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SEROEPIDEMIOLOGY OF INFECTIOUS BOVINE RHINOTRACHEITIS AND ISOLATION OF BOVINE HERPES VIRUS-1 FROM LOCAL BREED CATTLE IN SELECTED DISTRICTS OF SOUTH OMO ZONES, ETHIOPIA

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BY

MELKAMU TADESSE WORKENEH

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICENE AND AGRICULTURE

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A thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfilment of the requirements for the degree of Master of Sciences in Veterinary Epidemiology

BY

MELKAMU TADESSE WORKENEH (ID№ 5096/14)

JUNE, 2023

BISHOFTU, ETHIOPIA

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF CLINICAL STUDIES

As MVSc research advisors, we certify that we have read and evaluated the thesis prepared under our guidance by **Melkamu Tadesse Workneh** entitled: "**Seroepidemiology of Infectious Bovine Rhinotracheitis and Isolation of Bovine Herpes Virus-1 from local breed cattle in selected districts of South Omo Zones, Ethiopia**". We recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Epidemiology.

Submitted by: **Melkamu Tadesse Workneh** ______________ ______________

Name of Student Signature Date

Approved *for submittal to a* thesis assessment committee

1. **Dr. Zerihun Assefa** (DVM, MSc Assistant Professor) ________ ________

   Advisor Signature Date

2. **Dr. Hagose Asegedom** (DVM, MSc, PhD candidate) ________ ________

   Co-Advisor Signature Date

3. **Abebe Garuma** (BSC, MSc) ________ ________

   Co-Advisor Signature Date

4. **Dr. Haileleul Negussie** (DVM, MSc, PhD) ________ ________

   Department Head Signature Date

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPRTMENT OF CLINICAL STUDIES

As members of the examining board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by **Melkamu Tadesse Workneh,** entitled " **Seroepidemiology of Infectious Bovine Rhinotracheitis and Isolation of Bovine Herpes Virus-1 from local breed cattle in selected districts of South Omo Zones, Ethiopia** " and recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Epidemiology.

Dr. Geremew Haile (DVM, MSc)	________	________
Chairperson	Signature	Date
Dr. Rahimeto Abebe (DVM, MSc, PhD)	________	________
External Examiner	Signature	Date
Prof. Teshale Sori (DVM, MSc, PhD)	________	________
Internal Examiner	Signature	Date

AUTHOR DECLARATION

The work presented in this thesis was conducted from December 2022 to May 2023 at South Omo Zone, Ethiopia under the supervision of Dr. Zerihun Assefa (AAU), Dr. Hagos Asegedom, Abebe Garuma and Dr. Dereje Shegu (AHI). This thesis has been submitted in partial fulfillment of the requirements for the Master of Veterinary Science in Veterinary Epidemiology degree at Addis Ababa University College of Veterinary Medicine and Agriculture. I hereby declare that this thesis entitled 'Seroepidemiology of Infectious Bovine Rhinotracheitis and isolation of bovine herpes virus-1 from local breed cattle in selected districts of South Omo Zones, Ethiopia' is bonafide record research work done by me during the course of the research and that the thesis has not previously formed the basis for the award to me any degree, diploma associateship, fellowship or another similar title, of any other university or society.

Name: Melkamu Tadesse Signature _______________

College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

Date of Submission 16/ 06/2023

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LIST OF ABBREVIATIONS

AHI	Animal Health Institute
BoHV	Bovine Herpes Virus Type 1
C-ELISA	Competitive Enzyme-Linked Immunosorbent Assay
CPE	Cytopathic Effect
Dfl	Degree of Financial Leverage
DIVA	Differentiating Infected from Vaccinated Animals
DNA	DeoxyriboNucleic Acid
EEC	European Economic Community
EFSA	European Food Safety Authority
ELISA	Enzyme linked immunosorbent assay
EWCA	Ethiopian Wildlife Conservation Authority
HgCl2	Mercuric Chloride
I-ELISA	Indirect enzyme-linked immunosorbent assay
GDP	Gross Domestic Product
IBR	Infectious Bovine Rhinotracheitis
IPB	Infectious Pustula Balanoposthitis
IPV	Infectious Pustular Vulvovaginitis
gE	Immunoglobulin G
m.a.s.l	Meters Above Sea Level
MoA	Ministry of Agriculture
MDBK	Madine-Darby Bovine Kidney
NABC	Netherlands-African Business Council

NaOH	Sodium Hydroxide
OIE	Office International des Epizooties
PA	Peasant Association
PCR	Polymerase Chain Reaction
PFE	Pastoral Forum Ethiopia
pH	Power of Hydrogen
SNNPR	Southern Nations, Nationalities, and Peoples' Region
SOFED	South Omo zone Finance and Economy Development Department
STATA	Statistical Software Package
TCID	Tissue Culture Infectious Dose
UK	United Kingdom
USA	United States America
VN	Virus Neutralization
VTM	Viral transport media
WOAH	World Organisation for Animal Health

ABSTRACT

Infectious Bovine Rhinotracheitis (IBR) is a highly contagious viral disease of domestic and wild bovines and angulates caused by Bovine herpes virus 1 (BoHV-1). It causes a high economic loss in cattle industry worldwide. A cross-sectional study was conducted from December 2022 to May 2023 to determine the seroprevalence of the disease, assess the potential risk factors associated with the disease as well as to isolate the BHV-1 from local breed cattle in the selected districts of the South Omo zone, Ethiopia. A total of 400 blood sera and 24 nasal swaps were collected to determine the seroprevalence and isolation of the virus respectively. Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) was used to detect antibodies specific to Bovine Herpes Virus-1 (BoHV-1). The nasal swap samples were propagated on Madine-Darby Bovine Kidney Epithelial (MDBK, passage 84) cell line, monitored and inspected for the development of cytopathic effects (CPE). Descriptive statistics, chi-square (x^2) test, univariable and multivariable logistic regression were used to analyse the field and laboratory data. An overall individual animal seroprevalence of 81% (324 of 400) and herd-level seroprevalence of 88.29% (83 of 94) were recorded. Out of 24 samples were processed for virus isolation, virus specific cytopathic effects were observed only in 6 (25%) samples. Factors such as herd size (OR= 14.5, p=0.000), movement across a national border (OR=5.2, p=0.005), contact with wild animals (OR=3.99, p=0.005), retained placenta (OR=30.7, p= 0.005), and purchased, gifted or cattle rustling (OR=10.6, p=0.000) were found statistically associated with IBR seropositivity in multivariate analysis. However, abortion, dystocia, still birth, history of respiratory and ocular problem, sex, age, crossing Park and sanctuary boundary, were not found statistically significant. In general, the serological and cell culture results showed that BHV-1 was circulating among the herds in the study area. Given that the current study was limited in time, space and design, further works need to be conducted to generate additional information which helps reduce the transmission and socio-economic impacts of the IBR disease on domestic cattle.

Keywords: *BHV-1, Cattle, C-ELISA, IBR, Isolation, Seropositivity, Risk factor*

1. INTRODUCTION

Ethiopia ranks first in Africa's livestock resource map, and this sector accounts for around 45% of agricultural GDP, 18.7% of the national GDP and 16-19% of the total foreign exchange earnings of the country (Behnke and Metaferia, 2011). About 85-90% of mixed crop-livestock farmers and 21.6 million agro-pastoralists and pastoralists depend on livestock as a major economic activity for their livelihood (NABC, 2010).

Ethiopia’s pastoral and agro-pastoral area covers about 60% of the country’s total land mass of the country and support 12-15% (or 10-12 million people) of the country's. In Ethiopia, 97% of pastoralists live in the northeast, east, and south (Desta, 2013; Tofu *et al,* 2023). The pastoralist population of Ethiopia and their livestock regularly move across the borders into neighboring countries depending on levels of rainfall, grazing, and security. Estimates of the national livestock population should therefore be treated with caution, but approximate figures are available. The livestock population in pastoral areas of Ethiopia is estimated at 9.3 million cattle (30% of the national population), 12.4 million sheep (51.7% of the national population), 8.1 million goats (45% of the national population) and 1.8 million camels (almost 100% of the national population) (PFE, 2007).

At present, the sector's economic contribution to the country is minimal compared to its potential. One of the main causes of this mismatch between population size and production output from livestock in Ethiopia is undoubtedly the widespread occurrence of infectious and non-infectious diseases which drastically reduce the production and productivity of the animal through morbidity, mortality, and market restriction (MoA, 2021).

In recent times, global warming is reasonable for the recurrent occurrence of severe droughts and resulted in feed shortages and the introduction and spread of exotic animal diseases. Among them, Infectious Bovine Rhinotracheitis (IBR), a particularly prevalent and contagious respiratory disease in cattle, causes the dairy industry to suffer significant financial losses on a global level (Bosco *et al*., 2011; Gould *et al*., 2013).

IBR is a multi-organ disease caused by Bovine Herpes Virus-1 (BHV-1), and it affects both domestic and wild ruminants (Bowland *et al*., 2000; Muylkens *et al*., 2007). Bovine Herpes

Virus-1 is a virus of the genus Varicellovirus, subfamily *Alphaherpesvirinae* and family *Herpesviridae*, and is a highly contagious and infectious virus (Biswas *et al*., 2013; Newcomer and Givens, 2016). BHV-1 has different strains; the BHV-1.1 which is the respiratory subtypes, while strain BHV-1.2a and BHV-1.2b are the genital subtypes, and BHV-1.3 the encephalitic subtype (Muylkens *et al*., 2007).

The BHV-1 occurs worldwide and is endemic in a number of food-producing countries (Ackermann and Engels, 2006). Following infection, cattle are lifelong carriers of the virus with the potential of spontaneously reactivated viral shedding, especially at times of increased stress (Muylkens *et al.,* 2007). BoHV-1 infections can cause a variety of clinical symptoms and alterations to production parameters, from fever, decreased development, and decreased milk production to a higher chance of abortion and death (Graham, 2013; Nettleton and Russell, 2017).

The virus can spread from infected cattle mainly through inhalation, contact, and by naturally occurring mating or artificial insemination using virus-contaminated semen. IBR in cattle causes reproductive problems including subfertility indicated by higher service per conception and extended days open, retention of fetal membranes, abortion, metritis including oophoritis (Sibhat *et al*., 2018).

All breeds of cattle are equally susceptible, and the disease has become common in cattle older than 6 months old as a result of fading maternal antibody protection and increased population mixing of animals (Majumder *et al*., 2015; Constable *et al*., 2017). IBR doesn't naturally vary with the seasons. However, the feedlot cattle assembling during the fall and winter in temperate areas is what causes variation (Majumder *et al*., 2015). Increasing herd sizes, purchasing infected cattle, production system type, and taking part in agricultural competitions are some of the managemental and environmental risk factors that contribute to the development of BHV-1(Gay and Barnouin, 2009; Constable *et al*., 2017). The development of the disease has also been linked to inaccurate records of vaccination and uncontrolled movement of visitors and livestock into the farm (Boelaert *et al*., 2005).

Over the past 15 years, research has been carried out in numerous nations around the globe and reveals varying seroprevalence status ranging from 35.9–77.5% in Europe and 37–60.8% in Latin America (Raaperi *et al*., 2014). Recent, there aren't plenty of studies from

Sub-Saharan Africa, but some of the reports that are available include 48.3% in Southern Zambia (Mweene *et al.,* 2003), 69% in Ghana (Adu-Addai *et al.,* 2012), 74.5% in the Gauteng province of South Africa (Njiro *et al.,* 2011), 8.75 % in Sudan (Elhassan *et al.* (2015) and 17.4 % in Kenya (Kipyepo *et al.* 2020). In Ethiopia, previous seroprevalence studies conducted on IBR are limited to central, south-western, and north-central parts of the country primarily involving intensive production systems. The first report in Ethiopia was done by Lefevre (1975) with a prevalence rate of 41.8% in Harar and Sidama. Since then IBR seroprevalence of 58% was reported from Gobe area (Bekele *et al.,* 1989), 45.5% from Holeta (Sibahat *et al*., 2018), 26% from Desse and Kombolicha (Wedajo *et al.,*2021), 77.6 % from East and west Gojam (Zewde *et al.,* 2021) and 34.7 % from Bishoftu, Sebeta, Suluta, Sendafa, Holeta, Assela and Nekmte towns in Oromiya regional state and Bahir Dar and Gonder in Amhara regional state (Tesfaye *et al.,* 2022).

