemes in the 1980s since the Europe Union allowed IBR-free member states to request import conditions for cattle, semen, and embryos (Iscaro *et al*., 2021). To date, a variety of virus control programs have been performed or are in progress; features depend on epidemiological and economic issues (Thiry *et al*., 2006). Good management practices and hygienic measures should also be adopted. Isolate infected animals and providing adequate food and water will limit disease transmission and severity (Radostits *et al*., 2007). Effective monitoring and control measures are also required to avoid the risk of reintroducing BoHV-1 into BoHV-1 free herds/farms (Ackermann and Engels, 2006).

2.13.1 Vaccination

Conventional vaccines are widely used to prevent clinical signs of infectious bovine rhinotracheitis (Ackermann and Engels, 2006). Vaccination reduces the effective reproduction ratio, the infectious period of an infected animal, and the probability that a latently infected animal transmits virus after reactivation (Noordegraaf *et al*., 2000). Vaccines decrease the severity of the disease and reduce virus replication and transmission, but they are not able to prevent BoHV-1 infection followed by a state of latency. Four kinds of vaccines are available to prevent virus infection: modified live virus vaccines, inert (inactivated vaccines), subunit vaccines, and marker vaccines (Trapp e*t al*., 2003). Unfortunately, the use of vaccines is only

of temporary and limited value and all vaccines are not safe to be given to pregnant animals because it induces abortion and/or stillbirth (Meeusen *et al*., 2007). Vaccination with most modified live virus vaccines has the potential to produce latent infections (Biswas *et al.*, 2013). The best strategy is to use a well-planned vaccination program (Chase *et al*., 2017).

Modified live vaccine

The modified live vaccines induce a rapid immune response, long lasting immunity, and result in local and mucosal immunity (Yoo, 2010) Modified live vaccine generally induce both humoral and cellular immune responses (Jones and Chowdhury, 2010). These vaccines were able to induce protection against disease by 2–3 days after vaccination, although specific antibodies against BoHV-1 were not detected at that time. Safety is a concern for the vaccine because modified live vaccines, when applied intranasal, were able to establish latency (Jones and Chowdhury, 2010) with occasional reactivation and shedding (Ackermann and Engels, 2006). Modified live vaccine can also be pathogenic in young calves, because their immune system is not fully developed, and most the vaccines can be immunosuppressive (Jones and Chowdhury, 2010). However it provides protection from infection with virulent BoHV-1, and significantly reduces nasal shedding of the virus after vaccination (Petrini *et al.*, 2019). A single intranasal vaccination affords significant protection in addition to maternally derived antibodies, and the protection can be significantly prolonged by a booster intramuscular vaccination (Patel, 2005).

Inactivated vaccines

Inactivated vaccines have been developed because of the disadvantages of modified live vaccines (Nandi *et al*., 2009). Inactivated vaccines are safe for pregnant animals, stable in storage, and cause neither shedding of the virus, nor immunosupression, abortion nor latency, although they cannot prevent the development of latency following exposure to the wild-type virus (Patel, 2005). The vaccines usually produce only humoral immunity, but no cellular immune responses and relatively short-term memory (Jones and Chowdhury, 2007) however, the seroneutralizing response is higher with an inactivated vaccine (Kathiriya e*t al*., 2018). Inactivated vaccines have a better protective effect on virus re-excretion, following dexamethasone treatment, than attenuated vaccines (Nandi *et al*., 2009).

Subunit vaccine

The subunit vaccine contains one or more antigens of the virus (van Oirschot *et al*., 1996a) that induce protective immunity, and do not contain nucleic acid and other components that might cause unwanted side-effects (Yoo, 2010). Animals immunized with these proteins develop high levels of antibody and are protected from experimental challenge (Muylkens *et al*., 2007).

Marker vaccine

A marker vaccine is based on changes in one or more of the non-essential glycoproteins, which allow the differentiation of vaccinated animals from infected ones (Nandi *et al*., 2009). Glycoproteins gC, gE, gI, gG and gM are nonessential and thus may be deleted with little or no effect on virus production *in vitro* or *in vivo* (van Drunen Littel-van den Hurk, 2006).Viral mutants (gC-, gE-, gG-, Us9-deleted, thymidine kinase-deleted and LR gene mutant) of the virus have been constructed. Based on recent studies, both gE- and Us9-deleted viruses were found to be safe in calves because they do not reactivate from latency, and they are highly attenuated (Jones and Chowdhury, 2007). gC plays a role in viral attachment and is highly immunogenic, gG, gI and gE have a function in cell to cell spread mechanisms (Madavaraju *et al*., 2021).

2.13.2 Legislation

Legislation establishes the requirements for animals introduced and reared in AI centres. All animals introduced to the AI center must be isolated in their herd of origin, tested and be confirmed negative for BoHV-1 antibodies 30 days before movement (Anonymous, 2008). Bulls used for semen collection are tested serologically once a year (Anonymous, 2004b). EU legislation (Decision 2004/558/ CE) defines the requirements to be fulfilled in order to obtain approval for national BoHV-1 eradication programmes (Alkan *et al*., 2018). Legislation with respect to BoHV-1 for the international trade of cattle, bovine, semen, and embryos is constituted in EU-directives 64/432, 88/407, and 89/556 (Raaperi, 2012).

2.13.3 Test and Removal Strategy

The test and removal strategy has been successfully elaborated in several countries (Turin and Russo, 2003). Eradication of BoHV-1 with the culling of seropositive animals without vaccination has been the only successful method so far (Ackermann and Engels, 2006). This can only be considered if the seroprevalence of BoHV-1 is relatively low (Ackermann and Engels, 2006). A test and removal policy was implemented in many different European countries such as Finland and Switzerland (Biswas *et al*., 2013). For such countries, the national programme for the eradication of BoHV-1 was divided into four phases: (1) Prevention of transmission of the infection by restrictions on the trade of bovines and assessment of the prevalence of cattle with antibodies to BoHV-1. (2) Slaughtering animals with antibodies to BoHV-1 in order to eradicate BoHV-1 from breeding herds. (3) Detection and eradication of further BoHV-1 reservoirs (e.g., fattening cattle) (4) Monitoring programme and legal actions in order to maintain the favourable situation (Ackermann, 2006).

2.13.4 The DIVA Strategy (Differentiating Infected from Vaccinated Animals)

The method of depopulation is inefficient for eradication of BoHV-1 in countries with high seroprevalence (Jacevičius *et al*., 2008). If there are more than 15-20% BoHV-1-positive animals in a population, vaccination is the most realistic strategy to eradicate BoHV-1 (Kuijk, 2002). Marker vaccines are vaccines that allow serological differentiation between infected and vaccinated individuals (also called DIVA vaccines). This differentiation is based on the absence of one or more microbial proteins (mostly gE) in the vaccine that are present in the wild-type microorganism (Boelaert *et al*., 2005). Consequently, after infection, but not after vaccination, an antibody response to that specific protein(s) can be detected (Van Schaik *et al*., 2002).

Chapter 3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in Bahir Dar city and Bahir Dar Zuria distric from April 2022 to December 2022. Bahir Dar is the capital of Amhara National Regional State, The city is located approximately 565 km Northwest of Addis Ababa, having a latitude and longitude of 11°36′ N and 37°23′ E. The city receives average annual rainfall ranges from 1200 to 1600 mm and temperature 8–31 °C. The altitude of the area ranges between 1500 m–2300 m above sea level. In this areas small holder farming households produce milk mostly from indigenous cattle breeds. Cross bred dairying is being promoted by the regional government through distribution of pregnant heifers and use of artificial insemination due to the high milk demand and supply variation in the nearby urban and peri urban centers. The livestock population of the Bahir Dar city is composed of 17,616 bulls, 23,612 cows, 11,900 heifers and 12,284 calves (CSA, 2021).

Bahir Dar Zuria is part of the West Gojjam Zone, and the area is situated at an altitude ranging from 1700-2300 meter above sea level and has area coverage of 151,119 hectare. The area receives an average annual rainfall ranging from about 820 to 1250 mm. The minimum and maximum daily temperatures of the area are 10 and 32°C, respectively. Livestock production system is generally predominated by extensive system in which animals are allowed to forage freely during day time and kept in house during the night. Cattle are the mainstay of the household economy as they provide draught power for tillage for crop production, are the main sources of meat and milk, and provide income through the live animal market. The total cattle population in the district is estimated to be about 332,968 which are kept dominantly under the traditional smallholder farming system (CSA, 2021). The map of the study area that was generated is shown below in figure 5.

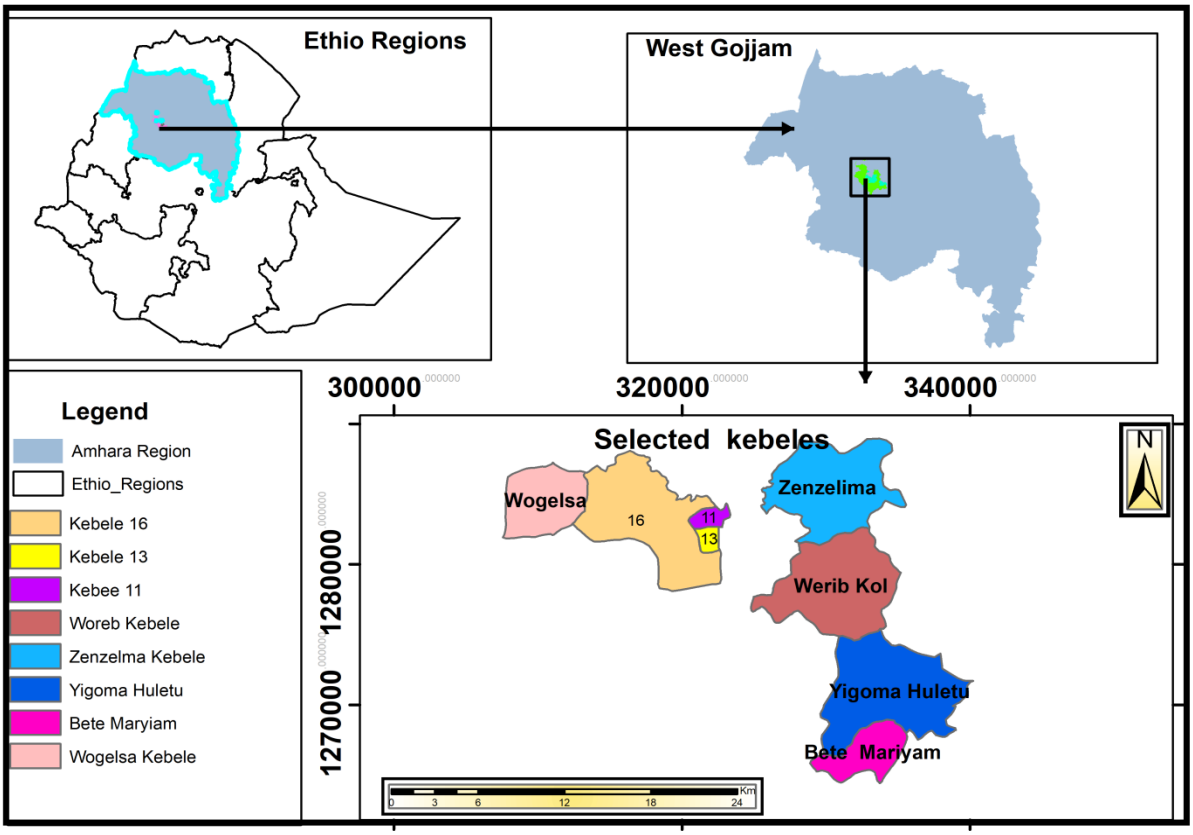


Figure 4. Location map of study areas

3.2 Study Design and Population

A cross-sectional study design was conducted from April 2022 to December 2022 in Bahir Dar city and Bahir Dar Zuria district to estimate seroprevalence and the associated risk factors of BoHV-1. Animals involved in this study were all indigenous zebu and Holstein Friesian-zebu cross, all age groups above six months (>6 month) managed under different management systems. In the sample collection period, information concerning putative risk factors believed to be associated with the seroepidemiology of BoHV-1 such as breed types, sex, age, herd size, origin and breeding method were recorded. Furthermore, the presence or absence of reproductive and respiratory disorders was registered.

The selected animals were divided into three age groups (≤ 2 years, 2 to 5 years and > 5 years old) according to dental formula (Torell *et al*., 2003). The age of each study animal was determined by dentation and consulting from animal owners and/or attendants. Parity status was categorized as primiparous (for cows/heifers having a single calf), multiparous for cows

(having two or multiple calves), and none (for heifers or cow that had not gave birth yet). Management of the cattle was categorized in accordance with either as extensively, semi-intensively, or intensively managed. Herd size was grouped into three categories, small herd size (less than 5 animals), medium herd size (6-10 animal) and large herd size (> 10 animal). The presence or absence reproduction and respiratory problems were collected according to observation and interview.

3.3 Sampling Method and Sample Size Determination

Before the commencement of the actual study, the list of *kebeles* and cattle producers was taken from the respective administrative District's livestock and Fishery office. The study sites across the three production systems (urban, peri urban and rural) were selected purposively based on the availability of dairy herds. A multistage stratified sampling method was used to select study *Kebeles*, villages, and households. Accordingly, a simple random sampling technique was employed to select *Kebeles* from each district, villages from each selected *kebeles*, and households from the selected village, and from households, with two or more dairy animals from the selected village. Finally, individual dairy cattle were sampled randomly for serum sample collection from each household. When selected cattle owners did not volunteer or migrate with his cattle to other areas, it was then replaced by other cattle owner mostly from the nearby area. Simultaneously, all relevant epidemiological data for individual animals were registered and recorded to capture potential risk factors associated with BoHV-1 seropositivity using data collection formats.

The sample size required for the study was calculated based on (Thrusfield, 2005),

$n = Z^2 * P_{exp} (1-P_{exp})/d^2$

Where; n = sample size, Z = 1.96, P_{exp} = expected prevalence,

d = absolute precision

Considering the expected prevalence of 50% (assuming no previous study in the present study area) with 95% confidence level and 5% absolute precision, the required sample size was estimated to be 384 individual cattle.

The estimated sample size was proportionally drawn from the two study districts (Bahir Dar Zuria and Bahir Dar City administration) based on their cattle proportion size. Consequently,

184 animals were sampled from Bahir Dar city including Bahir Dar city *kebele* 11, 13, 16, Zenzelma and Wereb *Kebele* while, 200 animals were selected from Bahir Dar Zuria district (Wegelsa,Yigomahuletu and Betemaryam *kebele*).

3.4 Data Collection Methods

3.4.1 Questionnaire Survey

A semi-structured questionnaire was prepared to obtain general information on the potential risk factors associated with the occurrence of bovine BoHV-1 in the study area. For this, verbal consent was obtained from the respondents, from which their animals tested for BoHV-1, and the objective of the survey explained to them before the start of the interview. Then, the questionnaire was administered for those selected individuals and the required information was collected. A total of 121 owners and/or attendants of cattle were interviewed parallel to blood collection using semi structured questionnaire which covers demographic data (including age, sex, marital status, educational level, location, Agricultural production system). Similarly, information related to possible associated risk factors of BoHV-1, a semi-structured questionnaire was also provided to animal owners. The individual animal's details such as sex, age, breed breeding method, reproductive disorders (abortion history, anestrus, repeat breeding, RFM, and still birth); parity status, animal origin and respiratory problems were registered during serum sample collection.

3.4.2 Blood Sample Collection

After proper restraining of animals, blood sample about 10 ml were collected from the jugular vein of each animal using 18 G needle and vacutainer tubes. The collected samples were settled overnight at room temperature and centrifuged the next morning at 1000 rpm for 10 minutes to separate the serum. After a separate layer formation, the serum part was decanted into sterile cryovials of 1.8 ml volume and transported on a cold chain to Bahir Dar Regional Animal Health Investigation and Diagnostic Laboratory, and stored at -20ºC until testing. Each sample was labeled with codes identifying each animal and herd.

3.4.3 Serological Diagnostic Procedures

Each of the 384 sera samples were subjected to serological examination by Competitive ELISA test (ID.vet, IBR, gB. competition, Grabels, France) with sensitivity, 100% and specificity, 100%). The serological analysis was performed by following the manufacturer's protocol and recommendations. Following the manufacturer's test procedure, briefly, first 50µl of dilution buffer 19 was added to each wells of antigen coated plate and then added 50µl of the positive and negative controls to plate wells of A1, B1 and C1, D1 respectively. Then 50µl of tested samples were added to the remaining wells and incubated at 37ºC for 2 hr. After 2hr incubation, each well was empty and washed three times with 300µl of the wash solution to remove the remain serum and dried with lent free towel. Ready to use conjugate (anti-gB Horse Radish Peroxidase (HRP)) (100µl) was added to each wells to fix the remaining free gB epitopes and then incubated at 37ºC for 30 minutes. After incubation, the same washed and dried protocol was applied and added 100µl of the substrate solution (TMB) to each wells and then incubated at room temperature for 15 minutes in dark place. Finally, 100µl of stop solution was added to each well to stop the reaction. Optical Density (OD) was read at 450 nm by ELISA reader (the reading and validation of the tests were carried out in accordance with the manufacturer's instructions)

The cut-off point was determined by the competition percentage (S/N %) of the test using the following calculation:

$$S/N\% = \frac{OD_{Sample}}{OD_{NC}}$$

Cut-off: S/N% ≤45% = the sample defined as positive

Cut-off: 45% <S/N% <55%= the sample would doubtful

Cut-off: S/N%≥55%= the sample said to be negative

3.5 Data Management and Analysis

Data generated by laboratory investigations and the questionnaire survey was entered, coded, and stored in Microsoft Excel Spreadsheet (Microsoft Excel, 2010). Then the final data analysis was performed using STATA/ version 17 for windows (Stata corp., college station, 2013). Descriptive and analytic statistics were employed. The prevalence of BoHV-1 was calculated by dividing the number of positive ELISA results by the total number of serum

samples tested. The animal level true prevalence were calculated by adjusting the corresponding apparent seroprevalence (AP) for specificity (Sp) and sensitivity (Se) of competitive ELISA using the following formula as indicated below. Both the sensitivity and specificity of ELISA were considered as 100%.

$$\text{Animal level true prevalence} = \frac{\text{Apparent prevalence}+\text{Specificity of the test}-1}{\text{Sensitivity of the test}+\text{Specificity of the test}-1}$$

After checking the multicollinearity between predictor variables using variance inflation factor (VIF), univariable logistic regression for proportion was used to reduce the non-important hypothesized risk factors with ($p<0.25$). This was further tested by multiple logistic regression for final conclusion with probability predictive limit less than 5% ($p<0.05$). Those significant predictor variables in the multivariable logistic regression model were selected as the final risk factors of BoHV-1. Odd ratio was used to assess the strength of association between exposure variable associated with seropositive of the disease. The goodness of fit of the model was determined using the Hosmer and Lemeshow test. The statistically significant association between variables and the seroprevalence was considered when the P-value was less than 0.05.

The effects of BoHV-1 on reproductive disorders, univariable logistic regression analyses were conducted considering BoHV-1 seroprevalence as an independent and each of the reproductive disorders as dependent variables.

3.6 Ethical Clearance

The study protocol was reviewed and approved by the Ethical Clearance Committee of Bahir Dar University, College of Agriculture and Environmental Sciences. Blood samples were collected by strictly following the standard operational procedure and minimizing any discomfort. Verbal informed consent was obtained from the animal owners during the interview and collection of blood samples from their cattle.

Chapter 4. RESULTS

4.1 Socio-demographic Characteristics of Respondents

Among the total dairy cattle owners interviewed, 93.4% and 8.4 % were male and female households, respectively. Among the participants, 16.5 % of respondents were from urban, 25.07 % from peri-urban, and the rest 57.8% were from rural site. Regarding the age, highest percentage of the respondents 40% were above 40 years old followed by 32.1% of (31-40 years old) and the rest 27.9% were 18-30 years old. As to the respondent's literacy rate, 27.8% of dairy cattle owners were illiterate, read and write (33.6%), elementary education (17.3%), secondary school completed (16.4 %) and the remaining 4.9 % were college and above. Majority of cattle owners occupation was farmer (74.5%) followed by private workers (19.8%) and least (5.7%) of the respondents job was governmental workers. Regarding profession, about 2.1 % of the respondents were professional, related to animal health and animal production. In the rural dairy production system, almost all dairy cattle were reared and kept with small ruminants, while dairy cattle not kept with small ruminants in case of urban production system; meanwhile, very few herds were kept along with sheep and goat in case of peri-urban production system. Indeed, in urban and peri-urban dairy production system, dairy production mainly performed as a primary source of income but in rural dairy production the dairy activity is a side line business and essentially done for household consumption.

4.2 The Overall Seroprevalence of BoHV-1

A total of 384 animals from 121 herds were involved for the study. Among these, 244 [63.54% (95% CI: 58.8-68.2)] dairy cattle were positive for the detection of BoHV-1 antibody while 99 herds [81.82% (95% CI: 74.8- 88.7)] had atleast one positive animal for BoHV-1 antibody. The true overall animal level seroprevalence of BoHV-1 in the study area was [63.54% (95% CI: 58.8- 68.2)] by considering 100% sensitivity and specificity of competitive ELISA.

4.3 Risk Factors for BoHV-1 Seroprevalence

4.3.1 Univariable Logistic Regression Analysis for Risk Factors for BoHV-1 Seropositivity

Univariable logistic regression was performed on the following possible risk factors for BoHV-1 seropositivity: study district, dairy production system, sex, age, breed, breeding method, origin of the animals, herd size and parity status. Sex was found to be non-significant risk factors (p=0.57) while the rest risk factors with $p < 0.25$ were selected as a candidate variable for multivariable logistic regression analysis (Table 5). Management system was omitted out during data analysis due to its high multicollinearity effect with dairy production system.

Comparatively higher Seroprevalence was recorded in Bahir Dar Zuria district (80.0%) compared to Bahir Dar City (45.6%). In addition, the seroprevalence of BoHV-1 in female and male dairy cattle was 62.6% and 65.7%, respectively. With respect to the age of cattle, the higher seroprevalence was obtained in age group of above 5 years of age (74.2%), followed by age group 2 to 5 years (64.0%) than age group below 2 years of age (45.6%) (Table 5).

Table 5. Univariable logistic analysis of potential risk factors for BoHV-1 in Bahir Dar City and Bahir Dar Zuria

Risk factor	Category	No. tested	No. positives (%)	COR (95%CI)	p-value
Study district	Bahir Dar city	184	84 (45.6)	1	-
	Bahir Dar Zuria	200	160 (80.0)	4.7 (3.03 -7.48)	<0.001
Dairy production system	Urban	88	27 (30.7)	1	-
	Peri-urban	96	57 (59.3)	3.3 (1.79-6.07)	<0.001
	Rural	200	160 (80.0)	9.0 (5.10-15.98)	<0.001
Sex	Female	276	173 (62.6)	0.8 (0.54-1.39)	0.57
	Male	108	71 (65.7)	1	-
Age	6 month to 2years	103	47 (45.6)	1	-
	2 year to 5 years	114	73 (64.0)	2.1 (1.23- 3.65)	0.007
	>5 year	167	124 (74.2)	3.4 (2.04-5.78)	<0.001
Animal origin	Homebred	277	155 (55.9)	1	-
	Purchased	107	89 (83.1)	3.9 (2.22 - 6.81)	<0.001
Breed	Holstein Friesian-zebu cross	166	70 (42.2)	1	-
	Indigenous zebu	218	174 (79.8)	5.4 (3.45 - 8.52)	<0.001
Breeding method	AI	202	103 (50.9)	1	-
	Natural mating	182	141 (77.4)	3.3 (2.12-5.15)	<0.001
Herd size	Small (1-5)	115	71 (61.7)	1	-
	Medium (6-10)	99	75 (75.8)	1.9 (1.06 -3.50)	0.029
	Large (>10)	170	98 (57.6)	0.8 (0.52 -1.36)	0.49
Parity status	None parity	82	22 (26.8)	1	-
	primiparous	57	37 (64.9)	1.5 (0.70- 3.14)	0.29
	multiparous	137	114 (83.2)	2.9 (1.61-5.23)	<0.001

4.3.2. Multivariable Analysis of Animal Level Risk Factors with BoHV-1 Seropositivity

In multivariable logistic regression analysis, study district and breeding method not significantly associated with BoHV-1 seropositivity. Based on dairy cattle location, the seroprevalence of BoHV-1 was significantly higher in rural dairy cattle production system (AOR= 4.2, 95% CI: 1.78–9.85; p<0.001) than in Peri-urban (AOR=2.2, 95% CI: 1.13–4.42; p=0.019) and urban dairy production system. The odd of BoHV-1 within age groups was increased as animals aged above five years were 3.2 times (AOR=3.2, 95% CI; 1.76- 5.86; p<0.001) more likely to be affected by BoHV-1 as compared to cattle below two years age. With respect to origin of cattle, purchased cattle were more likely at risk of acquiring BoHV-1 (AOR=2.9, 95% CI: 1.57–5.52; p=0.001) when compared to homebred cattle. The seroprevalence of BoHV-1 was also significantly associated with parity status and multiparous cows had higher odds ratio (AOR=2.9, 95% CI: 1.61–5.23; p<0.001) compared to nonparturated animals (Table 6).