Almost all of these studies in Ethiopia were carried out in intensive production system and highland areas. However, there is no report that shows an IBR status in pastoral and agro-pastoral areas of the country. Even though there is no single report of IBR in these settings, diseases that cause abortion, ocular problems, and respiratory problems are common in the area, according to some research findings that prompted the researcher to look for IBR in the area. On top of that, the nature of pastoral livestock production systems, such as free livestock mobility within the country, across borders of neighbouring countries and contact cattle with wild animals, sparked questions about IBR and the inception of this research. Moreover, evidence regarding IBR prevalence and potential risk factors precipitating the disease in South Omo are scarce.

General objective

The general objective of this study was to conduct a seroepidemiological study on Infectious Bovine Rhinotracheitis in South Omo Zone, Southern Nations, Nationalities, and Peoples' Region, Ethiopia.

The specific objectives were:

- ✓ To estimate the Seroprevalence of IBR in the South Omo Zone
- ✓ To assess the potential risk factors associated with IBR occurrence in the study area
- ✓ To isolate the bovine herpes virus 1 from local breed cattle in the study area

2. LITERATURE REVIEW

Infectious bovine rhinotracheitis (IBR), as its name indicates, is highly infectious; cattle are the natural hosts, and inflammation of the nose and trachea is a consistent feature (Nettleton and Russell, 2017).

2.1. Aetiology

Herpesviruses are prevalent viruses that can cause serious infections in humans as well as animals. Bovine herpesvirus type 1 (BHV-1) is an enveloped DNA virus that belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus Varicellovirus. It is linked to a variety of unique cattle disease syndromes, including infectious bovine rhinotracheitis (IBR), conjunctivitis, infectious pustular vulvovaginitis, balanoposthitis, abortion, encephalomyelitis, and mastitis (Fauquet *et al*., 2005).

Of eight herpes viruses, BoHV-1 is the most vital virus so far that naturally infects cattle. It is closely linked to the varicella-zoster (chickenpox) virus (human herpesvirus type 3) and pseudorabies virus (Suid herpesvirus type 1).The severe form of IBR was discovered in Europe during the winter of 1971-72, when it is thought to have originated in North America. Following the acute outbreaks, the disease has clinically evolved, and BoHV-1 infection is now present in a less severe form, with severe cases occurring on occasion. Aside from the economic costs of clinical BoHV-1 infection, a major risk is the disruption of international trade. (Gunn *et a*l., 2006).

2.2. Epidemiology of IBR

2.2.1. Host factors

The virus Herpesvirales, family Herpesviridae, and species Bovine herpesvirus-1 (BoHV-1) cause IBR. Domestic and wild cattle are both infected by the IBR virus (OIE, 2017). BoHV-1 may infect Artiodactyla (cattle, sheep, goats, water buffaloes, and camelids). Naturally, sensitive animal species include red deer (Cervus elaphus) (Frolich *et al.,* 2006), roe deer

(Capreolus capreolus), fallow deer (Dama dama) (Kalman and Egyed, 2005), and reindeer (Rangifer tarandus) (Lillehaug *et al.,* 2003).

Cattle, sheep, goats, water buffalo (Bubalus bubalis) (Fusco *et al.,* 2015; Raaperi *et al.,* 2014), and pig are some of the domestic species that are naturally susceptible. All breeds and ages of cattle are equally susceptible, and because maternal antibody protection decreases, the disease is more prevalent in cattle older than 6 months (Majumder *et al.,* 2015; Seyfi *et al*., 2016; Constable *et al*., 2017).

BoHV-1 is released in nasal fluid over a period of 10-17 days during acute primary infection, with a peak at 4-6 days post-infection. Cattle infected with BoHV-1.1 have higher viral titres in their nasal secretions (10-100-fold higher) than calves infected with BHV-1.2b. Bulls may shed BoHV-1 for several days to several weeks after primary preputial infection. Infected bulls may also shed significant levels of the virus in the semen (Nandi *et al*., 2009).

2.2.2. Environmental factors

Although there is no inherent seasonal variability in IBR disease, disease prevalence is greater in temperate countries during the months of fall and winter due to feedlot cattle assembly (Majumder *et al*., 2015). Increased herd size, purchasing infected cattle, participation in agricultural shows, and type of production system are all management and environmental risk factors that contribute to the spread of BHV-1 (Constable *et al*., 2017). Uncontrolled flow of people and livestock into the farm, as well as untrustworthy vaccination documents Journal Pre-proof 4 dates have also been linked to disease dissemination (Boelaert *et al.,* 2005).

The virus's inactivation in the environment is affected by elements such as humidity, temperature, light, pH, and the medium in which it lives, with low temperature and high relative humidity promoting life (OIE, 2017). The virus is stable for one month at 4°C. It deactivates in 21 minutes at 56°C, 10 days at 37°C, and 50 days at 22°C. The virus can live in feed for up to 30 days. Because the virus is enveloped, organic solvents such as ether, chloroform, and acetone are toxic to it. Many disinfectants are effective against the virus, including10% Lugol's iodine, 0.01% $HgCl_2$, 1% phenolic derivatives, 1% quaternary

ammonium bases, 0.5% NaOH, and 1% chlorinated lime,. Formalin (5%) inhibits1 within 1 min (Nandi *et al*., 2009).

2.2.3. Pathogen factors

The viral genome is made up of double-stranded DNA that encodes for around 70 proteins, 33 of which are structural and more than 15 are non-structural. The viral glycoproteins, which are found on the surface of the virion in the envelope, play a significant role in pathogenesis and immunity. BoHV-1 is classified into three subtypes: 1.1, 1.2a, and 1.2b. Subtypes 1.2 of BoHV may be less pathogenic than subtype 1.1. BoHV-5 has been reclassified as the previous BoHV-1.3, which may operate as a neuropathogenic agent in calves (Kathiriya *et al.,* 2018). BoHV-1 has antigenic and genetic affinities with the following ruminant alphaherpesviruses: BoHV-5, caprine herpesvirus 1, cervid herpesvirus 1 (red deer), cervid herpesvirus 2 (reindeer), and bubaline herpesvirus 1 and elk herpesvirus 1 (Thiry *et al*., 2006).

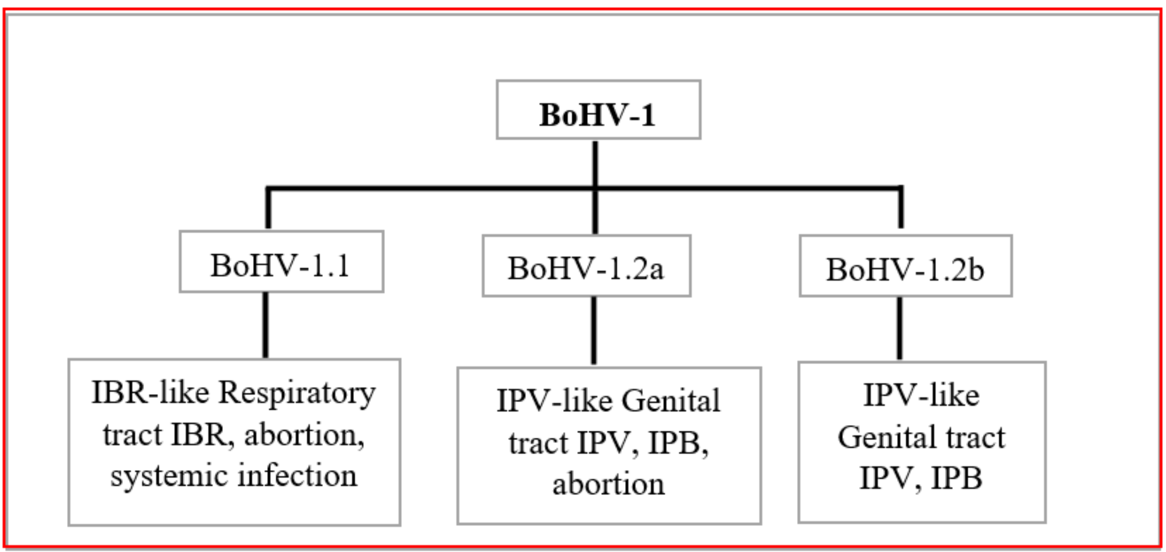

Figure 1: Diagram showing subdivision of BHV-1

Source: (Graham, 2007)

2.3. Global Status of IBR

Except in BHV-1-free countries, the virus is found globally (OIE, 2010). Over the last 15 years, studies conducted in various regions around the world found variable seroprevalence varying from 35.9 - 77.5% in Europe and 37-60.8% in Latin America (Raaperi *et al*., 2014). Recent findings of BHV-1 serop revalence in cattle from Sub-Saharan Africa are few, but include 48.3% in Southern Zambia (Mweene *et al.,* 2003), 69% in Ghana (Adu-Addai *et al*., 2012), and 74.5% in South Africa's Gauteng area (Njiro *et al*., 2011).

In the absence of management, infection is common at both the animal and herd levels. Raaperi *et al.,* (2014) looked into many different European prevalence surveys and discovered that herd-level prevalence ranged from 13.4% to 100% (mean 66.3%, median 70.4%) and animal-level prevalence ranged from 12.0% to 77.5% (mean 37.7%, median 38.4%). Calves had a lower infection rate than adults, although the incidence of seroconversion is higher in animals aged 24 months than in adults (Muylkens *et al*., 2007).

Table 1: Seroprevalence study of IBR in the different parts of the country

Study Country	**Study Animal**	**Diagnostic test**	**Prevalence (%)**	**Reference**
Kenya	Cattle	gB-ELISA	17.4	Kipyego *et al.,* 2020
Egypt	Cattle	I-ELISA	93.7	Hussein *et al.,* 2019
Morocco	Cattle	ELISA	50	Lucchese *et al.,* 2016
Iraq	Cattle	ELISA	4.7	Ahmed *et al.,* 2015
Iran	Cattle	ELISA/VN	33.97	Kargar *et al.,* 2001
India	Cattle	I-ELISA	65.87	Saravanajayam *et al.,* 2015
Colombia	Cattle	I-ELISA	57.5	Ortiz-González *et al.,* 2022
Nepal	Dairy cattle	I-ELISA	18.48	Jha,2005
China	Cattle	I-ELISA	69.2	Yan *et al.,* 2008
England	Cattle	I-ELISA	83.2	Williams and Winden,2014

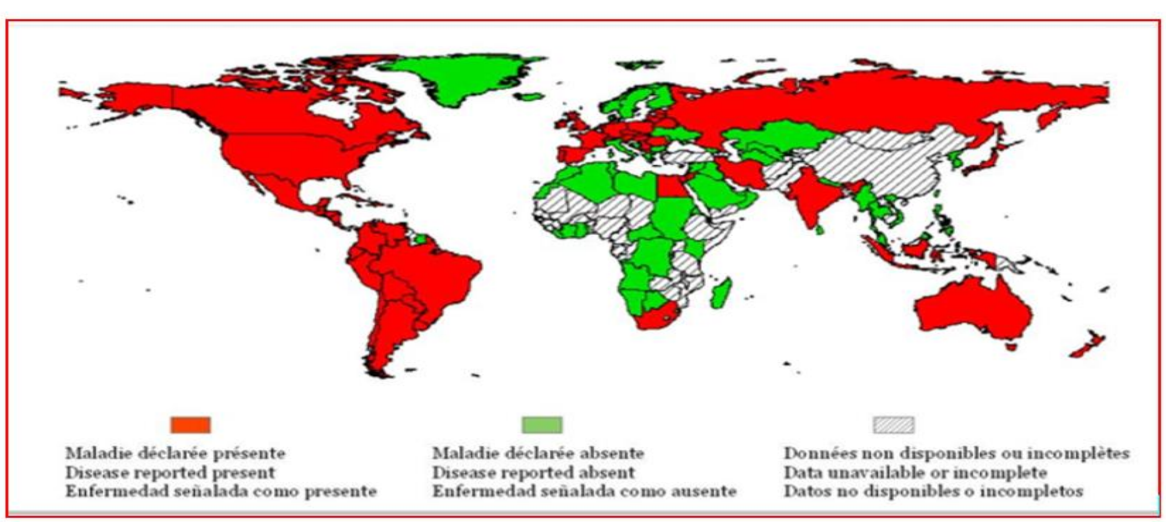

Figure 2: Distribution of Infectious BovineRhinotracheitis in 2004 (OIE, 2004)

2.4. IBR Status in Ethiopia

In Ethiopia, there were only a few works done on sero-epidemiology of IBR, which were limited to central, south, southwestern and north-central parts of the country (Lefevre, 1975; Bekele *et al*., 1989; Sibhat *et al*., 2018, Wedajo *et al*., 2021). During the last 3-4 decades, a prevalence of 41.8 and 67%, respectively has been reported in Harar and Sidamo provinces (Lefevre, 1975), and in Gobe and Ghibe in Central Ethiopia (Bekele *et al*., 1989). More recently, the study by Sibhat *et al*., 2018 revealed that an overall herd-level BHV-1 seroprevalence of 81.8% and individual animal-level seroprevalence of 41.0% were found in three major milk sheds in Addis Ababa, central, southern and southwestern parts of Ethiopia. Although a study in Kombolcha and Dessie districts reveals, that seropositivity was 25.6% (85/332) for IBR (Wedajo *et al*., 2021).