Table 6. Multivariable logistic regression analysis of the risk factors for seroprevalence of BoHV-1 Bahir Dar City and Bahir Dar Zuria

Risk factors	Category	No. tested	No. positives (%)	AOR (95% CI)	p-value
Dairy Production system	Urban	88	27 (30.7)	1	-
	Peri-urban	96	57 (59.3)	2.2 (1.13 –4.42)	0.019
	Rural	200	160 (80.0)	4.2 (1.78 –9.85)	< 0.001
Age	6 month up to 2 year	103	47 (45.6)	1	-
	2 years to 5 years	114	73 (64.0)	2.1 (1.10- 3.98)	0.023
	> 5years	167	124 (74.2)	3.2 (1.76- 5.86)	< 0.001
Animal origin	Homebred	277	155 (55.9)	1	-
	Purchased	107	89 (83.1)	2.9 (1.57 - 5.52)	0.001
Herd size	small	115	71(61.7)	1	-
	medium	99	75 (75.8)	2.1(1.06-4.26)	0.033
	large	170	98(57.6)	2.2 (1.15-4.29)	0.017
Breed	Holstein Friesian-zebu cross	166	70 (42.2)	1	-
	Indigenous zebu	218	174 (79.8)	2.4 (1.20- 5.21)	0.014
Parity status	None parity	82	22 (26.8)	1	-
	primiparous	57	37 (64.9)	1.5 (0.70- 3.14)	0.29
	multiparous	137	114 (83.2)	2.9 (1.61-5.23)	<0.001

AOR = Adjusted Odds Ratio, 1 = Reference.

4.3.3. Association of BoHV-1 Seropositivity with Reproductive and Respiratory Disorders

Seroprevalence of BoHV-1 among cows with cases of abortion, repeat breeding, anestrus, retained fetal membrane (RFM) and still birth was 93.3, 82.3, 95.0, 93.5 and 84.6%, respectively. The occurrence of reproductive disorders was significantly associated with BoHV-1 seropositivity. Cows infected with BoHV-1 had higher ($p < 0.001$) risk of abortion, repeat breeding, anestrus, retained fetal membrane, and still birth compared to apparently healthy animals. Animals with respiratory disorders, 84.7% (72/85) were relatively more affected with BoHV-1 than those who hadn't the sign 57.5% (172/299) (Table 7).

Table 7. Association of BoHV-1 seroprevalence with reproductive and respiratory disorders in Bahir Dar City and Bahir Dar Zuria

Disorders	Category	No. tested	BoHV1 positive	Prevalence (%)	OR (95% CI)	p-value
Abortion history	Aborted	45	42	93.3	10.7 (3.22-35.47)	<0.001
	Non aborted	231	131	56.7	1	-
Repeat breeding	Present	34	28	82.3	3.1 (1.2 - 7.82)	0.015
	Absent	242	145	59.9	1	-
anestrus	Present	40	38	95.0	14.2 (3.35-60.30)	<0.001
	Absent	236	135	57.2	1	-
RFM	Present	31	29	93.5	10.1 (2.37-43.58)	0.002
	Absent	245	144	58.7	1	-
Still birth	Present	26	22	84.6	3.6 (1.2 - 10.77)	0.022
	Absent	250	151	60.4	1	-
Respiratory disorder	Present	85	72	84.7	4.1 (2.17 - 7.70)	<0.001
	Absent	299	172	57.5	1	-

RFM = Retained Fetal Membrane, OR = Odds Ratio, 1 = Reference

Chapter 5. DISCUSSIONS

BoHV-1 is a worldwide disseminated pathogen displaying significant differences in regional incidence and prevalence with regards to the geographical positions and the breeding managements of the considered regions (Muylkens *et al.*, 2007). However, very little attention has been paid to this disease in Sub Saharan Africa and its potential role as an important co-infection causing immune suppression (Athingo, 2018). Based on serological surveys, several studies have aimed at identifying the risk factors for BoHV-1 seropositivity. In the present study on the sero-epidemiology of BoHV-1 in dairy cattle, a total of 384 serum samples were analyzed and 244 (63.54%) animals and 99 of 121 (81.82%) dairy herds were identified as seropositive which was more than some of the previously reported studies in the country. In Ethiopia some reports showed that the individual animal level seroprevalence of BoHV-1 ranges from 41.0 % (Berhanu Sibhat *et al*., 2018) up to 93.4% (Demeke Zewde *et al*., 2021) in which the current result falls between the range. These observed prevalences seem to be high for an area where BoHV-1 has never been reported and indicate the wide distribution of the virus in all study districts of northwest Ethiopia. The high seropositivity of animal against BoHV-1is probably due to lack of controls measures towards this infection.

The seroprevalence obtained in this study was just in line with the previous study in Southern India that reported the overall individual level prevalence of 63.54% (Krishnamoorthy *et al*., 2015). The current finding, is also very close to some of the previous similar studies conducted in some countries that have no control program for BoHV-1 infection such as 64.4% in North-Eastern Mexico (Segura-Correa *et al*., 2016), 64.1% in Hungary ((Raaperi, 2012), 62.8% in Morocco ((Lucchese *et al*., 2016) and 62% in Southern Brazil (Almeida *et al*., 2021), which might indicate that BoHV-1 is present in cattle around the world regardless of the different climate and management conditions of the herds in different countries.

According to the present study, the individual seroprevalence of BoHV-1 found was lower than the previous research works conducted in different parts of Ethiopia as 67% in Gobe and Ghibe (Berhanu Sibhat *et al*., 2018); 69.4% in West Gojjam, 71.7 % in Awi zone, and 93.4% in East Gojjam (Demeke Zewde *et al*., 2021). The result also lower than in similar studies conducted in unvaccinated dairy cattle of different countries as prevalence reported 67% in

Venezuela (Romero-Salas *et al*., 2013), 72.4% in Southern Mexico (Romero-Salas *et al*., 2013), 67% in Peru (Dora *et al*., 2013), 99.92% in Spain (Dias *et al*., 2013a), 66.12% in India (Singh and Yadav, 2010), 69 % in Ghana (Adu-Addai *et al*., 2012), 72.1% in Turkey (İnce and Şevik, 2022), 74.5% in Gauteng province of South Africa (Njiro *et al*., 2011), 77.7 % in Southern Italian Apennines (Rinaldi *et al*., 2007), and 93.75% in Egypt (Hekal *et al*., 2019) have been reported, indicating a wide distribution of the virus.

On the other hand, the current finding is higher than similar studies conducted in Ethiopia such as 25.6% in Dessie and Kombolcha (Tadeg Wedajo *et al*., 2021), 41.0% in Addis Ababa, Central and Southern Ethiopia, 41.8% in Harar and Sidamo provinces (Berhanu Sibhat *et al*., 2018), apart from Ethiopia the current finding also higher than similar studies in other countries of the world; 12% in Ireland (Cowley *et al*., 2011), 16.7 % in Cameroon (Athingo, 2018), 22% in Estonia (Raaperi *et al*., 2010), 24.19 % in Algeria (Kaddour *et al*., 2019), 25.9% in Tunisia ((Raaperi, 2012), 35% in Belgium (Boelaert *et al*., 2005), 35.8% in china (Yan *et al*., 2008), 37% in Uruguay (Guarino *et al*., 2008), 39.76% in in Fars Province, Southern Iran (Hashemi *et al*., 2022b), 17.4% in Kenya (Kipyego *et al*., 2020), 43 % in Ecuador (Carbonero *et al*., 2011), 42.5% in South West England (Woodbine *et al*., 2009a), 48.3% in Southern Zambia (Mweene *et al*., 2003) and 50% Morocco (Lucchese *et al*., 2016).

The seroprevalence of BoHV-1 variation in between the present and previous reports in Ethiopia and other parts of the world, could be accounted to the difference in the type of cattle management practices, agro-ecological and geographical variations, difference in herd size, types of breeding method, year of study, the type of test used and herd size (Orjuela, 2020).

Among the dairy cattle production system, a significantly higher prevalence (80.0%) of BoHV-1 was recorded in rural dairy cattle production as compared to peri-urban (59.3%) and urban (30.7%) dairy cattle production systems. This finding was in accordance with (Athingo, 2018) who reported high prevalence rate in rural area of Cameroon and (Segura-Correa *et al*., 2016) also reported a high (49.28%) sero prevalence in the rural dairy production system in Mexico. The likely reason for high prevalence of seropositivity in rural dairy production might be due to the fact that cattle in the rural dairy production system communally grazed and frequently come in contact with other animals thus perpetuating the transmission of the

disease (Bronsvoort *et al*., 2004) leading to the presently detected high prevalence rate of antibodies to BoHV-1. Besides, the uncontrolled free grazing on other animal species (sheep and goats) in the rural production system is a common practice that may increase the risk of disease spread among herds. Sheep and goats are known to be carriers of this enzootic disease and this induces the transmission and spread of the disease might increase (Biswas *et al*., 2013; Van Schaik *et al*., 2002).

With respect to age, a significant statistical association was found between the seroprevalence of BoHV-1 and cattle age groups. Animals aged above five years (>5 years old) were 3.2 times (AOR=3.2, 95% CI: 1.76–5.86; p<0.001) more likely to be affected by BoHV-1 as compared to cattle below two years (<2 years), which is in accordance with the study conducted in Northwestern Ethiopia (East Gojjam and Awi study districts) (Tadeg Wedajo *et al*., 2021) and similar report in Yucanta Mexico (Solis-Calderon *et al*., 2003), in India (Rajkhowa *et al*., 2004) and in Northeast Algeria (Kaddour *et al*., 2019). The possible reason might be repeated subclinical infection and also their greater exposure to the infective agent and loss maternal immunity (Kipyego *et al*., 2020).

In case of animal origin, significant differences in seroprevalence of BoHV-1 in this study were observed between animals purchased (83.1%) and homebred (55.9%). Purchased animals were 2.9 times more likely to suffer BoHV-1 (AOR= 2.9, 95 % CI: 1.57–5.52; p=0.001) than homebred cattle. This finding agreed with (Martinez-Ibeas *et al*., (2015)) who found purchased animal in Ireland were 3 times more likely to be seropositive for BoHV-1 than homebred cattle. In the same study in Netherlands, showed that purchased animals had a 3.5-fold greater chance of being positive than those animals that were homebred (Dias *et al*., 2013b). This might be due to the respiratory form of the disease that is associated with frequent introduction of cattle from various parts of the country and management practices of cattle (Ampe *et al*., 2012). However, this finding contradicts the result of (Segura-Correa *et al*., (2016)) who reported 71.58% and 46.0% prevalence of BoHV-1 in home and purchased cattle, respectively.

Seroprevalence of BoHV-1 was observed to increase with increasing herd size; herds keeping ten animals and more were found to have a higher prevalence than those keeping less. Similar

trends were found by studies elsewhere although the number of animals considered per herd was not the same (Solis-Calderon *et al*., 2003) and large herds are probably maintained by the purchase of replacement animals. Introduction of latently infected heifers or cows into such herds particularly through intra-regional trade would probably result in high herd prevalence following reactivation of the virus as a result of stress associated with transportation and mixing with other animals outside of their herds of origin (Dias *et al*., 2013a).

This study showed significant association of breed of animal with the occurrence of BoHV-1 as Indigenous Zebu being more frequently infected than Holstein Friesian-zebu cross with which did not agree with the findings of many researchers whom they reported the absence of a relationship between the disease and breed, suggests that, under the same circumstances, both local and cross breed cattle breed have an equal chance of acquiring BoHV-1 infection (Elhassan *et al*., 2011). The possible reason for higher seroprevalence indigenous zebu cattle could be the practice of natural bull mating with bulls of unknown health status and high contact rate with unknown herds that causes the rapid spread of the disease (Romero-Salas *et al*., 2013).

When the reproductive stage of the female was considered, seropositivity for the disease proportionally increased as the number of calving increases based on the parity. Female cattle with no parity had the lowest seroprevalence (26.8 %), whereas multiparous cows showed the highest prevalence (83.2%) with statistical significance of ($p<0.001$). This finding was in agreement with the reports of (Demeke Zewde *et al*., 2021) who found (91.5%) prevalence for multiparous, and (56.3%) for nonparturated dairy cattle, being higher in multiparous cows with two fold of risk of BoHV-1 for multiparous than nonparturated animal. This finding also consistent with the report from Pakistan that animals having parity more than two times were more prone to infections (Batool *et al*., 2022). This might be due to the repeated exposure to breeding by the infection is able to occur in the bull or AI service (Gould *et al*., 2013).

Furthermore, BoHV-1 seropositivity in cattle in the current study was associated with different reproductive disorders. Higher prevalence of BoHV-1 in this study was found in animals which had history of reproductive problems such as rabortion (93.3%), repeat breeding (82.3%), anestrus (95%), retained fetal membrane (93.5%) and still birth (84.8%).

These disorders could be the results of infection with virulent strains of BoHV-1.1 and 1.2a in cattle (Ackermann and Engels, 2006). The significant association of the BoHV1 seropositivity with the occurrence of reproductive disorders in this study was approved by previous findings in Ethiopia as 100% abortion cases, 57.6% repeated breeding cases, 80.0% anestrus, 59.8% retention of fetal membrane case were seropositive for BoHV-1 (Tadeg Wedajo *et al*., 2021) and 65.15% repeat breeding, 100% abortion and 100% Retained fetal membrane cases were seropositive for BoHV-1in Southern India (Krishnamoorthy *et al*., 2015). Similarly, 79.69 %, 76.32% and 76.09% BoHV-1 prevalence in abortion, repeated breeding Retained placenta cases consecutively reported in Gujarat, Indian unvaccinated dairy cattle (Kathiriya *et al*., 2018). These reports showed that BoHV1 disease is a significant cause of reproductive disorders in dairy cattle worldwide.

Indeed, in the present study there was a significant association between BoHV-1 and respiratory disorders including ocular and nasal discharges. The association between BoHV-1infection and respiratory problem in cattle has been also described in other studies. The current finding was in accordance with finding of Ethiopian worker (Demeke Zewde *et al*., 2021) who found similar results; serological survey of bovine respiratory diseases in dairy herds undertaken in Sudan and Iran reported related findings (Hashemi *et al*., 2022a). BoHV-1 in cattle might have affected the respiratory airways and been misdiagnosed as parasitic pneumonia, as pointed out previously (Zacarías *et al*., 2002). Since the BoHV-1 infection in adult cattle is usually subclinical, these animals may be acting as reservoirs and therefore as a source of infection for younger animals (Dora et al., 2013; Jones et al., 2011a).

High seroprevalence of BoHV-1 in this study has been a strong indicator that the virus was circulating in indigenous cattle's in the area. Most of the risk factors in this study were significantly associated with the seropositivity of BoHV-1. Yet, no vaccination for BoHV-1 in Ethiopia has been delivered; the wide distribution and high sero-prevalence of BoHV-1 in this study has been a strong indicator that the virus was circulating in indigenous cattle's in the area. The distribution of virus and risk factors identification are important in order to establish measures for epidemiological prevention and control programs against this economically important disease to minimize its infection and dissemination.

Chapter 6. CONCLUSIONS AND RECOMMENDATIONS

The present study revealed that the animal level seroprevalence (63.54%) and the herd level sero prevalence (81.82%) of BoHV-1 in dairy cattle was higher, which was the first report in the study area. This high prevalence directly implies large productivity and production loss of dairy cattle in the study area. The fact that animals were not vaccinated and that all age-groups had high prevalence indicates that the BoHV-1 are naturally circulating in this population. This study has investigated the possible potential risk factors that are significantly associated with the seropositivity of BoHV-1. Among the potential risk factors investigated, dairy production system, age of the animal, herd size, breed, animal origin and parity status of the cow were significant predictors of BoHV-1 in the study area. There was also a significant association between the prevalence of BoHV-1 and reproductive and respiratory disorders in dairy cows. This suggests that BoHV-1 is causing a significant economic loss through impairing reproductive and respiratory functions.

Moreover, such a high prevalence of BoHV-1 in the study area might impede the region's dairy development initiative. This necessitates an evidence based BoHV-1 disease control and prevention strategy in the study area. Based on the above conclusion, the following recommendations are forwarded.

- As BoHV-1 is widely circulating in the study area, regular vaccination is warranted
- Regular screening of dairy herds about BoHV-1could help to take the necessary measures in tackling the spread of the virus
- A strict biosecurity measure should be placed in dairy farms
- Precautionary measures should be taken during animal marketing in which newly purchased animals shall be quarantined and screened before joining the herd.
- Bull screening should be practiced in herds practicing natural breeding
- Awareness creation to cattle producers is needed so that they can take their precautionary measures to protect their herds from BoHV-1

7. REFERENCES

Ackermann, M., (2006). Pathogenesis of Gammaherpesvirus infections. *Veterinary microbiology* **113**, 211-222.

Ackermann, M., Engels, M., (2006). Pro and contra IBR-eradication. *Veterinary microbiology* **113**, 293-302.

Adeli, E., Pourmahdi Borujeni, M., Haji Hajikolaei, M.R., Seifi Abad Shapouri, M., (2017). Bovine Herpesvirus-1 in Khouzestan province in Iran: seroprevalence and risk factors. *Iranian journal of ruminants health research* **2**, 47-56.

Adu-Addai, B., Koney, E., Addo, P., Kaneene, J., Mackenzie, C., Agnew, D., (2012). Importance of infectious bovine reproductive diseases: an example from Ghana. *The Veterinary Record* **9**, 171, 47.

Albayrak, H., Tamer, C., Ozan, E., Muftuoglu, B., Kadi, H., Dogan, F., Cagirgan, A., Elhag, A., Akman, A., Kurucay, H.N., (2020). Molecular identification and phylogeny of bovine herpesvirus-1 (BoHV-1) from cattle associated with respiratory disorders and death in Turkey. *Med. Weter* **76**, 358-361.

Alkan, F., Bilge-Dagalp, S., Karapınar, Z., Timurkan, M., Coskun, N., Burgu, I., (2018). Long-term study (2005–2010) on the vaccination with BoHV-1 glycoprotein E-deleted marker vaccine in selected two dairy herds in Turkey. *Tropical animal health and production* **50**, 353-363.

Almeida, Í.C.d., Almeida, Y.V., Donatele, D.M., Clipes, R.C., Barioni, G., Zanini, M.S., Filippo, P.A.D., (2021). Seroprevalence and associated factors of infectious bovine rhinotracheitis and bovine viral diarrhea in dairy cows in the Caparaó region, Espírito Santo, Brazil. *Ciência Rural* **19**, 51.

Ampe, B., Duchateau, L., Speybroeck, N., Berkvens, D., Dupont, A., Kerkhofs, P., Thiry, E., Dispas, M., (2012). Assessment of the long-term effect of vaccination on transmission of infectious bovine rhinotracheitis virus in cattle herds hyperimmunized with glycoprotein E–deleted marker vaccine. *American journal of veterinary research* **73**, 1787-1793.

Athingo, R.N., (2018). *Spatial sero-survey of respiratory tract viral infections in cattle at the wildlife-livestock interface in the Mnisi communal farming area of South Africa.* Doctoral dissertation, University of Pretoria,

Bahari, A., Gharekhani, J., Zandieh, M., Sadeghi-Nasab, A., Akbarein, H., Karimi-Makhsous, A., Yavari, M., (2013). Serological study of bovine herpes virus type 1 in dairy herds of Hamedan province, Iran. In: *Veterinary Research Forum* **7**, 111.

Batool, A., Rabbani, M., Awan, F.N., Raza, S., Firyal, S., Azeem, S., (2022). Serologic Evidence of Bovine Herpes Virus-1 in Cattle and Buffalo Population of Punjab, Pakistan. Parity **2**, 191.

Berhanu Sibhat, Gelagay Ayelet, Eystein Skjerve, Endrias Gebremedhin, Kassahun Asmare, (2018). Bovine herpesvirus-1 in three major milk sheds of Ethiopia: Serostatus and association with reproductive disorders in dairy cattle. *Preventive veterinary medicine* **150**, 126-132.

Bilge-Dagalp, S., Can-Sahna, K., Yildirm, Y., Karaoglu, T., Alkan, F., Burgu, I., (2008). Effects of bovine leucosis virus (BLV) infection on the bovine viral diarrhoea virus (BVDV) and bovine herpes virus 1 (BHV1) seroprevalences in dairy herds in Turkey. *Rev Med Vet* **159**, 385-390.

Biswas, S., Bandyopadhyay, S., Dimri, U., H. Patra, P., (2013). Bovine herpesvirus-1 (BHV-1)–a re-emerging concern in livestock: a revisit to its biology, epidemiology, diagnosis, and prophylaxis. *Veterinary Quarterly* **33**, 68-81.

Boelaert, F., Bironf, P., Soumare, B., Dispas, M., Vanopdenbosch, E., Vermeersch, J., Raskin, A., Dufey, J., Berkvens, D., Kerkhofs, P., (2000). Prevalence of bovine herpesvirus-1 in the Belgian cattle population. *Preventive Veterinary Medicine* **45**, 285-295.

Boelaert, F., Speybroeck, N., de Kruif, A., Aerts, M., Burzykowski, T., Molenberghs, G., Berkvens, D., (2005). Risk factors for bovine herpesvirus-1 seropositivity. *Preventive veterinary medicine* **69**, 285-295.

Brock, J., (2021). *Decision support using advanced modelling concepts in Epidemiology: enhancing future bovine herpesvirus type 1 eradication in Ireland*. Doctoral Dissertation, Jena, Friedrich-Schiller-Universität Jena, 2021.

Brock, J., Lange, M., Guelbenzu-Gonzalo, M., Meunier, N., Vaz, A.M., Tratalos, J.A., Dittrich, P., Gunn, M., More, S.J., Graham, D., (2020). Epidemiology of age-

dependent prevalence of Bovine Herpes Virus Type 1 (BoHV-1) in dairy herds with and without vaccination. *Veterinary research* **51**, 1-13.

Can, M.F., Ataseven, V.S., Yalcin, C., (2016). Estimation of production and reproductive performance losses in dairy cattle due to bovine herpesvirus 1 (BoHV1) infection. *Veterinarski Arhiv* **86**, 499-513.

Carbonero, A., Saa, L., Jara, D., García-Bocanegra, I., Arenas, A., Borge, C., Perea, A., (2011). Seroprevalence and risk factors associated to Bovine Herpesvirus 1 (BHV-1) infection in non-vaccinated dairy and dual purpose cattle herds in Ecuador. *Preventive veterinary medicine* **100**, 84-88.

Castrucci, G., Martin, W., Frigeri, F., Ferrari, M., Salvatori, D., Tagliati, S., Cuteri, V., (1997). A serological survey of bovine herpesvirus-1 infection in selected dairy herds in northern and central Italy. *Comparative immunology, microbiology and infectious diseases* **20**, 315-317.