Table 2: Prevalence of the IBR in Ethiopia

Study Area	Study Animal	Sample Size	Seroprevalence	Reference
Bishoftu, Sebeta, Suluta, Sendafa, Holeta, Assela , Nekmte, Bahir Dar and Gonder *	Dairy cow	1,114	34.7	Tesfaye *et al.*, 2022
East and west Gojam, Awi	Cattle	442	77.6	Zewde *et al.*, 2021
Dessie	Dairy cow	183	21.1	Wedajo *et al., 2021*
Kombolcha	Dairy cow	149	30.9	Wedajo *et al., 2021*
Central Ethiopia	Holstein-Friesians (HF), Jersey and HF-Zebu crossbred cattle	555	30.8	Sibhat *et al., 2018*
Southern Ethiopia		629	45.5	Sibhat *et al., 2018*
Western Ethiopia		195	55.9	Sibhat *et al., 2018*
Gobe	Cattle	988	58	Bekele *et al., 1989*
Ghibe	Cattle	604	81	Bekele *et al.*, 1989
Harar and Sidama	cattle		41.8	Lefevre, 1975

Note * study was carried out from 2018 to July 2020

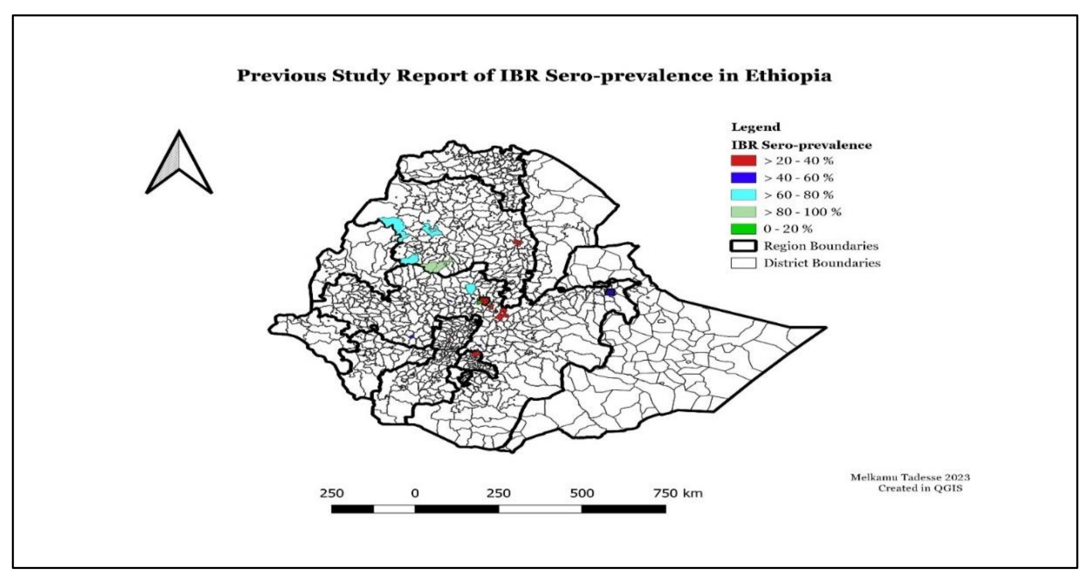

Figure 3: Previous Study Area Report of IBR Seroprevalence in Ethiopia

Source: (*Wedajo et al.,* 2021*;* Zewde *et al.,* 2021 and Tesfaye *et al.,* 2022)

In Ethiopia, as the below figures show, there is a difference in the seroprevalence of IBR over the years. The highest overall animal level seroprevalence of BoHV-1 antibodies were 77.6% in indigenous breeds of extensively and semi-intensively grazing cattle in North-western parts of Ethiopia (i.e. East Gojam, West Gojam, and Awi Zone). And the lowest Sero-positivity was 25.6% (85/332) for IBR in dairy cattle in Dessie and Kombolcha towns in the South Wollo zone of Amhara region, north-central Ethiopia.

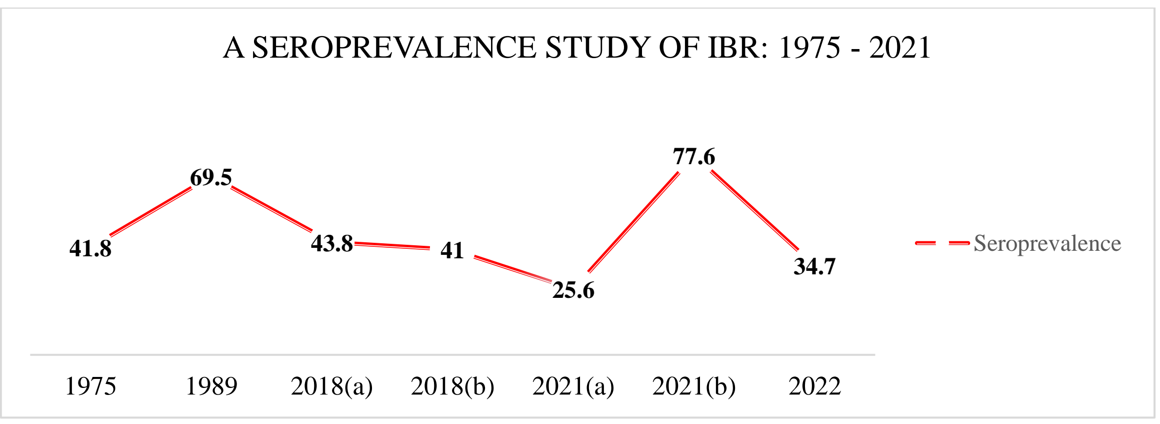

Figure 4*:* Temporal patter of Seroprevalence study of IBR 1975 – 2021

Source: (Wedajo *et al.,* 2021*;* Zewde *et al.,* 2021 and Tesfaye *et al.,* 2022)

2.5. Transmission

The routes of transmission can be horizontal or vertical, BoHV-1 is typically transmitted through direct contact between an at-risk animal and an infected animal excreting virus in oro-nasal or vaginal secretions. Aerosol distribution does occur, however it is thought to be restricted to a few metres in most circumstances (EFSA, 2006). Infected semen is another method of viral transfer, and vertical transmission may occur in pregnancy. Following a primary or reactivated infection, BoHV-1 may be shed in sperm, with the potential for transmission via artificial or natural insemination. BoHV-1 adsorbed to the zona pellucida may potentially be transferred during embryo transfer. BoHV-1 has been isolated from milk and faeces, however neither source is thought to represent a substantial source of transmission route in the field (EFSA, 2006). Biswas *et al.,* (2013) stated that IBR is transmitted directly through respiratory, ocular, or genital secretion during contact with acutely infected animals and latently infected animals, in which reactivation of the virus

takes place, and indirectly through contaminated fresh or frozen semen from infected bull semen, humans, and contaminated material.

2.6. Clinical Signs of IBR

The clinical signs may vary widely and have been grouped as: respiratory form, genital form, ocular form and encephalomyelitis form. The most economically significant clinical entity caused by BoHV-1 is IBR/IPV/IBP (Muylkens *et al.,* 2007). Depending on the exposure, the incubation period ranges from 2 to 4 days. Nasal secretion, which is initially serous, increases, food intake declines, and as the temperature goes over 41°C, the animal begins to salivate profusely. Milk production suddenly decreases, the animals stop eating, and nasal secretions become seromucous, then mucopurulent. Conjunctivae redden, ocular discharge increases, and while initially serous, it gradually turns mucopurulent. If subsequent infections are averted by the introduction of antimicrobial medications four days after the first symptoms, the temperature drops, as do pulse and respiration rates. If bacterial infections arise, when secondary bacterial infections occur, the temperature rises again or remains steady. This stage of infection can be fatal (Biswas *et al.,* 2013).

Abortions frequently occur in the course of a clinical episode of IBR when virus isolation from the respiratory tract is easy. The clinical entity is different from those abortions associated with viral infections where no overt IBR is noticed. Because some time elapses between fetal death and abortion, the foetuses are in general in various stages of autolysis. The general behaviour of the aborting animals is hardly changed, only milk production is reduced if abortions occur during lactation. If treatment of the animals suffering from a retained placenta is carried out correctly, metritis can be avoided (Graham, 2013).

Infections of the upper (e.g., conjunctivitis) and lower respiratory (e.g., bronchitis) and genital tracts (prepuce, vulva, and caudal vagina) mucosal surfaces cause pain and misery in the animals (Kathiriya *et al.,* 2018). Secondary clinical indications of infection such as eye discharge, salivation, and fever result from the progression of the infection in these areas. Secondary infection results in a change in the consistency of the discharges (from serous to mucopurulent) as well as increased local pathology such as necrotic lesions in the nose and pustules and ulcers in the genital tracts. The existence of these lesions causes pain and

suffering in the animals, and their behaviour reflects this, as they lose appetite, become weak, and exhibit pain responses to typically non-painful management (Gunn *et al*., 2006).

Additionally, the genital tract can be affected by the virus, which can lead to balanoposthitis and pustular vulvovaginitis. Postmortem examinations reveal tracheitis, laryngitis, and rhinitis. Mortality is low, and most infections run a subclinical course. BoHV-1 may be a factor in multifactorial disease like "shipping fever," which can be brought on by secondary bacterial infections that worsen respiratory conditions. (OIE, 2017).

2.6.1. Latency and reactivation

Latency is believed to develop in almost all animals that are infected with high or low doses of attenuated or virulent BHV-1 (Biswas *et al*., 2013). The terminal sensory neurons that innervate the diseased mucosae quickly initiate neuro invasion during the main respiratory tract infection. The trigeminal nerve is used by the virus as it ascends into the central nervous system (CNS), where it largely establishes latent infection in the trigeminal ganglion, where an initial, transient lytic infection is visible. During latency, viral gene expression is turned down and viral DNA replication is undetectable. The BoHV-1 latency-related transcript, which blocks the start of viral replication, is one of the virally encoded genes that keeps the virus in its latent condition. The onset of latency occurs concurrently with the emergence of the virus's immunological response, which unquestionably contributes to the virus's confinement in the latent state within ganglia. There is some data supporting the tonsils' potential role as small sites of latency, as well as the potential role of peripheral blood leukocytes (Favier *et al.,* 2014). The BoHV-1 causes latent infection in immune privileged sites following productive viral infection (Nuotio *et al*., 2007; Nettleton and Russell, 2017 and OIE 2017).

2.7. Pathogenesis

Direct contact with infected cattle, semen, or aerosol is the route of infection (Sáez *et al.,* 2003). Initially, BoHV-1 causes rapid virus multiplication in the upper respiratory tract, with peak virus titres occurring between three and six days later. The nasal swabs may consistently generate an infectious virus for ten days. The virus can be found in ocular swabs for up to eight days after it has been transmitted to the eyes. Clinical symptoms such as pyrexia (a temperature of 40 to 42°C), inappetence, dullness, and ocular and nasal discharges

appear two to three days after infection. The discharge begins off serious but by day five, mucoid and later mucopurulent discharge have developed along with nasal ulcers. Clinical symptoms can continue for up to 10 days, and given the mucosal damage the animal has had, there is typically a remarkable recovery after that (Smith, 2014).

Following the entry into epithelial cells, BoHV-1 initiates the lytic replication cycle, which corresponds to the successive expression of viral genes and causes both creations of new progeny viruses and cell death. The increase in cell ballooning and intranuclear inclusions are signs cytopathic effect (CPE) of the BoHV-1. Cell death occurs during the BoHV-1 replication cycle through the processes of necrosis and apoptosis (Muylkens *et al.,* 2007).

Secondary bacterial infections can affect how severe a disease is, and pneumonia and purulent rhinotracheitis can consequence in death. The respiratory tract is the only place where the virus can replicate, however, inside the animal, there can be neuroinvasion and perhaps undetectable low-level viremia (Smith, 2014).

2.8. Economic Importance of IBR

2.8.1. Diseases impact

BoHV-1 may result in production losses by causing respiratory (IBR) and venereal disease (IPV, IPB); reduced abortion and fertility (Graham, 2013) and a decrease in milk yield. There is a scarcity of data available to quantify the associated losses in the field. The yearly damages in the UK due to the disease and its treatment were predicted to be up to £3.1 million in 2005, with mortality/premature culling accounting for the greatest proportion of these, followed by weight loss (Bennett and Ijpelaar, 2005).

A larger research in Ireland projected a 250 L/year decrease in production for multiparous cows in herds that tested positive for antibodies in bulk milk tank samples (Sayers, 2017). Apart from decreased milk production, small effects on herd fertility and mortality were seen, adding to the increasing proof that asymptomatic BoHV-1 infection might result in continuous losses in dairy herds (Sayers, 2017). During a subclinical bovine herpesvirus 1 infection on a dairy farm, losses due to a decline in milk output were estimated to be roughly 9.5 L over a 14-day infectious period (Biswas *et al.,* 2013). Outbreaks in sperm collection

centers can be extremely costly, necessitating the euthanasia of all bulls in the facility (Raaperi *et al*., 2014).

Data from 133 herds in the Netherlands were modeled, and an average loss of 0.92 kg of milk per cow per day was found over a 9-week period following infection (OIE, 2017). Data modeling for a herd in the UK with a subclinical outbreak revealed a 2.6 kg/day loss in milk supply in seropositive cows compared to seronegative cows over a two-year period (Noordegraaf *et al*., 2000). The presence of IBR in the country can cause restriction of the international livestock trade (Biswas *et al.,* 2013).

2.8.2. Disease prevention and control measures impact

In general, credible economic analysis published data for disease are scarce (Bennett and Ijpelaar, 2005). In Duch to date, program expenses have been projected at Dfl 219 million (€99.5 million). Vaccination accounted for 62.5% of these costs, with the remainder owing to diagnosis (10.5%), monitoring (7.8%), and culling (19.2%). Additional costs for dealing with the remaining 5% seropositive animals included testing (€2.7 million) and culling (€25 million), with a 397-week payback period (Bennett and Ijpelaar, 2005).

2.9. The Morbidity and Mortality Rates of the Disease

Case morbidity varies according to a number of parameters, including the virulence of the BoHV-1 strain, the host's resistance factors/immune condition, and any concurrent bacterial infection (Muylkens *et al*., 2007). Infection outcomes can range from very low in subclinical pictures to high (up to 90%), particularly in naive populations, with morbidity and case mortality rates typically higher in neonatal and suckling calves than in adults (Patel, 2005; EFSA, 2006; Nandi *et al.,* 2009; Graham, 2013; Raaperi *et al*., 2014). According to modelling studies from the Netherlands, approximately 5% of infected cows are clinically affected (Noordegraaf *et al*., 2000). As sub clinical infections are becoming more prevalent (Muylkens *et al.,* 2007).

IBR infection can be associated with considerable mortality even though it may have a subclinical course, especially since the early 1970s, when BoHV-1.1 strains were introduced

to Europe. Early outbreaks in the UK and Ireland in 1989/1990 had case fatality rates as high as 8% (OIE, 2017). In California, a case-mortality rate of 3% was recorded for the initial description of IBR (Graham, 2013). The Netherlands' modeling studies assumed a mortality rate of roughly 2% among clinically affected animals (Noordegraaf *et al*., 2000).

The research conducted in Ireland looked at the probable variables linked with herd infection (positive for BoHV-1 bulk milk antibody detection) and found no link between infection and mortality across different age groups (calves, young stock, adults). However, in BoHV-1 ELISA positive herds, whole-herd mortality counts increased by a factor of 1.001 ($p = 0.023$) (Sayers, 2017).

2.10. Diagnosis

There are numerous direct (agent identification) and indirect (immune response) test methods for BoHV-1 that are listed in the WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2017). Kits are widely accessible on the market. Using paired serum samples, serological testing can be performed to diagnose acute infection and prove an individual is free of infection for international trade, and determine infection prevalence for seroepidemiological purposes, and support eradication programs and subsequent surveillance (EFSA, 2006).

Table 3: Test Methods for Diagnosis of BoHV1 and their purpose

Method	**Purpose**					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Conformati on of clinical cases	Prevalence of infection – surveillanc e	Immune status in individual animals or population post-vaccination
Identification of the agent 1						
virus Isolation	–	+	+	++	–	–
Rea-time PCR	–	+	+	+++	–	–
Detection of immune response						
ELISA	+++	+++	+++	++	+++	+++
VN	++	++	++	+	++	++

Key: +++ = recommended for this purpose; ++ = recommended but has limitation;

+ = suitable in very limited circumstance; - = not appropriate for this purpose.

PCR = polymerase chain reaction; ELISA = enzyme –linked immunosorbent assay;
VN=virus neutralisation

Source: (EFSA, 2006; OIE, 2017)

In the majority of labs, semiquantitative, fast, highly sensitive PCR has replaced fluorescent antibody tests and virus isolation in cell culture. Small levels of virus can be found in diagnostic samples like bovine semen and in other diagnostic sample using real-time PCR assays. ELISAs are used for determining antibodies, and some labs also offer serum neutralization (SN) testing. These tests have excellent specificity, and extensive research has focused on increasing their sensitivity to identify latent virus carriers with few detectable antibodies (Nettleton and Russell, 2007). Repeat testing is frequently necessary with ELISAs due to the range of uncertainty regarding the cut-off value (Saha *et al.,* 2010).

2.11. Differential Diagnosis

There are different respiratory disease reproductive diseases and respiratory diseases that need to be differentiated from IBR including Neopora caninum, *Hisophilus somni*, *Pasteurella multocida, Trueperella pyogenes*, Mannheimia species, Mannheimia species, and bovine viral diarrhea (Nettleton and Russell, 2007).

2.12. Treatment

There are no antiviral medications available to treat BoHV-1 infection. Antibiotics and supportive medications, on the other hand, can be administered to decrease subsequent bacterial infection (OIE, 2017).

2.13. Control and Preventions

2.13.1. Vaccination

There are a different live and inactivated vaccines available, including those with DIVA characteristics (depending on the gene for glycoprotein E being deleted), the use of which is critical in the approved control and eradication programs in place in a number of countries (Raaperi *et al*., 2014).

2.13.2. Biosecurity measures

It is generally accepted that biosecurity measures are successful at preventing the entry of pathogens. The risk factors for spreading of BoHV-1 are well-known (Raaperi *et al.,* 2014), and they fall broadly into the categories of animal trade (and mobility), persons, fomites, and semen, eggs, and embryos, as well as airborne spread (Muylkens *et al.,* 2007). There are solutions to deal with the routes of introduction. Quarantine can lower the danger associated with trade when used in conjunction with appropriate serological testing, especially if knowledge of the status of introduced animals and their source herds is added. It is possible to prevent or limit animal contact with members of other herds by taking precautions including enforcing a no-return policy, abstaining from shows (or implementing quarantine), and installing proper boundary fencing. Spread of aerosols can happen over incredibly tiny distances. According to Mars *et al.,* (2000), the R0 is said to drop below 1.0 at a distance of 4.4 m.

Limiting visitors and their interaction with the cattle, using suitable disinfecting techniques, and/or providing boots and gear that are specialized to farms can all help reduce risks related to fomites and persons. Bulls joining semen-collection facilities that have been certified for intracommunity commerce must adhere to quarantine and subsequent monitoring regulations, and semen and embryos imported from other nations must also follow these standards (Mars *et al*., 2000).

2.13.3. Restrictions on the movement of animals and products

The main measure of limitation concerns the mobility of carriers of latent infections. Application of serological screening makes this possible. Since the measures served as the foundation for the authorized movement controls that support control and eradication programs, they are thought to be effective. However, due to the occurrence of seronegative latent carriers and the insufficient sensitivity of diagnostic tests to detect antibodies to gE, quarantine and surveillance protocols might not always be totally effective (Raaperi *et al.*, 2014).

2.13.4. Test and slaughter

In areas of low BoHV-1 seroprevalence, culling of seropositive animals without vaccination (test and slaughter strategy) has been the most effective method for eradication. Additionally, it is advised to gradually remove all seropositive cattle from a normal breeding stock and replace them with seronegative offspring in order to develop an IBR-free breeding population. (Ackermann and Engels, 2006). Scandinavian countries (Denmark, Finland, Norway, and Sweden), as well as Austria, Switzerland, and some Italian regions, have effectively accomplished IBR eradication by employing this technique (Iscaro *et al.,* 2021).

3. MATERIAL AND METHODS

3.1. Study Area

The current study was carried out in the South Omo Zone, Southern Nations, Nationalities, and Peoples' Region (SNNPR) of Ethiopia. South Omo zone consists of 11 districts and is located between 4° 27’- 6° 26’ North and 34º 57’–37º 49’ East, Kenya is its southern neighbour, Southwest by South Sudan, on the North by Konta, on the West by South West Ethiopia region, and on the East by the Oromia region. The border is well known on the west by the Ilemi Triangle (claimed by Ethiopia, Kenya, and Sudan). In the South Omo Zone, Jinka Town serves as the administrative center (SOFEDD, 2022).

The South Omo zone has a total area coverage of 24,249 km2 and its altitude ranges from 380 to 3,300 m.a.s.l. (DAO, 2003). It has an arid and semi-arid climate with annual temperature that ranges from 19 - 42°C and an average annual rainfall of 1100mm which exhibits a bimodal rainfall (long and short rainy seasons). March through May is the long rainy season, whereas mid-September through mid-November is the short rainy season. (DAO, 2003).

The livelihood of these pastoralist communities is mainly the rearing of livestock and the use of their products and the agropastoralists are dependent on both livestock products and crop cultivation. The livestock production system is predominantly extensive, where animals are allowed to forage freely during the daytime and kept in open enclosures during the night. Even though different mingles at the village are used for each species, livestock shared grazing areas and watering points. In order to reduce labor demand, mobile herds are sometimes kept together with five or more village herds. Still, this practice makes it easier for the disease to spread from infected to susceptible herds (Addis *et al.,* 2013).

The Omo River, which empties into Lake Turkana on the western side and flows south, and the Omo National Park (Annex1), which is also situated in the lower Omo Valley, are the sources of this zone's name. On the eastern side of the Omo River, there is a place called Tama Wildlife Reserve and Mago National Park (Annex2). The eastern boundary of this area contains Lake Chew Bahir, which is bordered by Stephanie Wildlife Sanctuary. Mount Smith (2560 meters) and Mount Mago (2538 meters) are two prominent mountains. At least 12 distinct ethnic groups can be found there, and there could be as many as 21 different languages (SOFEDD, 2022).