Chase, C.C., Fulton, R.W., O'Toole, D., Gillette, B., Daly, R.F., Perry, G., Clement, T., (2017). Bovine herpesvirus 1 modified live virus vaccines for cattle reproduction: Balancing protection with undesired effects. *Veterinary microbiology* **206**, 69-77.

Chen, X., Wang, X., Qi, Y., Wen, X., Li, C., Liu, X., Ni, H., (2018). Meta-analysis of prevalence of bovine herpes virus 1 in cattle in Mainland China. *Acta tropica* **187**, 37-43.

Cowley, D., Clegg, T.A., Doherty, M.L., More, S.J., (2011). Aspects of bovine herpesvirus-1 infection in dairy and beef herds in the Republic of Ireland. *Acta Veterinaria Scandinavica* **53**, 1-9.

Craig, N., Fletcher, S.L., Daniels, A., Newman, C., O'Shea, M., Tan, W.S., Warr, A., Tait-Burkard, C., (2022). Direct Lysis RT PCR in Cell Culture Supernatant Allows for Fast and Accurate Quantification. Viruses **14**, 508.

Crook, T., Benavides, J., Russell, G., Gilray, J., Maley, M., Willoughby, K., (2012). Bovine herpesvirus 1 abortion: current prevalence in the United Kingdom and evidence of hematogenous spread within the fetus in natural cases. J*ournal of veterinary diagnostic investigation* **24**, 662-670.

CSA., (2021). Central statistical agency federal democratic republic of Ethiopia agricultural sample survey report on livestock and livestock characteristics Addis Ababa, Ethiopia. (2): 2020-2021. Statistical Bulletin 2.

Dangas, G.D., Weitz, J.I., Giustino, G., Makkar, R., Mehran, R., (2016). Prosthetic heart valve thrombosis. Journal of the American College of Cardiology **68**, 2670-2689.

Demeke Zewde, Tadele Tadesse, Sisay Alemu, (2021). Sero Status and Presumed Risk Factors Assessment for Bovine Herpesvirus-1 in North Western, Ethiopia. *Austin J Vet Sci & Anim Husb* **8**, 1080.

Dias, J., Alfieri, A., Ferreira-Neto, J., Gonçalves, V., Muller, E., (2013a). Seroprevalence and risk factors of bovine herpesvirus 1 infection in cattle herds in the state of Paraná, Brazil. *Transboundary and Emerging Diseases* **60**, 39-47.

Dora, R.-S., Concepcion, A.-A., Felipe, M.-P., Zeferino, G.-V., Anabel, C.-R., Mariel, A.-D., (2013). Seroprevalence and risk factors associated with infectious bovine rhinotracheitis in unvaccinated cattle in southern Veracruz, Mexico. *African journal of microbiology research* **7**, 1716-1722.

Elashmawy, A.M., Ali, S.S., El-Habbaa, A.S., Bastawecy, I.M., (2019). Seroprevalence and molecular detection of isolated BoHV-1 among farm animals. *Benha Veterinary Medical Journal* **37**, 137-143.

Elhassan, A.M., Fadol, M., El-Hussein, A., (2011). Seroprevalence of bovine herpes virus-1, bovine herpes virus-4 and bovine viral diarrhea virus in dairy cattle in Sudan. *Pak Vet J* **31**, 317-320.

Ellis, J.A., (2009). Update on viral pathogenesis in BRD. Animal Health Research Reviews **10**, 149-153.

Farooq, S., Kumar, A., Chaudhary, S., Batra, K., Maan, S., (2019a). Bovine herpesvirus 1 (BoHV-1): A review on latency and persistence of infection in cattle. *Journal of Pharmacognosy and Phytochemistry* **8**, 875-879.

Farooq, S., Kumar, A., Chaudhary, S., Maan, S., (2019b). Bovine Herpesvirus 1 (BoHV-1) in Cattle and Buffalo: A Review with Emphasis on Seroprevalence in India. *Int. J. Curr. Microbiol. App. Sci* **8**, 28-35.

Fry, L.M., Bastos, R.G., Stone, B.C., Williams, L.B., Knowles, D.P., Murphy, S.C., (2019). Gene gun DNA immunization of cattle induces humoral and CD4 T-cell-mediated

immune responses against the Theileria parva polymorphic immunodominant molecule. Vaccine **37**, 1546-1553.

Fulton, R., d'Offay, J., Dubovi, E., Eberle, R., (2016). Bovine herpesvirus-1: Genetic diversity of field strains from cattle with respiratory disease, genital, fetal disease and systemic neonatal disease and their relationship to vaccine strains. Virus research **22**, 115-121.

Ganguly, S., Mukhopadhayay, S., Paul, I., (2008). Studies on seroprevalence of infectious bovine rhinotracheitis in cattle population of West Bengal. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **29**, 12-16.

García, M.G., Casas, A.J.A., Martínez, A.C., Rodríguez, C.B., Bocanegra, I.G., Maldonado, J.L., Gómez, J., Remujo, J.A.P., (2009). Seroprevalence and risk factors associated with bovine herpesvirus type 1 (BHV1) infection in non-vaccinated cattle herds in Andalusia (South of Spain). *Spanish journal of agricultural research* 56, 550-554.

Givens, M., (2018). Risks of disease transmission through semen in cattle. Animal **12**, 165-171.

Glazov, E.A., Horwood, P.F., Assavalapsakul, W., Kongsuwan, K., Mitchell, R.W., Mitter, N., Mahony, T.J., (2010). Characterization of microRNAs encoded by the bovine herpesvirus 1 genome. *Journal of General Virology* **91**, 32-41.

Gonzalez-Garcia, M., Maldonado, J., Gómez-Pacheco, J., Arenas-Casas, A., Carbonero-Martínez, A., Borges-Rodríguez, C., Bocanegra, I.G., Perea-Remujo, J., (2009). Seroprevalence and risk factors associated with bovine herpesvirus type 1 (BHV1) infection in non-vaccinated cattle herds in Andalusia (South of Spain). *Spanish Journal of Agricultural Research* **7**, 550-554.

Gould, S., Cooper, V.L., Reichardt, N., O'Connor, A.M., (2013). An evaluation of the prevalence of Bovine herpesvirus 1 abortions based on diagnostic submissions to five US-based veterinary diagnostic laboratories. J*ournal of Veterinary Diagnostic Investigation* **25**, 243-247.

Graham, D.A., (2013a). Bovine herpes virus-1 (BoHV-1) in cattle a review with emphasis on reproductive impacts and the emergence of infection in Ireland. *Irish veterinary journal* **66**, 1-12.

Graham, D.A., (2013b). Bovine herpes virus-1 (BoHV-1) in dairy cattle a review with emphasis on reproductive impacts and the emergence of infection in Ireland and the United Kingdom. *Irish veterinary journal* **67**, 22-45.

Guarino, H., Nunez, A., Repiso, M., Gil, A., Dargatz, D., (2008). Prevalence of serum antibodies to bovine herpesvirus-1 and bovine viral diarrhea virus in beef cattle in Uruguay. *Preventive veterinary medicine* **85**, 34-40.

Gupta, P.K., (2019). Bovine Herpesvirus. Recent Advances in Animal Virology, **12**, 37.

Hashemi, M., Bakhshesh, M., Khezri, M., Gharagouzlouian, M.M., Tavakoli, G., (2022a). A two-year serological study of bovine viral diarrhea virus, bovine alphaherpesvirus 1 and bovine parainfluenza virus type 3 in Qazvin dairy cattle farms, Northwestern of Iran. *Veterinarski arhiv* **92**, 1-10.

Hashemi, M., Bakhshesh, M., Manavian, M., (2022b). Bovine viral diarrhea virus and bovine herpes virus-1 in dairy cattle herds in Fars province, Southern Iran: seroprevalence and evaluation of risk factors. Archives of Razi Institute **77**, 1621-1629.

Hekal, S.H.A., Al-Gaabary, M.H., El-Sayed, M.M., Sobhy, H.M., Fayed, A.A.A., (2019). Seroprevalence of some Infectious transboundry diseases in cattle imported from Sudan to Egypt. *Journal of Advanced Veterinary and Animal Research* **6**, 92.

İnce, Ö.B., Şevik, M., (2022). Risk assessment and seroprevalence of bovine herpesvirus type 1 infection in dairy herds in the inner Aegean Region of Turkey. Comparative Immunology, Microbiology and Infectious Diseases **10**, 741.

Iscaro, C., Cambiotti, V., Petrini, S., Feliziani, F., (2021). Control programs for infectious bovine rhinotracheitis (IBR) in European countries: an overview. *Animal Health Research Reviews* **22**, 136-146.

Jacevičius, E., Šalomskas, A., Milius, J., Petkevičius, S., Mockeliūnas, R., Jacevičienė, I., Lelešius, R., Pridotkas, G., (2008). Prevalence and control measures of infectious bovine rhinotracheitis in Lithuania. Starptautiskās zinātniskās konferences raksti, Dzīvnieki. Veselība. Pārtikas higiena., Jelgava **23**, 49-53.

Jones, C., (2009). Regulation of innate immune responses by bovine herpesvirus 1 and infected cell protein 0 (bICP0). Viruses **1**, 255-275.

Jones, C., (2014). Reactivation from latency by alpha-herpesvirinae submfamily members: A stressful situation. *Curr Top Virol* **12**, 99-118.

Jones, C., (2016). Latency of bovine herpesvirus 1 (BoHV-1) in sensory neurons. *Herpesviridae* **1**, 24.

Jones, C., Chowdhury, S., (2007). A review of the biology of bovine herpesvirus type 1 (BHV-1), its role as a cofactor in the bovine respiratory disease complex and development of improved vaccines. *Animal Health Research Reviews* **8**, 187-205.

Jones, C., Chowdhury, S., (2010). Bovine herpesvirus type 1 (BHV-1) is an important cofactor in the bovine respiratory disease complex. Veterinary Clinics: *Food Animal Practice* **26**, 303-321.

Jones, C., da Silva, L.F., Sinani, D., (2011a). Regulation of the latency reactivation cycle by products encoded by the bovine herpesvirus 1 (BHV-1) latency-related gene. *Journal of neurovirology* **17**, 535-545.

Jones, C., da Silva, L.F., Sinani, D., Ståhl,. (2011b). Regulation of the latency reactivation cycle by products encoded by the bovine herpesvirus 1 (BHV-1) *Journal of neurovirology* **18**, 635-645.

Kaddour, A., Bouyoucef, A., Fernandez, G., Prieto, A., Geda, F., Moula, N., (2019). Bovine herpesvirus 1 in the northeast of Algiers, Algeria: Seroprevalence and associated risk factors in dairy herd. Journal of advanced veterinary and animal research **6**, 60.

Kampa, J., Alenius, S., Emanuelson, U., Chanlun, A., Aiumlamai, S., (2009). Bovine herpesvirus type 1 (BHV-1) and bovine viral diarrhoea virus (BVDV) infections in dairy herds: self clearance and the detection of seroconversions against a new atypical pestivirus. *The Veterinary Journal* **182**, 223-230.

Kampa, J., Ståhl, K., Moreno-López, J., Chanlun, A., Aiumlamai, S., Alenius, S., (2004). BVDV and BHV-1 infections in dairy herds in northern and northeastern Thailand. *Acta Veterinaria Scandinavica* **45**, 181-192.

Kathiriya, J., Sindhi, S., Mathapati, B., Bhedi, K., (2018). Seroprevalence of infectious bovine rhinotracheitis (bhv-1) in dairy animals with reproductive disorders in saurashtra of Gujarat, India. *International Journal of Current Microbiology and Applied Sciences* **7**, 1371-1376.

Kaur, G., Chandra, M., (2016). Herpesvirus in bovines: Importance of bovine herpesvirus type 1, In: *Herpesviridae*. IntechOpen.**3**, 56-69

Keuser, V., Schynts, F., Detry, B., Collard, A., Robert, B., Vanderplasschen, A., Pastoret, P.-P., Thiry, E., (2004). Improved antigenic methods for differential diagnosis of bovine, caprine, and cervine alphaherpesviruses related to bovine herpesvirus 1. *Journal of clinical microbiology* **42**, 1228-1235.