According to the 2022 South Omo Zone Finance and Economy Development Department (SOFEDD, 2022), the zones has recorded a livestock population of 4,757,960 cattle, 6,265,051 goats, 2,484,370 sheep, 3010 camel, 2,005,632 poultry, 355,353 donkey, 52,179 horses, and 41,036 mules; the human population was 642,358.

The national park in the zones was mainly established to conserve the large numbers of plains’ animals in the area, namely the big four which are African buffalo, giraffe, lions and African elephant. Also recorded were greater-kudu, lesser-kudu, tiang, warthog, duiker, Lelwel’s hartebeests, Beisa oryx, wild dog, Grant’s gazelle, leopards, gureza, gerenuk, common baboon and varies species of primates (EWCA, 2021).

The study area is situated 739 Km (Benna-Tsemay), 780 km (Hamer), 956 km (Dacenech), and 860 km (Nyangatom) south of Ethiopia's capital Addis Ababa. (Figure 5). The district practice is predominantly pastoralism with little rain-fed and irrigated agriculture (CSA, 2018).

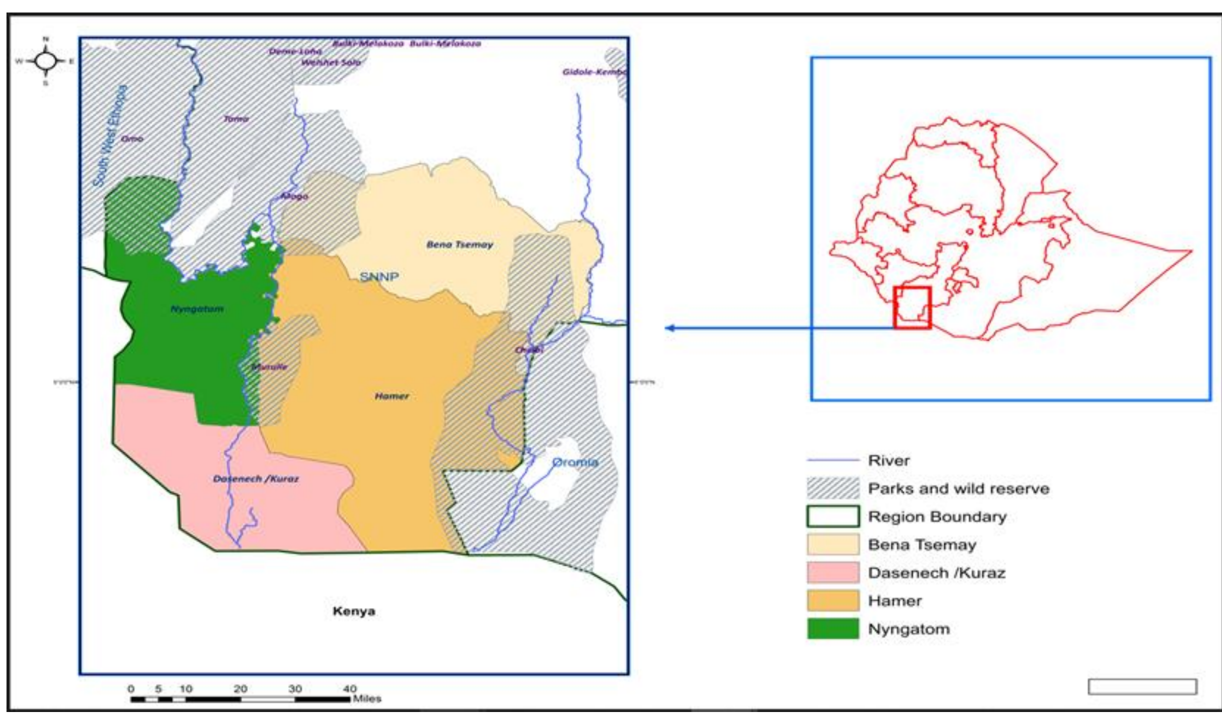

Figure 5: The location and key features of the study area and its surrounding regions

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3.2. Study Population

The current study was conducted in a local breed of cattle from four districts of the South Omo Zone. The study included both female and male animals older than six months. Vaccination history was not considered during sampling, as there has never been an IBR vaccination program in Ethiopia. Risk factors believed to be associated with the epidemiology of IBR were investigated in the study. These include species, age, sex, herd size, movement pattern, origin, and clinical condition of the animal. Herd of different sizes (small, medium and large) were visited.

3.3. Study Design

A cross-sectional study design was conducted from December 2022 to May 2023, with the aim to determine the seroprevalence of IBR and to identify associated risk factors in four selected districts of South Omo Zone. Additionally, nasal swaps were collected from animals with a history of reproductive, respiratory, and ocular problems for virus isolation. To assess the potential risk factor associated with the disease, a structured questionnaire was prepared (Annex 3), translated to local language and herd owners or keepers were interviewed during the fieldwork to capture herd and animal-level factors of IBR. The data collected included animal and herd level history of reproductive problems like abortion, stillbirth, retained placenta, and dystocia; respiratory problems, and ocular problems. Other variables such as age, sex, herd size animal origin (purchase, gift, rustling), contact history with parks and wildlife sanctuaries, and animal movements were also assessed for their association with the occurrence of IBR. The demographic information of volunteers was also recorded using a pretested structured questionnaire. Animal age was taken from the owner and during analysis age was classified according to Ayana *et al.* (2021) as Young (between 6 months and 2 years) and adult (greater than 2 years).

3.4. Sampling Method and Sample Size Determination

The South Omo zone was purposively selected based on the availability of susceptible cattle population, frequent reports of abortion and stillbirth, geographical location and proximity to the border, the possibility of contact with wild animals in nearby parks and sanctuary, no previous IBR study reports in the zone. Districts (Dasanech, Gnangatom, and Hammer) were also purposively selected considering their boarding with Kenya and South-Sudan.

However, peasant associations (PA) were randomly selected. Accordinglly, from Dasenech, Gnangatom, Hammer and Bennatsemay district, 2, 3, 3 and 4 PA were randomly selected from the lists of PAs in the selected district., Herds were randomly selected and finally, individual animal from the selected herd were also selected using simple random technique (Figure 6). Accordingly, about 94 herds; 19 large, 20 medium, and 55 small size were sampled in this study (Annex 4). Herd size is based on the South Omo Zone classification of herd size i.e. smallholder (< 20 Cattle), medium (20-50 cattle), and large (> 50 cattle), and 2, 5 and 10 cattle are sampled respectively (SOFEDD, 2022).

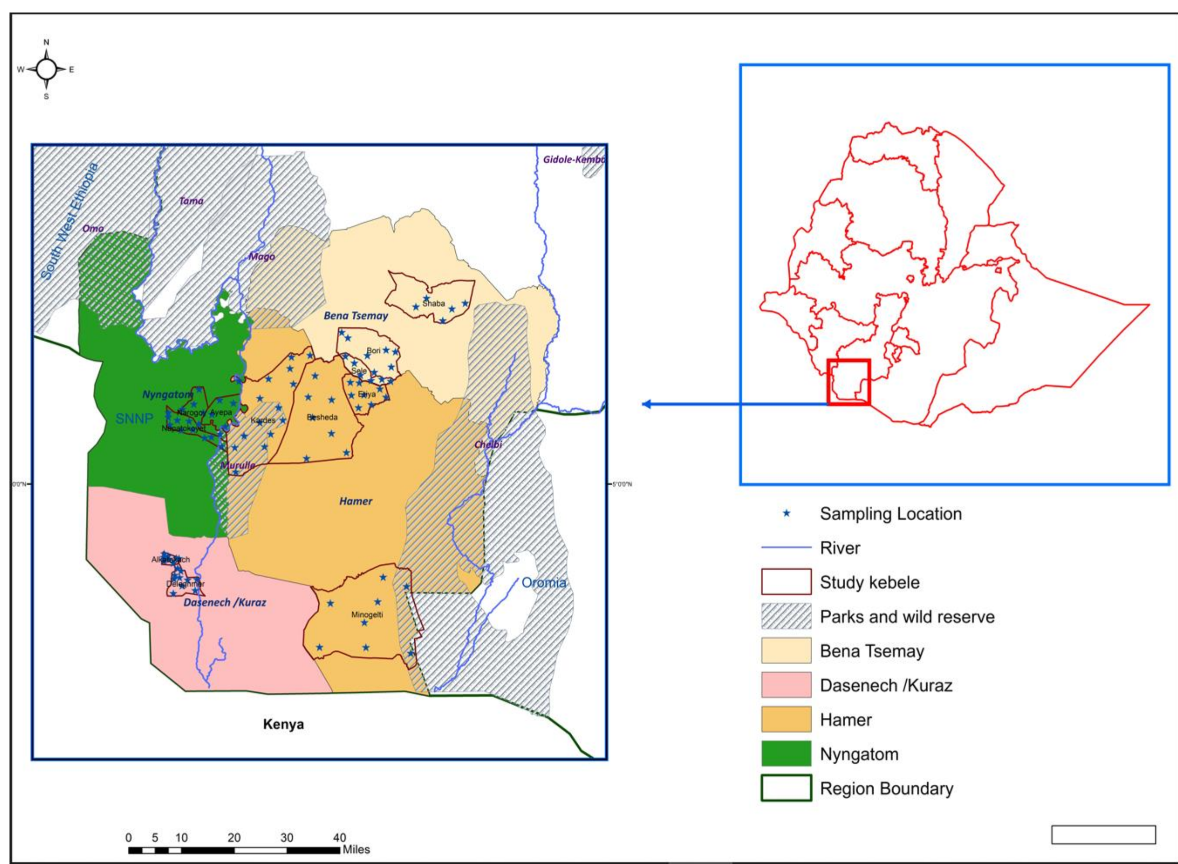

Figure 6: The location and key features of the study point and its surrounding regions

The sample size for this study was estimated using the formula given by Thrusfield (2008), and as a previous IBR study was not carried out in the study area, 50% expected IBR prevalence was considered to calculate the sample size with 5% absolute precision. Accordingly, the formula used to calculate the required sample size was as follows

$$n = \frac{(Z^2) \times Pexp\,(1-Pexp)}{d^2}$$

Where, n= the required Sample size; Z = 1.96; Pexp = the expected prevalence of IBR in the area which is 50%, and d = the desired absolute precision at a 95% confidence interval. Based on the above formula, 384 cattle were to be sampled, and a more or a total of 400 sera samples were collected.

3.5. Sample Collection, Handling, and Transportation

Four hundred blood whole blood were collected from the jugular vein of the randomly selected animals (Annex 5). During sampling, around 10 mL of blood sample was taken using a plain vacutainer tube in a sterile condition. Each vacutainer tube containing the sample was labelled using animal ID, age, sex, and sampling date by permanent marker but other variables for each sampled animal were filled in other worksheet formats. To separate sera from the blood, the tube containing blood was set tilted and left overnight for clotting at room temperature. The separated sera were then flowed into a cryovial tube to obtain clear serum. The cryovial tube containing serum was coded similarly to the vacutainer tube by a permanent marker and then the sera sample was chilled in an icebox containing an icepack and transported to Animal Health Institute (AHI) in a cold chain system and finally stored at –20ºC until laboratory analysis was resumed. 50 nasal swabs from animals with respiratory and reproductive problems were collected and Viral Transport Media (VTM) was added to each collected swabs and transported to AHI with a cold chain system and finally stored at –90ºC until the viral isolation process started (Annex 6).

3.6. Laboratory Testing

3.6.1. Serological test

At the AHI serology section, all collected sera samples were tested using Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) for the detection of BHV-1 antibody. The C-ELISA is a confirmatory test with a sensitivity of 93% and specificity of 99% (Annex 7).

During testing, all procedures were strictly followed based on the manufacturer's protocol (ID screen IBR gB Competition IDvet, 310, rue Louis Pasteur- Grabels- France). About 50 μl diluent was added to all wells and then 50 μl sera (test samples, negative and positive control) was added as per plate layout into the BoHV-1 antigen pre-coated plate. Samples and controls in the Seal plate were evaluated and incubated for 2 hour at 37ºC. The plates

were washed 3 times. Then 100 µl of the conjugate will be added to each well. The plate was carefully shaken to help the correct homogenization of the components mixed on each well. The plate was sealed and incubated for 30 min at +37ºC and Washed 3 times. Finally, 100 µl of the substrate solution was added, to each well. The plate was kept for 15 min at room temperature, in a dark place, and stopped by 100 µl /well of the stop solution. The Optical density (OD) of each well at 450 nm was read within the following 5 min after the addition of the stop solution. The data interpreted for the sample less than or equal to 45 % are considered positive, greater than and less than 55% are considered doubtful, and greater than or equal to 55% are considered negative (Annex 8).

3.6.2. Isolation of BHV-1

Sample preparation: From 50 swabs samples collected, 24 swab samples were selected from IBR strong seropositive cattle. Selected swab samples were vortexed and macerated well in the transport medium itself used for sample collection. The resulting suspensions were then transferred to a centrifuge tube and centrifuged at 3000rpm for 10 minutes using a refrigerated centrifuge. The supernatants were collected, filtered using 0.45um Millipore syringe filters, and ready to be inoculated into the suitable cell lines or stored at - 20 °c until inoculation.

Cell culture preparation: Madine-Darby Bovine Kidney Epithelial cells (MDBK, passage 84) obtained from Athens Veterinary Diagnostic Laboratory, University of Georgia, USA, were revived from liquid nitrogen and re-cultured in a $25cm^2$ tissue culture flask. The confluent flask was then sub-cultured to multiple $25cm^2$ TC flasks and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2% Glutamax and 1% Antibiotic-Antimycotic solution at 37ºC in a humidified incubator at 5% CO_2. According to the number of samples to be isolated, cells were subcultured to 24-wells tissue culture plates until they become 80-90% confluent.

Virus isolation: The isolation of BHV 1 was carried out as per the standard protocol given in the OIE manual (Adsorption method) (Annex 9). Sample suspensions prepared and stored at -20ºC were thawed and 0.1ml of the samples was inoculated into each well of subconfluent MDBK cell cultures in 24-wells TC-flasks. After 60min adsorption at 37ºC, the serum-free medium was added to each well including five uninoculated wells on each plate as a negative control, and incubated at 37ºC in a humidified incubator at 5% CO_2. Cells were monitored every 24 hrs post-infection and inspected for cytopathic effects (CPEs) using an inverted

microscope. The cultures were freeze-thawed and the resulting lysates were inoculated into fresh cultures until the third passage. The sample that did not show a cytopathic effect (CPE) by the third passage was discarded and treated as negative. Samples that produced CPE by the third passage were further passaged for virus propagation. The CPE comprised rounding of cells 48-72 hrs. post-infection (PI) with the characteristic "bunch of grapes" like aggregation developed by 72 hr, finally leading to the complete destruction of the cell sheet by 96-120 hrs. Each time the cell culture is freeze– thawed for 3times and clarified by centrifugation, and the supernatant is used for inoculation of fresh monolayers (Edwards *et al.,* 1990) (Annex 10).

3.7. Data Management and Analysis

Both laboratory test results and data collected through the questionnaires from the study area were first entered and stored in a Microsoft Excel spreadsheet program and coded for analysis. The descriptive and analytical statistic was utilized to analyze the data by STATA Version 17, 2022 software for Windows. Herd and individual animal-level data were organized categorically as described in Annex 11. Initially, the data were checked for accuracy, coded errors, and analysed using descriptive statistics. A Herd with at least one seropositive animal was considered positive. Accordingly, a herd-level prevalence was calculated by dividing the number of positive herds by the total number of herds in the study and multiplied by 100. Similarly, the prevalence of IBR was calculated by dividing the total positive animals by the total number of sampled and multiplying by 100. Chi-square (x^2) test was used for testing relationships between IBR occurrence with each demographic characteristic, and associated risk factors. Then, univariable logistic regression analyses were conducted to establish the association of the putative risk factors with occurrence of IBR at 95% confidence intervals (CI) was considered. All risk factors which showed significant association with IBR positivity ($P < 0.25$) at univariable analysis were considered for multivariable logistic regression analysis to determine the independent association between risk factors and the occurrence of IBR at 95% CI. Results were considered statistically significant when the P value is < 0.05 at a 95% confidence interval.

3.8. Ethical Consideration

Ethical clearance for this study was obtained from the College of Veterinary Medicine and Agriculture (CVMA), Addis Ababa University, minutes of animal research ethical review committee (minutes number and date of review: VM/ERC/02/15/022, 23/12/2022) with certificate reference number VM/ERC/11/02/15/2023 (Annex 12). Before starting the study, participants were made aware of its objectives, and the livestock owners provided their written signed consent for Questionnaire and photograph (Annex 13 and 14). To minimize stress, discomfort, and injury proper restrained techniques were used before sampling, blood collecting needles were used as it is recommended for cattle (16 gauge) and the most important thing is the engagement of the herd owner/keeper who has a close relationship with the animals to restrain them smoothly while taking blood and swab samples.

4. RESULTS

4.1. Seroprevalence of Infectious Bovine Rhinotracheitis in the Study Area

In the present study, a total of 400 sera samples were collected from 94 herds that were not vaccinated against IBR. Out of 400 sera samples tested using I-ELISA, 324 (81%, 95% CI: 76.8 - 84.5) were found to be positive for bovine herpes virus 1 antibody, at individual level. At the herd level, 83 (88.29%, 95% CI: 79.9 - 93.4) herds were IBR seropositive. The highest individual level seroprevalence (91.92 %; n=91) was recorded in Benna-tsemay district followed by Nyangatom district (82.18 %; n=83) and lowest prevalence was recorded in Dacenech (67.21 %; n= 41). The highest herd level seroprevalence was observed in Nyangatom (95%; n=19) followed by Benna-tsemay (94.4%; n=17), Dacenech (88.8%; n= 16) and Hamer (81%; n=31) (Table 4).

Table 4: Individual animal and herd level seroprevalence of IBR among the selected districts in the South Omo Zone using I-ELISA

District	Animal level				Herd level			
	No. sample	No +ve (%: 95 % CI)	$\chi2$	P-value	No. sample	No +ve (%: 95 % CI)	$\chi2$	P-value
Nyangatom	101	83(82.2:73.4-88.5)			20	19(95:70.1-99.4)		
Hamer	139	109(78.4:70.7-84.5)			38	31(81.57:65.6-91.1)		
Benna-Tsema	99	91(91.92:84.5-95.9)	15.9	0.001	18	17(94.4:67.5-99.3)	3.19	0.363
Dacenech	61	41(67.2:54.4-77.9)			18	16(88.8:63.3-97.4)		
Total	400	324(81:76.8-84.5)			94	83(88.29:79.9-93.4)		

CI: Confidence interval, $\chi2$: Chi-square, p-value: Probability value

Across the 12 pastoral villages visited in the four study districts, the individual animal-level seroprevalence ranged from 62.16 – 96.97 % with the highest prevalence recorded in Bori (96.97 %; n= 32) PA of Benna-Tsemay district followed by Beshada (93.94 % ; n = 31) PA of Hamer district, and the lowest seroprevalence was Delegnmer (62.16%;n= 23) PA of Dacenech district. The corresponding herd level prevalence of IBR ranges from 62.5 to 100%. Lowest herd seroprevalence at village level was observed in Eriya (62.5%; n=5) PA of Hamer district. However, majority of the herd's seroprevalence was found to be 100% (Figure 7).

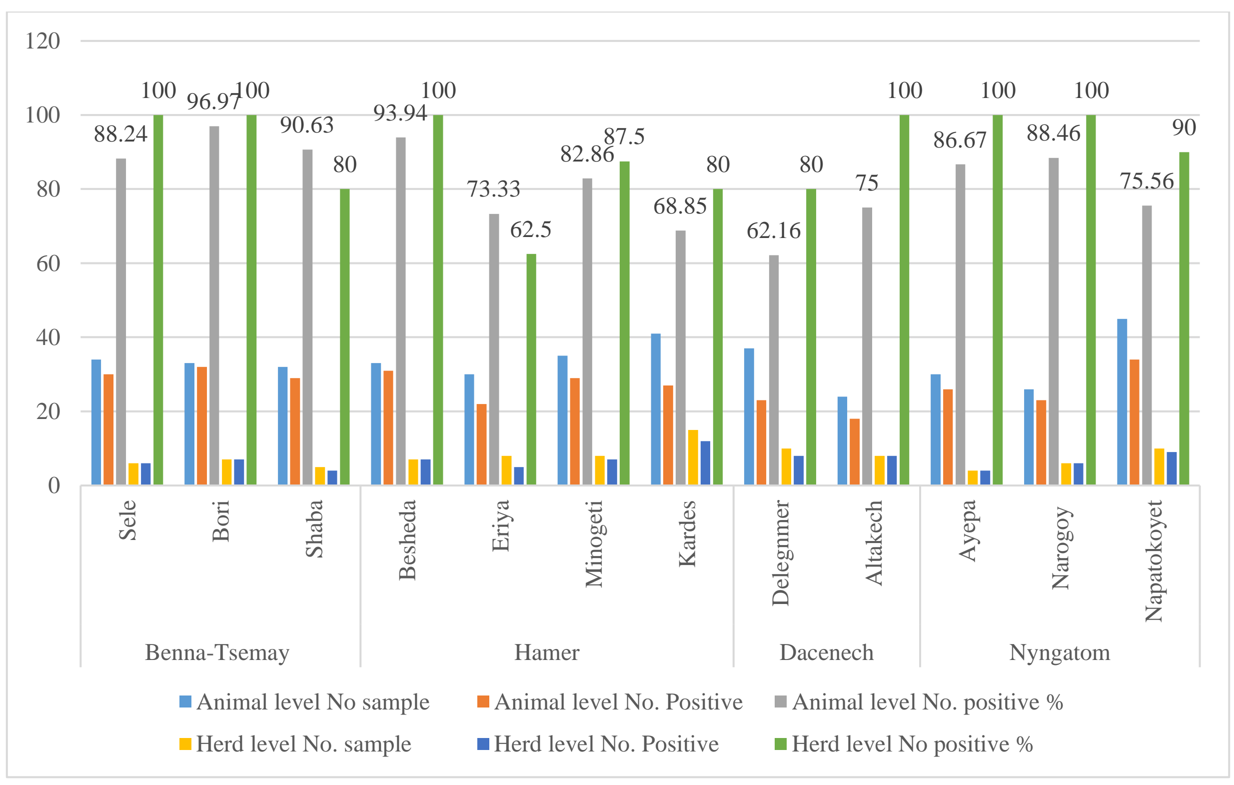

Figure 7: Animal and herd-level seroprevalence of IBR among villages in four districts of South Omo Zone

4.2. Socio-demographic Status of the Respondents

Out of 94 respondents interviewed, the principal herd respondents were comprised of men (61.7 %), while women as principal herd respondents in this population were fewer (38.3 %). Most of the principal respondents were mature working-age (35.1 %), and a few of them were of working age (11.75%). Regarding education status majority of the respondents had basic writing and reading (57.45%) and the least had joined secondary school 1 (1.06 %). The sociodemographic variables used in the current study were not significantly associated with the seroprevalence of IBR (P > 0.05) (Table 5).