Kipyego, E.S., Gitau, G., Vanleeuwen, J., Kimeli, P., Abuom, T.O., Gakuya, D., Muraya, J., Makau, D., (2020). Sero-prevalence and risk factors of infectious bovine rhinotracheitis virus (type 1) in Meru County, Kenya. *Preventive veterinary medicine* **17**, 48-63.

Krishnamoorthy, P., Patil, S., Shome, R., Rahman, H.,(2015). Seroepidemiology of infectious bovine rhinotracheitis and brucellosis in organized dairy farms in southern India.**18**, 125-159.

Kuijk, H., (2002). Experiences of selection eradication, based on IBR-marker vaccine in the Netherlands. *Magyar Allatorvosok Lapja* **124**, 398-408.

Kuotsu, K., Kuotsu, N., Kipu, K., (2019a). Bovine herpes virus-1 (BoHV-1) in cattle-a review with emphasis on epidemiological parameters influencing the prevalence of bovine herpes virus-1 in cattle in India.**84**, 541-594.

Kuotsu, K., Sathiyabama, K., Kuotsu, N., Kipu, K., (2019b). Sero-prevalence of bovine herpes virus-1 (BHV-1) infection in cattle in organized farm **34**, 167-177.

Levings, R.L., Roth, J.A., (2013). Immunity to bovine herpesvirus 1: I. Viral lifecycle and innate immunity. *Animal health research reviews* **14**, 88-102.

Lucchese, L., Benkirane, A., Hakimi, I., El Idrissi, A., Natale, A., (2016). Seroprevalence study of the main causes of abortion in dairy cattle in Morocco. *Vet Ital* **52**, 3-19.

Madavaraju, K., Koganti, R., Volety, I., Yadavalli, T., Shukla, D., (2021). Herpes simplex virus cell entry mechanisms: An update. *Frontiers in Cellular and Infection Microbiology* **10**, 617578.

Mahmoud, M., Allam, A., (2013). Seroprevalence of bovine viral diarrhea virus (BVDV), bovine herpes virus type 1 (BHV-1), parainfluenza type 3 virus (PI-3V) and bovine respiratory syncytial virus (BRSV) among non vaccinated cattle. *Global Veterinaria* **10**, 348-353.

Mahmoud, N., Salem, G., (2012). isolation and molecular identification of bhv-1 from cattle suffering from respiratory signs. Kafrelsheikh *Veterinary Medical Journal* **10**, 119-140.

Majumder, S., Ramakrishnan, M., Nandi, S., (2015). Infectious bovine rhinotracheitis: An Indian perspective. *International Journal of Current Microbiology and Applied Sciences* **4**, 844-858.

Malla, J.A., Chakravarti, S., Gupta, V., Chander, V., Sharma, G.K., Qureshi, S., Mishra, A., Gupta, V.K., Nandi, S., (2018). Novel polymerase spiral reaction (PSR) for rapid visual detection of bovine herpesvirus 1 genomic DNA from aborted bovine fetus and semen. Gene **44**, 107-112.

Mars, M., De Jong, M., Franken, P., Van Oirschot, J., (2001). Efficacy of a live glycoprotein E-negative bovine herpesvirus 1 vaccine in cattle in the field. Vaccine **19**, 1924-1930.

Martinez-Ibeas, A.M., Power, C., McClure, J., Sayers, R.G., (2015). Prevalence of BoHV-1 seropositive and BVD virus positive bulls on Irish dairy farms and associations between bull purchase and herd status. *Irish Veterinary Journal* **68**, 1-9.

McGeoch, D.J., Davison, A.J., Dolan, A., Gatherer, D., Sevilla-Reyes, E.E., (2008). Molecular evolution of the Herpesvirales, In: *Origin and evolution of viruses. Elsevier* **29**. 447-475.

McGeoch, D.J., Rixon, F.J., Davison, A.J., (2006). Topics in herpesvirus genomics and evolution. Virus research **117**, 90-104.

Meeusen, E.N., Walker, J., Peters, A., Pastoret, P.-P., Jungersen, G., (2007). Current status of veterinary vaccines. Clinical microbiology reviews **20**, 489-510.

Minh, L.Q., (2011). *Studies on the bovine herpesvirus 1 US3 protein kinase (BHV-1pUS3)*. Dctoral dissertation, Greifswald, University, 2011.

Muylkens, B., Farnir, F., Meurens, F., Schynts, F., Vanderplasschen, A., Georges, M., Thiry, E., (2009). Coinfection with two closely related alphaherpesviruses results in a highly diversified recombination mosaic displaying negative genetic interference. *Journal of virology* **83**, 3127-3137.

Muylkens, B., Thiry, J., Kirten, P., Schynts, F., Thiry, E., (2007). Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Veterinary research **38**, 181-209.

Mweene, A., Fukushi, H., Pandey, G., Syakalima, M., Simuunza, M., Malamo, M., Nambota, A., Samui, K., Tsubota, T., Nakazato, Y., (2003). The prevalence of bovine herpesvirus-1 in traditional cattle in Southern Province, Zambia. Revue scientifique et technique (International Office of Epizootics) **22**, 873-877.

Nandi, S., Kumar, M., Manohar, M., Chauhan, R., (2009). Bovine herpes virus infections in cattle. Animal Health Research Reviews **10**, 85-98.

Nardelli, S., Farina, G., Lucchini, R., Valorz, C., Moresco, A., Dal Zotto, R., Costanzi, C., (2008). Dynamics of infection and immunity in a dairy cattle population undergoing an eradication programme for Infectious Bovine Rhinotracheitis (IBR). *Preventive Veterinary Medicine* **85**, 68-80.

Nettleton, P., Russell, G., (2017). Update on infectious bovine rhinotracheitis. In Practice **39**, 255-272.

Newcomer, B.W., (2021). Infectious Agents: Infectious Bovine Rhinotracheitis. Bovine Reproduction **41**, 753-757.

Njiro, S., Kidanemariam, A., Tsotetsi, A., Mnisi, M., Lubisi, B., Potts, A., Williams, R., Katsande, T., Baloyi, F., Moyo, G., (2011). A study of some infectious causes of reproductive disorders in cattle owned by resource-poor farmers in Gauteng Province, South Africa. *Journal of the south african veterinary association* **82**, 213-218.

Noordegraaf, A.V., Jalvingh, A.W., de Jong, M.C., Franken, P., Dijkhuizen, A.A., (2000). Evaluating control strategies for outbreaks in BHV1-free areas using stochastic and spatial simulation. *Preventive Veterinary Medicine* **44**, 21-42.

Ortiz-González, A.D., Buitrago, H.A.L., Bulla-Castañeda, D.M., Lancheros-Buitrago, D.J., Garcia-Corredor, D.J., Díaz-Anaya, A.M., Tobón-Torreglosa, J.C., Ortiz-Ortega, D., Pulido-Medellín, M.O., (2022). Seroprevalence and risk factors associated with bovine herpesvirus 1 in dairy herds of Colombia. *Veterinary World* **15**, 1550.

Owen, D.J., Crump, C.M., Graham, S.C., (2015). Tegument assembly and secondary envelopment of alphaherpesviruses. Viruses **7**, 5084-5114.

Patel, J., (2005). Characteristics of live bovine herpesvirus-1 vaccines. *The Veterinary Journal* **169**, 404-416.

Paulus, C., Nitzsche, A., Nevels, M., (2010). Chromatinisation of herpesvirus genomes. Reviews in medical virology **20**, 34-50.

Petrini, S., Iscaro, C., Righi, C., (2019). Antibody responses to bovine alphaherpesvirus 1 (BoHV-1) in passively immunized calves. Viruses **11**, 23.

Petrini, S., Martucciello, A., Righi, C., Cappelli, G., Torresi, C., Grassi, C., Scoccia, E., Costantino, G., Casciari, C., Sabato, R. (2022). Assessment of Different Infectious Bovine Rhinotracheitis Marker Vaccines in Calves. Vaccines 2022, **10**, 1204.

Piacenti, A., King, D., Seal, B., Zhang, J., Brown, C., (2006). Pathogenesis of Newcastle disease in commercial and specific pathogen-free turkeys experimentally infected with isolates of different virulence. *Veterinary pathology* **43**, 168-178.

Raaperi, K., (2012). *Epidemiology, impact on herd health and control of bovine herpesvirus 1 in Estonian dairy cattle herds*. Eesti Maaülikool university, Doctoral dissertation.

Raaperi, K., Bougeard, S., Aleksejev, A., Orro, T., Viltrop, A., (2012). Association of herd BRSV and BHV-1 seroprevalence with respiratory disease and reproductive performance in adult dairy cattle. *Acta Veterinaria Scandinavica* **54**, 1-10.

Raaperi, K., Nurmoja, I., Orro, T., Viltrop, A., (2010). Seroepidemiology of bovine herpesvirus 1 (BHV1) infection among Estonian dairy herds and risk factors for the spread within herds. *Preventive veterinary medicine* **96**, 74-81.

Raaperi, K., Orro, T., Viltrop, A., (2014). Epidemiology and control of bovine herpesvirus 1 infection in Europe. *The Veterinary Journal* 201, 249-256.

Radostits, O., Blood, D., Gay, C., (2007). A text book of diseases of cattle sheep, pig, goats and horses 10th edition. Bailliere Tindall, London **21**, 1210-1230 .

Rajkhowa, S., Rajkhowa, C., Rahman, H., Bujarbaruah, K., (2004). Seroprevalence of infectious bovine rhinotracheitis in mithun (Bos frontalis) in India **37**, 209-227.

Rinaldi, L., Pacelli, F., Iovane, G., Pagnini, U., Veneziano, V., Fusco, G., Cringoli, G., (2007). Survey of bovine herpes virus 1 coinfection in cattle. Parasitology research **100**, 359-364.

Romero-Salas, D., Ahuja-Aguirre, C., Montiel-Palacios, F., García-Vázquez, Z., Cruz-Romero, A., Aguilar-Domínguez, M., (2013). Seroprevalence and risk factors associated with infectious bovine rhinotracheitis in unvaccinated cattle in southern Veracruz, Mexico. *African journal of microbiology research* **7**, 1716-1722.

Romero-Salas, D., Cruz-Romero, A., Aguilar-Domínguez, M., Ibarra-Priego, N., Barradas-Piña, F., Domingues, L.N., Castro-Arellano, I., Lohmeyer, K., León, A., (2018).

Seroepidemiology of Bovine Herpes Virus-1 Infection in Water Buffaloes from the state of Veracruz, Mexico. *Trop Biomed* **35**, 541-552.

Russell, T., Bleasdale, B., Hollinshead, M., Elliott, G., (2018). Qualitative differences in capsidless L-particles released as a by-product of bovine herpesvirus 1 and herpes simplex virus 1 infections. *Journal of Virology* **92**, 1259-1218.

Sayers, R.G., (2017). Associations between exposure to bovine herpesvirus 1 (BoHV-1) and milk production, reproductive performance, and mortality in Irish dairy herds. *Journal of Dairy Science* **100**, 1340-1352.

Seal, K.H., Bertenthal, D., Miner, C.R., Sen, S., Marmar, C., (2007). Bringing the war back home: Mental health disorders among 103 788 US veterans returning from Iraq and Afghanistan seen at Department of Veterans Affairs Facilities. *Archives of internal medicine* **167**, 476-482.

Segura-Correa, J., Zapata-Campos, C., Jasso-Obregón, J., Martinez-Burnes, J., López-Zavala, R., (2016). Seroprevalence and risk factors associated with bovine herpesvirus 1 and bovine viral diarrhea virus in North-Eastern Mexico. *Open veterinary journal* **6**, 143-149.

Segura-Correa, J.C., Solorio-Rivera, J.L., Sánchez-Gil, L.G., (2010a). Seroconversion to bovine viral diarrhoea virus and infectious bovine rhinotracheitis virus in dairy herds of Michoacan, Mexico. *Tropical animal health and production* **42**, 233-238.

Segura-Correa, J.C., Solorio-Rivera, J.L., Sánchez-Gil, L.G., (2010b). Seroconversion to infectious bovine rhinotracheitis virus in dairy herds of Michoacan, Mexico. Tropical animal health and production **43**, 341-2438.