Table 5: Socio-demographic status of the respondents among four districts in the South Omo Zone

Variables	Sampled herd n (%)	Seronegative n (%)	Seropositive n (%)	χ2	p-value
Gender					
Female	36 (38.3 %)	3 (8.33 %)	33(91.67 %)	0.6408	0.423
Male	58 (61.7 %)	8(13.79%)	50 (86.21 %)		
Age					
15 – 24 (Working Age)	11 (11.7 %)	1(9.09 %)	10 (90.9 %)	4.8976	0.179
25-54(Primary working Age)	22 (23.4 %)	2 (9.09 %)	20 (90.9 %)		
55- 64 (Mature working Age)	33 (35.1 %)	7 (21.21 %)	26 (78.79 %)		
65 and over (Elder)	28 (29.8 %)	1 (3.57 %)	27 (96.43 %)		
Education Status					
Illiterates	25 (26.6 %)	2 (8 %)	23 (92 %)	1.2521	0.741
Basic Write and Read	54 (57.45 %)	8 (14.81 %)	46 (85.19 %)		
Primary school	14 (14.89 %)	1 (7.14 %)	13 (92.86 %)		
Secondary School	1 (1.06 %)	0(0 %)	1(100 %)		

p-value: Probability value , χ2: Chi square

4.3. Risk Factors for IBR Prevalence in the Study Area

The univariable logistic regression analysis indicated that living in the Benna-Tsema district (COR = 5.5, 95% CI: 2.3 - 13.6, p = 0.000) and Nyangatom (COR = 2.24, 95% CI: 1.1 - 4.7, p = 0.031) , large herd size (COR = 24.3, 95%CI: 10.4 - 56.4, p =0.000), being female (COR = 2.1, 95% CI: 1.2 - 3.6, p =0.012) and adult in age (COR = 3.4, 95%CI: 2.0 - 5.7, p = 0.000) were significantly associated with the occurrence of IBR as shown in Table 6.

Table 6: Univariable logistic regression analysis of some selected risk factors with IBR seropositivity

Risk Factor	Level	No. Sample	No +ve (95%CI)	COR (95% CI)	p-value
District	Nyangatom	101	83 (82.2:73.4-88.5)	2.24 (1.1-4.7)	0.031
	Hamer	139	109(78.4:70.7-84.5)	1.7 (0.9-3.5)	0.094
	Benna-Tsema	99	91(91.9:84.5-95.9)	5.5 (2.3-13.6)	0.000
	Dacenech	61	41(67.21:54.4-77.9)	Ref	
Herd Size	Large	190	183(96.3:92.4-98.3)	24.3 (10.4 - 56.4)	0.000
	Medium	100	84(84:75.4-89.9)	4.8 (2.5 - 9.4)	0.000
	Smallholder	110	57(51.8:42.4-61)	Ref.	
Sex	Female	321	268(83.5:78.9-87.2)	2.1 (1.2 - 3.6)	0.012
	Male	79	56(70.8:59.8-79.8)	Ref	
Age	Adult	243	215(88.5:83.7-91.9)	3.4 (2 - 5.7)	0.000
	Young	157	109(69.4:61.7-76.2)	Ref	

COR: Crude Odds Ratio, CI: Confidence interval, p-value: Probability value, Ref: Reference

The proportion of cattle with reproductive disorders was higher in seropositive cattle as compared to seronegative ones (Figure 8) and reproductive and respiratory problems were statistically significant ($P < 0.05$) but respiratory problems were protective factor (COR = 0.54, 95%CI: 0.32 - 0.9, p =0.019) (Table 7).

Table 7: Univariable logistic regression analysis of risk factors for IBR seropositive based on clinical condition

Risk Factor	Level	No. sample	No. positive	$\chi 2$	P-value	COR 95% CI	P-Value
History of ocular problem	No	306	252(82.4:77.6-86.2)	1.54	0.213		
	Yes	94	72(77.60:66.9-85.4)				
History of respiratory problem	No	185	159(85.9:80.1-90.3)	5.47	0.019	0.54 (0.32-0.9)	0.019
	Yes	215	165(76.7:70.6-81.9)			Ref	
History of reproductive problem	No	234	179(76.5:70.6-81.5)	7.43	0.006	2.12 (1.23-3.67)	0.007
	Yes	166	145(87.5:81.3-91.6)				

COR: Crude Odds Ratio, CI: Confidence interval, p-value: Probability value, Ref: Reference, $\chi 2$: Chi square

Proportions of cattle with a history of reproductive problems were higher in seropositive cattle as compared to seronegative ones. However, cattle with a history of ocular and reproductive problems are lower in seropositive cattle as compared to seronegative ones in the study area, as illustrated in Figure 8.

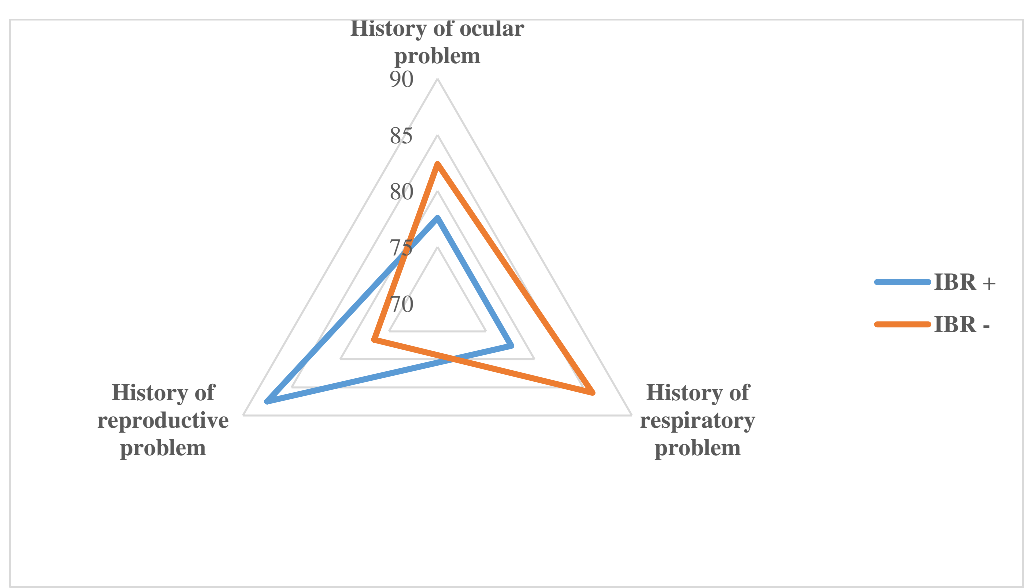

Figure 8: Relationship of IBR Sero-status with cattle's health status

The movement pattern of cattle in the study area was analysed after classifying the pattern into four categories. The percentage of seropositive cattle with movement categories of limited movement within districts, crossing parks and sanctuary, crossing regional boundary, and crossing national border were found to be 50 %, 94.62 %, 93.51 %, and 89.47 % respectively. Univariable logistic regression analysis revealed that all movement patterns and origin of animal being purchased from auction market/obtained as a gift, cattle rustled were significantly associated with IBR seropositivity ($p<0.05$). Univariable logistic regression result also revealed that crossing parks and sanctuary district (COR =9.9, 95% CI: 5.2 - 19.2, p = 0.000), crossing regional boundary (COR =10.7, 95% CI: 5.9 - 19.2, p = 0.000), and crossing national border (COR =3.3, 95%CI: 1.8 - 6.3, p=0.000), presence of contact with wild animal (COR=9.7, 95% CI: 5.5 - 17, p=0.000), and animals sourced from Purchase/gift/rusting (COR =10.5, 95% CI: 4.46 - 25, p=0.000), were statistically significantly associated with seropositivity of IBR. The probabilitility of getting IBR infection in purchased/gift/rustled cattle was 10.5 times more likely to be positive for the disease than home breed cattle (Table 8).

Table 8: Univariable logistic regression analysis on the basis of the movement pattern of cattle

Risk Factor	Level	No. sample	No. positive	COR (95% CI)	p-value
Limited movement in District	Yes	108	54 (50:40.6-59.4)	0.08 (0.45-0.14)	0.000
	No	292	270 (92.47:88.8-94.9)	Ref	
Cross parks and sanctuaries boundary	Yes	223	211 (94.62:90.7-96.9)	9.9 (5.2-19.2)	0.000
	No	177	113 (63.84:56.4-70.6)	Ref	
Cross-regional boundary	Yes	262	245 (93.51)	10.7 (5.9-19.5)	0.000
	No	138	79 (57.25)	Ref	
Across-national border	Yes	133	119 (89.47:82.9-93.6)	3.3 (1.8-6.3)	0.000
	No	267	205 (76.7:71.3-81.5)	Ref	
Contact with wild animals	Yes	276	225 (92.4:88.6-94.9)	9.7 (5.5-17)	0.000
	No	124	69 (55.6:46.7-64.2)	Ref	
Animal Origin	Purchased/gift/ cattle rustling	160	154 (96.3:91.8-98.3)	10.5(4.46-25)	0.000
	Home cattle	240	170 (70.8:64.7-76.3)	Ref	

COR: Crude Odds Ratio, CI: Confidence interval, p-value: Probability value, Ref: Reference

4.3.1 Association of reproductive disorders with IBR seropositivity

Some selected reproductive disorders like abortion, stillbirth, retained placenta, and dystocia were analysed using univariable logistic regression and a seroprevalence of 97.7 % (n=87), 89.6 % (n=29), 98.6 % (n=149), and 77.1 % (n=35) respectively were recorded. IBR seropositivity was statistically associated with history of abortion (COR=11.8, 95% CI=2.8 - 49.3, $p<0.05$) and retained placenta (COR=30.9, 95 % CI = 7.4 - 149.6, $p<0.05$) (Table 9).

Table 9: Univariable logistic regression analysis of some selected risk factors on the basis of reproductive problem

Risk Factor	Level	No. sample	No. positive	COR (95% CI)	p-value
History of Abortion	Yes	87	85(97.7:91.1-99.4)	11.8 (2.8-49.3)	0.000
	No	234	183(78.2:72.4-83)	Ref	
History of stillbirth	Yes	29	26(89.6:71-96)	0.52 – 6.1	0.35
	No	292	242(82.8:78-86)	Ref	
History of Retained Placenta	Yes	149	147(98.6:94.7-99.6)	30.9(7.4-149.6)	0.000
	No	172	121(70.3:63-76.7)	Ref	
History of Dystocia	Yes	35	27(77.1:60.2-88.3)	0.26 – 1.5	0.287
	No	286		Ref	

COR: Crude Odds Ratio, CI: Confidence interval, p-value: Probability value, Ref: Reference

The selected reproductive disorders (abortion, still birth, retain placenta and dystocia) were also analysed using a multivariable logistic regression. However, only retained placenta was observed statistically significant with an odds ratio of 30.7. That means IBR seropositive cattle had developed retained placenta 30.7 times higher than the negative once (Table 10).

Table 10: Association of selected reproductive disorders with IBR seropositivity using multivariable logistic regression

Risk Factor	Level	COR (95% CI)	p-value	AOR	95% CI	p-value
History of Abortion	Yes	11.8 (2.8-49.3)	0.000	1.01	0.15 - 6.5	0.987
	No	Ref		Ref		
History of stillbirth	Yes	0.52 – 6.1	0.35			\
	No	Ref				
History of Retained Placenta	Yes	30.9(7.4-149.6)	0.000	30.7	5.3 – 177.9	0.000
	No	Ref		Ref		
History of Dystocia	Yes	0.26 – 1.5	0.287			
	No	Ref				

AOR: Adjusted Odds Ratio, CI: Confidence interval, p-value: Probability value, Ref: Reference

4.3.2. Multivariable logistic regression analysis of risk factors associated with IBR

Risk factors which showed significant association with IBR seropositivity using univariable logistic regression analysis ($p < 0.05$) were subjected to a further analysis using multivariable logistic regression model. Accordingly, risk factors such as herd size, animal origin, movement across-national border, and contact with wild animals showed statistically significant association ($p < 0.05$) with IBR seropositivity. Animals sampled from medium and large herd sizes had significantly higher odds of being seropositive as compared to their counterparts from small herd size. Animals with limited movement were more likely at less risk of being IBR seropositive and was statistically significant (AOR = 0.034, 95 % CI: 0.006 – 0.2; P = 0.000). In addition, the odds of animals crossing national boundaries to be positive for IBR infection increased by 5.2 times more than animals that didn't cross. Contact with a wild animal was significantly associated with the seroprevalence of IBR. Cattle that were originally from purchased, gift, and cattle rustling had 10.6 times odds to have IBR antibodies compared to home cattle (Table 11).