Serem, E., (2019). *Sero-prevalence and Risk Factors of Infectious Bovine Rhinotracheitis in the Smallholder Dairy Farms of Naari Sub-location of Meru County, Kenya*. Doctoral dissertation, University of Nairobi.

Singh, R., Yadav, S., (2010). Seroprevalence of bovine herpes virus-1 in Uttar Pradesh. Haryana Vet **49**, 54-55.

Solis-Calderon, J., Segura-Correa, V., Segura-Correa, J., Alvarado-Islas, A., (2003). Seroprevalence of and risk factors for infectious bovine rhinotracheitis in beef cattle herds of Yucatan, Mexico. *Preventive Veterinary Medicine* **57**, 199-208.

Ståhl, K., Rivera, H., Vågsholm, I., Moreno-López, J., (2002). Bulk milk testing for antibody seroprevalences to BVDV and BHV-1 in a rural region of Peru. *Preventive Veterinary Medicine* **56**, 193-202.

Stults, A.M., Sollars, P.J., Heath, K.D., Sillman, S.J., Pickard, G.E., Smith, G.A., (2022). Bovine Herpesvirus 1 Invasion of Sensory Neurons by Retrograde Axonal Transport Is Dependent on the pUL37 Region 2 Effector. *Journal of virology* **96**, 1486-1421.

Tadeg Wedajo, Muleta Lemma, Alemayehu Yilma, Tefera Asgedom, Hagos Reda, Abadi Amare, (2021). Seroprevalence of infectious bovine rhinotracheitis and brucellosis and their effect on reproductive performance of dairy cattle. *Journal of Veterinary Medicine and Animal Health* **13**, 106-113.

Takacs, I., (2013). Effect of us1gene mutation on pseudorabies virus gene expression. bmc molecular biology **14**, 1-12.

Thiry, J., Keuser, V., Muylkens, B., Meurens, F., Gogev, S., Vanderplasschen, A., Thiry, E., (2006). Ruminant alphaherpesviruses related to bovine herpesvirus 1. Veterinary research **37**, 169-190.

Thrusfield, M. (2005). Veterinary epidemiology 2nd ed. Black Well Science (Oxford).

Tibary, A., (2014). Infectious Agents: Infectious Bovine Rhinotracheitis. Bovine Reproduction **101**, 541-544.

Tikoo, S.K., Campos, M., Babiuk, L.A., (1995). Bovine Herpesvirus 1 (Bhv-1): Biology, Pathogenesis, and Control. Advances in virus research **45**, 191-223.

Torell, R., Bruce, B., Kvasnicka, B., Conley, K., (2003). Methods of determining age of cattle. Cattle Producer's Library: CL712. University of Nevada, Reno, NV **9**, 489-523. http://www. unce. unr. edu/publications/files/ag/other/cl712. pdf (accessed on 4 July 2022).

Trapp, S., König, P., Beer, M., (2003). Conventional and marked BHV-1 vaccines in Germany: a brief review. Berliner und Munchener Tierarztliche Wochenschrift **116**, 208-215.

Tuncer-Göktuna, P., Alpay, G., Öner, E.B., Yeşilbağ, K., (2016). The role of herpesviruses (BoHV-1 and BoHV-4) and pestiviruses (BVDV and BDV) in ruminant abortion cases in western Turkey. *Tropical animal health and producti*on **48**, 1021-1027.

Turin, L., Russo, S., (2003). BHV-1 infection in cattle: an update. CABI Reviews, **27**, 7 .

Valas, S., Brémaud, I., Stourm, S., Croisé, B., Mémeteau, S., Ngwa-Mbot, D., Tabouret, M., (2019). Improvement of eradication program for infectious bovine rhinotracheitis in France inferred by serological monitoring of singleton reactors in certified BoHV1-free herds. *Preventive veterinary medicine* **171**, 104743.

van Drunen Littel-van den Hurk, S., (2006). Rationale and perspectives on the success of vaccination against bovine herpesvirus-1. *Veterinary microbiology* **113**, 275-282.

Van Schaik, G., Schukken, Y., Nielen, M., Dijkhuizen, A., Barkema, H., Benedictus, G., (2002). Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. *Preventive veterinary medicine* **54**, 279-289.

Van Schaik, G., Schukken, Y., Nielen, M., Dijkhuizen, A., Benedictus, G., (2001). Epidemiology: Risk factors for introduction of BHV1 into BHV1-free Dutch dairy farms: A case-control study. Veterinary Quarterly **23**, 71-76.

Varela, A., Holz, C., Cibulski, S., Teixeira, T., Antunes, D., Franco, A., Roehe, L., Oliveira, M., Campos, F., Dezen, D., (2010). Neutralizing antibodies to bovine herpesvirus types 1 (BoHV-1) and 5 (BoHV-5) and its subtypes. Veterinary Microbiology **142**, 254-260.

Verbruggen, E., (2021). *the effect of calcium on the replication of bovine herpesvirus 1 (bhv-1) in the respiratory mucosa*. Doctoral dissertation, Ghent University.

Waldeck, H., van Duijn, L., Mars, M.H., Santman-Berends, I.M., Biesheuvel, M.M., van Schaik, G., (2021). Risk Factors for Introduction of Bovine Herpesvirus 1 (BoHV-1) Into Cattle Herds: A Systematic European Literature Review. *Frontiers in Veterinary Science* **59**, 1212.

Wathes, D.C., Oguejiofor, C.F., Thomas, C., Cheng, Z., (2020). Importance of viral disease in dairy cow fertility. Engineering **6**, 26-33.

Woodbine, K.A., Medley, G.F., Moore, S.J., Ramirez-Villaescusa, A.M., Mason, S., Green, L.E., (2009a). A four year longitudinal sero-epidemiological study of bovine herpesvirus type-1 (BHV-1) in adult cattle in 107 unvaccinated herds in south west England. BMC Veterinary Research **5**, 1-12.

Woodbine, K.A., Medley, G.F., Moore, S.J., Ramirez-Villaescusa, A.M., Mason, S., Green, L.E., (2009b). A longitudinal sero-epidemiological study of bovine herpesvirus type-1

(BHV-1) in dairy cattle in unvaccinated herds in south east England. BMC Veterinary Research **7**, 17-24.

Wrathall, A., Simmons, H., Van Soom, A., (2006). Evaluation of risks of viral transmission to recipients of bovine embryos arising from fertilisation with virus-infected semen. Theriogenology **65**, 247-274.

Yadav, V., Singh, S.P., Kumar, R., Diwakar, R., Kumar, P., (2018). A Review on Current Status of Infectious Bovine Rhinotrichitis in India. *International Journal of Current Microbiology and Applied Sciences* **7**, 411-426.

Yan, B., Chao, Y., Chen, Z., Tian, K., Wang, C., Lin, X., Chen, H., Guo, A., (2008). Serological survey of bovine herpesvirus type 1 infection in China. Veterinary microbiology **127**, 136-141.

Yang, B., (2019). *Replication characteristics of BoHV-4 in respiratory and genital mucosae and lack of association with subclinical endometritis*. doctoral dissertation, Ghent University.

Yazici, Z., Albayrak, H., Ozan, E., Gumusova, S., (2015). Serological status of bovine herpes virus type 1 in cattle in small scale private farms in the Central Black Sea Region, Turkey. *Pak Vet J* **35**, 101-102.

Yesilbag, K., Bilge-Dagalp, S., Okur-Gumusova, S., Gungor, B., (2003). Studies on herpesvirus infections of goats in Turkey: Prevalence of antibodies to bovine herpesvirus 1. Revue de médecine vétérinaire **154**, 772-774.

Yoo, H.S., (2010). Infectious causes of reproductive disorders in cattle. Journal of Reproduction and Development **56**, 53-60.

Zacarías, E., Benito, A., Rivera, H., (2002). Seroprevalencia del virus de la rinotraqueitis infecciosa en bovinos criollos de Parinacochas, Ayacucho. Revista de Investigaciones Veterinarias del Perú **13**, 61-65.

Zaher, K.S., Syame, S.M., Elhewairy, H.M., Marie, H.S., (2014). Investigation of bovine respiratory disease complex in Egypt with emphasis on some viral and bacterial pathogens. *Life Sci. J* **11**, 56-62.

Zhang, S., Cheng, A., Wang, M., Xiang, J., Zhu, D., Jia, R., Luo, Q., Cui, H., Zhou, Y., Wang, Y., (2010). Bioinformatics Analysis and Characteristics of Glycoprotein K

Encoded by the Newly Identified UL53 Gene of Duck Enteritis Virus. In: 2010 4th International Conference on Bioinformatics and Biomedical Engineering, 1-8.

Zhou, Y., Li, X., Ren, Y., Hou, X., Liu, Y., Wei, S., Dai, G., Meng, Y., Hu, L., Liu, Z., (2020). Phylogenetic analysis and characterization of bovine herpesvirus-1 in cattle of China, 2016–2019. Infection, Genetics and Evolution **10**, 4-16.

8. APPENDICES

The purpose of this questionnaire is, to gather information regarding the sero epidemiology of BoHV-1 in dairy cattle

Appendix. 1. Demographic information

Code No----------

1. Name of the owner----------
2. Address----------district----------Kebele----------village----------

3. Sex of the owner: a) male---------- b) female----------

4. Age of the owner (respondent)----------

5. Educational level: a) illiterate a) read and write b) elementary school c) Secondary school complete d) College/ degree holder

6. Marital Status: a) married b) single b) divorced d) widowed--------

8. System of Agricultural production a) livestock c) mixed crop- livestock c) modern production system

9. Dairy production as a source of income a) primary b) secondary/side line activity

10. Animal marketing system a) free contact of animals at market b) no contact of animal at market

11. Contact and/or graze with other small ruminants

Appendix table 1. Household herd composition

Herd composition		local	cross	Remark
Calf > 6 month	Male			
	Female			
cow				
Heifer				
Bullock				
Bull				
Equine				
Total Herd				

Appendix 2.Questionnaires to evaluate risk factors

1. Location a) Urban b) peri-urban c) rural

2. Sex of the animal 1) Male 2) Female

3. Age of the animal ----------

4. Total number of cattle in the herd (Herd size) 1) 1-5 2) 6-10 3) >10

5. (For farm) type 1) urban dairy production 2) peri-urban dairy production 3) Small-holder (rural dairy production)

6. Source of breeding service 1) Artificial insemination 2) Natural breeding /bull service

7. Origin (sources) of the animal 1) Homebred (raise from young ones on my farm) 2) Purchased

8. Parity status of the cow/heifer a) primiparous (1^{st}parity) b) multiparous (two and above parity c) none parity

I. Reproductive and respiratory disorder questionnaires as to evaluate as a risk

1. History of abortion 1) present 2) absent

2. For question 1, your answer if present, then in which trimester abortion most commonly occurs present in which trimester most commonly occur 1) 1-3 2) 4-6 3)7-9

3. Repeat breeding 1) present 2) absent

4. Retained fetal membranes 1) present 2) absent

5. History of Still Birth 1) present 2) absent

6. Blind calf birth 1) present 2) absent

7. Abnormality calf birth 1) present 2) absent

8 .Respiratory disorders 1) present 2) Absent

9. If respiratory disorder present, then 1) ocular nasal discharge 2 sneezing/ coughing 3) both

Appendix. 3 case definition

Rural dairy production system: it is part of the subsistence farming system that contributes up to 98% of the total milk production of in Ethiopia, and includes pastoralists, agro-pastoralists, and mixed crop-livestock producers.

Per-urban dairy production system: developed in areas where the population density is high and agricultural land is shrinking due to urbanization.

Urban dairy production system: The herd is dominated with improved/cross breed dairy cattle and the production system is market oriented and milk production is for sales.

None parity: No parturition
Primiparous: the term primiparous pertains to a female that delivered an offspring at one time (single parturition).

Multiparous: A cow that has given birth more than once (two or more parturition)

Herd: group of animals of one kind kept together under human control*;* have similar resource of feeding, drinking and etc.

Apoptosis: encoded suicide program which allow the elimination of the cell that have been produced in excess, developed improperly, or sustained genetic damage.

Latency is characterized by a restricted gene expression profile, the lack of production of infectious virions with the ability to reactivate at later times, generating new infectious virus

Abortion: the termination of a pregnancy by removal or expulsion of an embryo or fetus.

Retained fetal membrane: a lack of expulsion of the placenta within the first 24 hour after calving

Still birth: calve loss or calves born dead or dying within 24 hours of parturition

Abortion: is the expulsion of dead fetus of recognizable size at any stage of gestation

Repeat breeding: cows' failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities

Appendix table 2. Age determination of cattle by teeth eruption

Types of teeth	Age at eruption	references
i. Upper teeth		(Torell et al., 2003)
Central incisor	8-12 month	
Lateral incisor	9-13 month	
Canine(cuspid)	16-22 month	
First molar	13-19 month	
Second molar	23-31 month	
ii. Lower teeth		
First molar	14-18 month	
Second molar	25-33 month	
Canine (cuspid)	17-23 month	
Lateral incisor	10-16 month	
Central incisor	6-10 month	

Appendix table 3. Blood sample collection format

S.N	Owner's name	address	sex	Age	Breed		Tag no.	Management system			remark
					Local	cross		extensive	Semi intensive	Intensive	

Appendix. 4. Competitive ELISA testing procedure

1. add

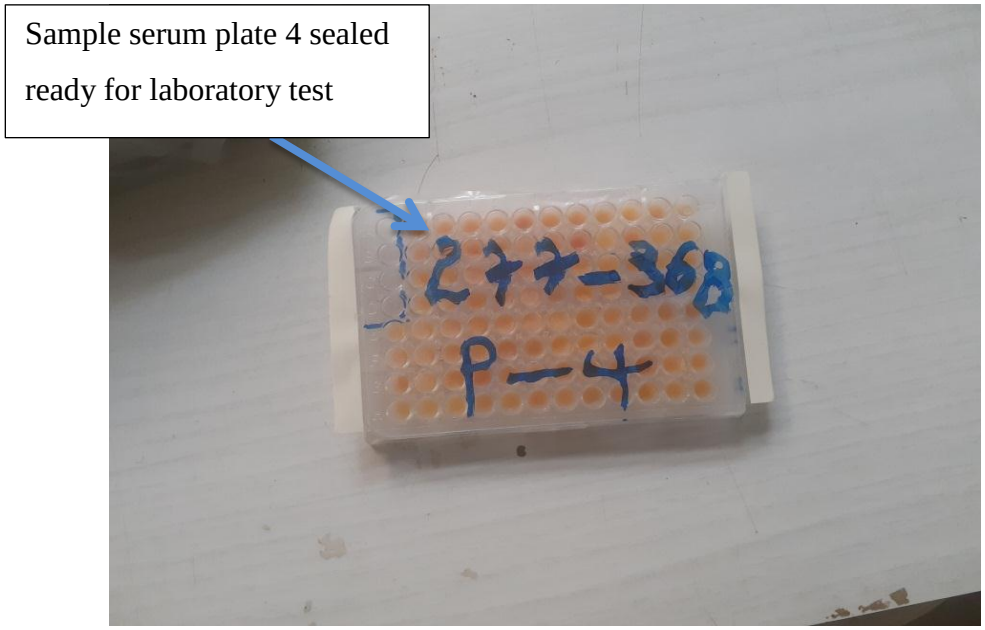
50 µl of the dilution buffer 19 to each wall

50 µl of the positive control to wells A1 and B1

50 l of the negative control to wells C1 and D1

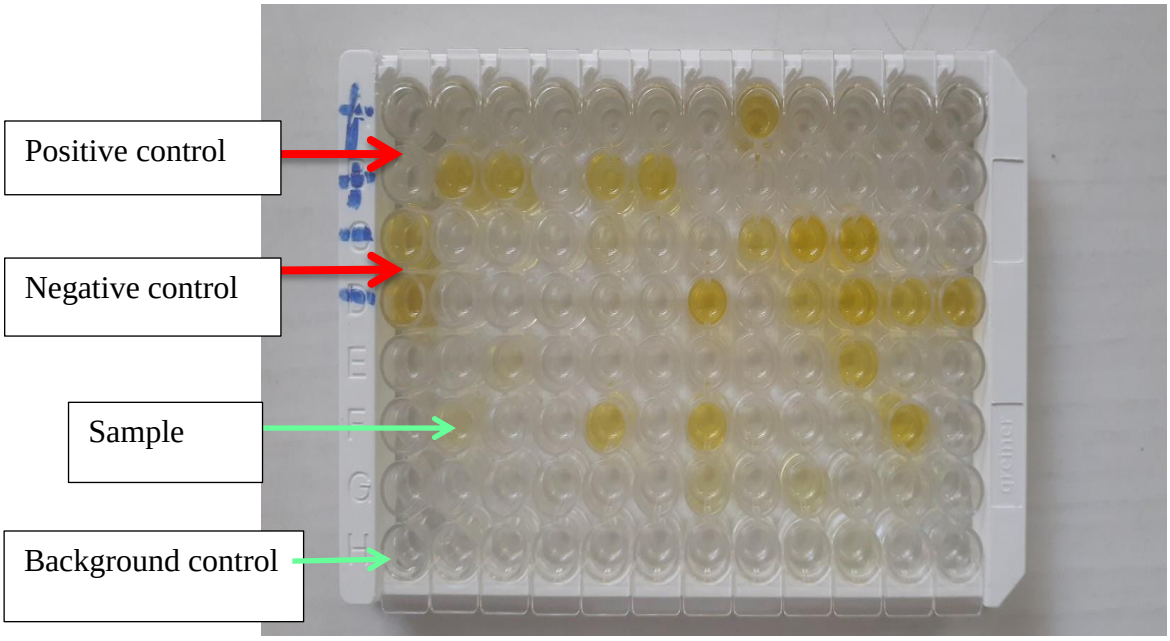
50µl of each sample to be tested to the remaining wells

2. Incubate 2h±5 minute at 37°C (±2°C)
3. Empty the wells. Wash each well 3 times with approximately 300 µl of wash solution. Avoid drying of the wells between washing.
4. Add 100 µl of the ready-to-use conjugate to each well.
5. Incubate 30 minute ±3 minute at 37 ºC (2± ºC)
6. Empty the wells. Wash each well 3 times with approximately 300 µl of wash solution. Avoid drying of the wells between washings.
7. Add 100 µl of the substrate solution to each well.
8. Incubate 15 minute ± 2 minute at 21 ºC (± 5 ºC) in the dark.
9. Add 100 µl of the stop solution to each well in order to stop reaction.
10. Read and recorded the O.D (optic density) at 450 nm by micro plate reader

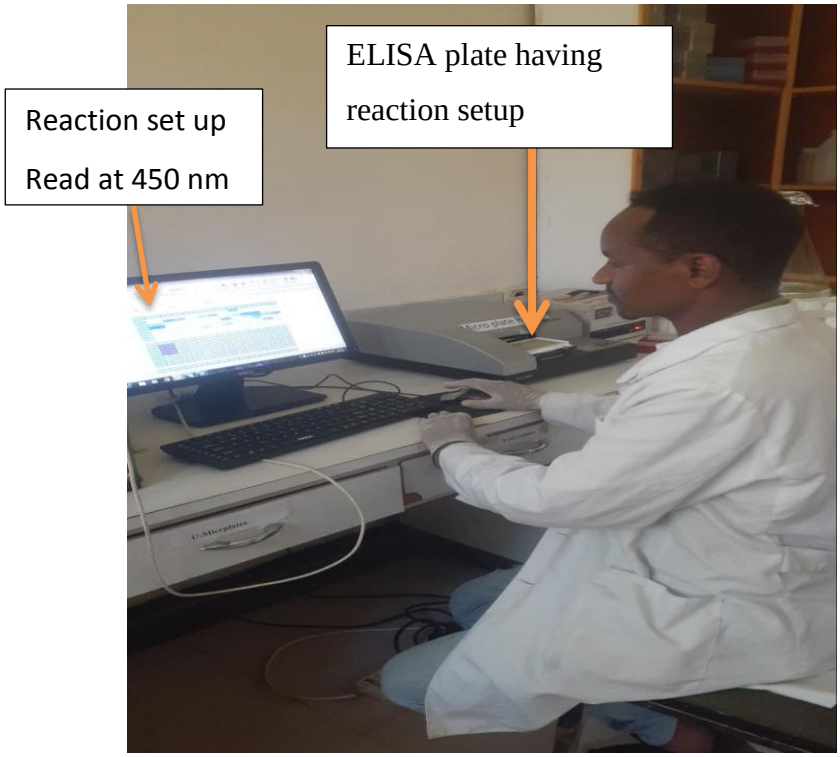


Appendix figure 1. Serum samples ready for laboratory test (c-ELISA)

Appendix figure 2. Competitive -ELISA process at the serology laboratory



Appendix figure 3. ELISA Plate having reaction set up.



appendix figure 4. ELISA Plate having reaction set up read at micro plate reader

Appendix table 4. Test result

IBR	OD Mean		plate 4			Validation						
Posc	0.0715					ODNc>0.7 Ok						
Neg c	1.658					ODPC/ODNC<0.3 Ok						
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.072	0.085	0.061	0.075	0.07	0.078	0.057	1.53	0.081	0.064	0.071	0.084
B	0.071	1.798	1.35	0.09	0.904	1.328	0.066	0.071	0.07	0.074	0.07	0.071
C	1.035	0.06	0.06	0.065	0.21	0.063	0.101	0.737	1.921	1.952	0.064	0.1
D	2.281	0.054	0.061	0.102	0.1	0.092	1.706	0.067	0.603	1.532	1.026	1.546
E	0.063	0.063	0.247	0.056	0.067	0.066	0.07	0.086	0.08	0.954	0.075	0.066
F	0.069	0.335	0.065	0.061	0.934	0.063	1.025	0.127	0.067	0.09	1.357	0.068
G	0.064	0.061	0.075	0.09	0.067	0.06	0.337	0.095	0.306	0.095	0.124	0.082
H	0.066	0.084	0.079	0.072	0.07	0.068	0.059	0.061	0.076	0.145	0.073	0.076

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos cc	5.12666	3.67913	4.52352	4.22195	4.70446	3.43788	92.2799	4.8854	3.86007	4.28227	5.06634
B	Pos cc	108.444	81.4234	5.42823	54.5235	80.0965	3.9807	4.28227	4.22195	4.46321	4.22195	4.28227
C	Neg cc	3.61882	3.61882	3.92039	12.6659	3.79976	6.09168	44.4511	115.862	117.732	3.86007	6.03136
D	Neg cc	3.25694	3.67913	6.15199	6.03136	5.54885	102.895	4.04101	36.3691	92.4005	61.8818	93.2449
E	3.79975875	3.79976	14.8975	3.37756	4.04101	3.9807	4.22195	5.18697	4.82509	57.5392	4.52352	3.9807
F	4.16164053	20.2051	3.92039	3.67913	56.3329	3.79976	61.8215	7.65983	4.04101	5.42823	81.8456	4.10133
G	3.86007238	3.67913	4.52352	5.42823	4.04101	3.61882	20.3257	5.72979	18.456	5.72979	7.47889	4.94572
H	3.98069964	5.06634	4.76478	4.34258	4.22195	4.10133	3.5585	3.67913	4.58384	8.74548	4.4029	4.58384

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

Pos	77
Neg	15

Appendix. 5. Validation and interpretation of the test

Sera samples were tested for the presence of BoHV-1 antibodies using competitive ELISA kit (ID.vet, IBR, gB. competition, Grabels, France) according to manufacturer's instructions. The optical density (OD) of the samples was measured at 450 nm.

The S/N percentage of BoHV-1 antibody was calculated for each sample as follows:

S/N %=(OD sample/OD $_{NC}$) x100

Validation: the test is valid if

The mean value of the negative control OD (OD_{NC}) is greater than 0.7

$$OD_{NC} > 0.7$$

-the mean value of the positive control (OD_{PC}) is less than 30% of the OD_{NC}.

$$OD_{PC}/OD_{NC} < 0.3$$

Based on the manual, the samples would be considered positive if the value of S/N% ≤ 45%. The samples with the S/N% greater or equal to 45% and less than 55% were considered doubtful, and the samples with S/N% greater than or equal to 55% were considered negative