Table 11: Multivariable logistic regression model for IBR sero-prevalence and associated risk factors

Risk Factor	Level	AOR	95% CI	p-value
District	Nyangatom	1.68	0.32 – 8.89	0.54
	Hamer	2.1	0.59 – 7.2	2.1
	Benna-Tsema	2.8	0.6 – 13.3	0.2
	Dacenech	Ref		
Sex	Female	0.95	0.34 – 2.6	0.93
	Male	Ref		
Age	Adult	0.38	0.13 – 1.13	0.08
	Young	Ref		
Herd Size	Large	14.7	4.9 - 44.5	0.000
	Medium	4.5	1.78 - 11.6	0.002
	Smallholder	Ref		
History of ocular problem	No	-		
	Yes			
History of respiratory	No	0.76	0.34 – 1.73	0.53
	Yes	Ref		
Limited movement in District	Yes	0.034	0.006 - 0.2	0.000
	No	Ref		
Cross-regional boundary	Yes	0.15	0.01 – 2.17	0.164
	No			
Cross parks and sanctuaries boundary	Yes	2.9	0.32 – 26.5	0.34
	No	Ref		
Cross-national boundary	Yes	5.2	1.65 - 16.9	0.005
	No	Ref		
Contact with a wild animal	Yes	3.99	1.58 - 10.06	0.003
	No	Ref		
Origin	Purchased/gift/cattle rustling	10.6	3.39 - 33.4	0.000
	Home breed	Ref		

AOR: Adjusted Odds Ratio, CI: Confidence interval, p-value: Probability value, Ref: Reference

4.4. Isolation of Bovine Herpes Virus 1 (BHV 1)

Out of 24 samples processed for BHV-1 isolation, 6 (25%) showed cytopathic effects like cell rounding, swelling, detachment, and floating on the MDBK cell line (Table 12) and (Figure 8).

Table 12: Swabs that show cytopathic effects on MDBK cell line

District	Kebele	No sample	Test employed % of CPE Positive Viruses in on MDBK cell line with respect to district
Nyngatom	Ayepa	2	-
	Narogoy	2	1 (50 %)
	Napatokoyet	2	-
Dacenech	Delegnmer	2	-
	Altakech	2	-
Hamer	Besheda	2	1 (50 %)
	Eriya	2	1(50 %)
	Minogeti	2	-
	Kardes	2	-
Benatsemay	Sele	2	2 (100 %)
	Bori	2	-
	Shaba	2	1(50 %)
	Sele	2	-
Total		24	6 (25%)

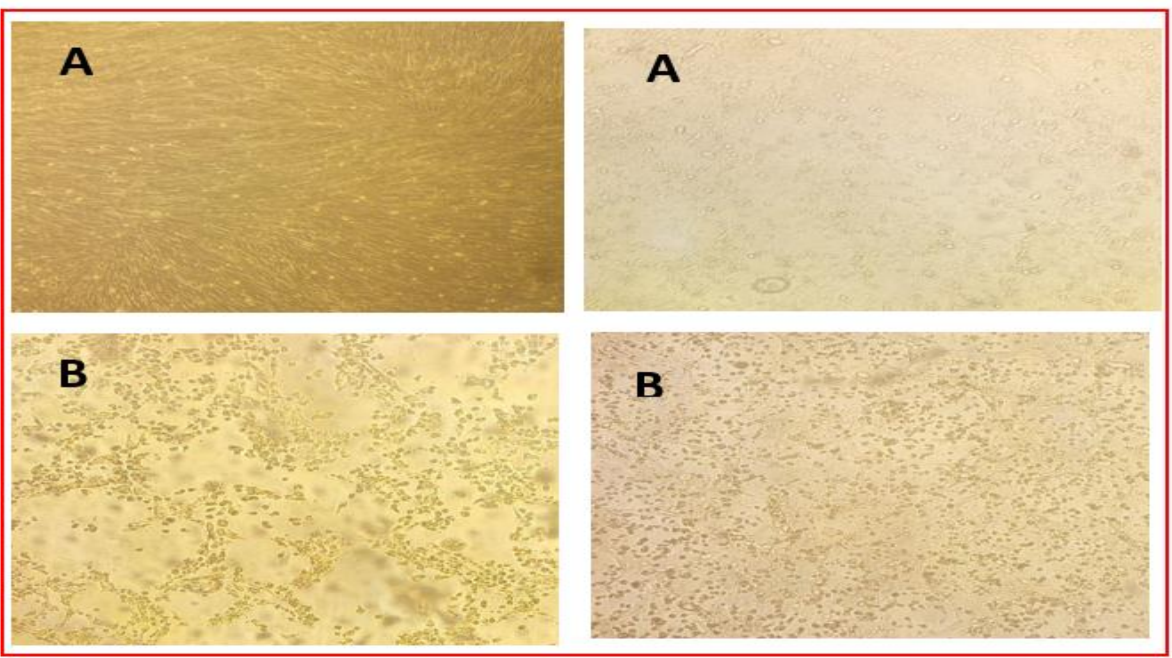

Figure 9: Control and MDBK cells with cytopathic effects on cell line

A) Control and non-inoculated MDBK cells

B) MDBK cells showing CPE characterized by cell rounding, aggregation, and detachment of cells.

5. DISCUSSION

IBR is a globally distributed disease with significant geographical variation in prevalence. In Ethiopia, a few serological surveys have been conducted and they are limited to highland areas and urban dairy farms and information on seroepidemiological study of IBR in pastoral and agropastoral regions in Ethiopia is lacking. This study revealed the first seroepidemiological study of IBR and isolation of Bovine Herpes Virus 1 (BoHV-1) from cattle population reared in free grazing areas in selected districts of South Omo zone.

The result of the present study showed the overall seroprevalence of 81% (95% CI: 76.8 - 84.5) in South Omo, Ethiopia. The seroprevalence ranges from 67.21 % in Dacenech to 91.92 in Benna-Tsemay which is higher than the previous findings reported in Ethiopia by Bekele *et al*. (1989) in Gobe and Ghibe (67 %), Sibhat *et al.*(2018) in Central, Southern and South-western part (41 %), Tesfaye *et al.*(2021) in selected district in Oromia and Amhara (34.7%), Wedajo *et al.* (2021) in Kombolcha and Desie (25.6 %), and Zewde *et al.* (2021) in North western Ethiopia (77.6%). The higher prevalence in the current findings might be due to the movement of cattle within and across the national border, contact with wild animals, type of breeding method, differences in herd sizes variation in management and/or agro-ecology of the study area.

Similarly, the current finding was higher than other international findings conducted by, Jha (2005) in Nepal (50.8 %), Koppad *et al.* (2007) in Karnataka (21%), Yaa *et al.* (2008) in China (36 %), Raaperi *et al.*(2010) in Estonia (22 %), William and Winden (2014) in UK (69.2 %), Elhassan *et al.* (2015) in Sudan (8.75 %) and Kipyepo *et al.* (2020) in Kenya (17.4%).

On the contrary, higher than the current finding was reported by Hussein *et al.* (2019) in Egypt (93.75 %) from cattle imported from Sudan, Giraldo *et al.* (2013) in Caquetá Colombia (90 %) and by Dias *et al.* (2013) in Spain (99.92%). These variations may be attributed to the reactivation of latent infection as a result of the stress during long transportation of animals, where the animal with reactivated virus can shed the virus without clinical disease and infect other contact animals (Lazic *et al.* 2003), great majority of animals that are seropositive to BoHV-1 considered latently infected one and can shed the virus when reactivation occur in response to any stressors (Nuotio *et al.* 2007).

Regarding the overall herd level seroprevalence, it was recorded to be 88.29 % which ranges from 62.5 % to 100 % across the villages in the study area. This was slightly higher compared to the studies in other parts of Ethiopia conducted by Sibhat *et al.* (2018) in central, southern, and south-western part (81.8 %) and Tesfaye *et al.(*2021) in selected districts of Oromia and Amhara regions (75.5%). The difference in the prevalence recorded in the different study areas may be associated with the variances in climate change, management and/or geographical variables (Kampa *et al.* 2004), type of breeding methods, differences in herd sizes (Ackermann and Engels, 2006) , animal density (Raaperi *et al.*, 2010), frequent infected animals' introduction to the herds (Abad *et al.,* 2016).

The results of the univariate analysis model of this study revealed that a number of risk factors were associated with IBR seropositivity. Those risk factors include district, herd size, sex and age of the cattle; history of abortion, retained placenta movement pattern of the herd, contact with wild animals and animal origin.

In the current study, the seroprevalence was found to vary among the studied districts. The Benna-Tsemay district had significantly higher seroprevalence (91.92 %) as compared to Nyangatom (82.12 %), Hamer (78.42) and Dacenech (67.21 %). The reason behind this may be higher contact pattern of domestic animals with wild animals, a high density of cattle in the grazing area of Benna-Tsemay district rangeland due to adjacent districts affected with drought may lead to the viral spread and increase the chances of IBR infection in susceptible animals. In line with this Abdelfattah (2022) who discovered that small ruminants reared on the same farm were linked to BHV-1 infection in cattle, and also similar study conducted by Handel *et al*.(2011) showed that IBR can interact with four different herpesviruses found in other animals, including goats and buffaloes.

Benna -Tsemay and Hamer district borders directly with Mago National Park, and while Nyangatom border with Omo National Park. In these districts, there is frequent interaction between domestic and wild animals at watering and grazing points, which could facilitate the disease spill over from wildlife to domestic cattle and therefore it could contribute to the highest seroprevalence of IBR observed in this districts. The current result was in agreement with reports of European countries in the Netherlands, where red deer can come into contact with cattle and potentially can become infected with BoHV-1 (Mollema *et al.*, 2005). This might in agreement with an explanation given in many studies by Kálmán and Egyed (2005); Thiry *et al.* (2006); Intisar *et al*.(2009); Scicluna *et al.*(2010) who reported that the virus

infects mostly cattle but also many other species of domestic and wild artiodactyls including deer.

The lowest seroprevalence observed in Dacenech district might be due to the absence of contact pattern of cattle with wild animals, a lower number of sampled animals, and smaller herd sizes having more less sedentary as a result of private and public development projects in the area which could have played in restricting livestock mobility in the district.

Among the villages visited in the four study districts, lower individual animal-level seroprevalence was observed in Kardes village (68.85 %) in Hamer district and in Delgnmer village (62.16 %) in Dacenech district. This low prevalence might be due to the ethnic conflict of these villages with their neighbouring district that could have resulted in restriction of animal movement. A lowest herd-level seroprevalence (62.5%) was also obtained in Eriya village and this might be due to smaller herd size groups being sampled from the village.

With regard to sex, higher seroprevalence of IBR was recorded in females (83.49 %; n = 321) than in males (70.89 %; n = 79). This was in line with the finding of Derrar *et al.* (2019), Thakur *et al.* (2017), and Jain *et al.* (2006) who reported a higher prevalence in females than males. This higher prevalence might be due to stress factors related to female animals such as pregnancy, calving, start of lactation or due to frequent exposure to infection during breeding and it could also be because of sampling of more females than males (Selim and Abdelhady, 2020, Benaissa *et al.*, 2021). In line with this, Bosco *et al.* (2011) and Gould *et al.* (2013) stated that natural service by infected bulls increase risk of IBR to females. Similarly, a significant association between sex and IBR seropositivity was observed in this study, which agrees with the findings of Thakur *et al.* (2017), and Muñoz *et al*. (2020) but did not agree with the findings of Derrar *et al.* (2019) who reported the absence of a relationship between the disease and sex, thereby indicating that virus presentation is independent of the sex of the cattle.

The prevalence of IBR significantly varied depending on the age of the animals, with adult cattle having the highest prevalence (88.5%) than the young ones (69.4%). The odds of IBR occurrence in adult animals were 3.4 times greater than the occurrence in young animals. These findings were in agreement with previous works done by Saravanajayam *et al.* (2015), Segura-Correa *et al.* (2016), Adeli *et al.* (2017), and Kipyego *et al.* (2020). As infected cattle remain infected for life, it is not surprising that higher seropositivity was observed in aged

cattle. Contrary to this finding, O'Grady *et al.* (2008) found no differences in age categories in a study on introducing beef bulls into a performance testing station in Ireland, and similarly, Brock *et al.,* (2020) reported that outbreaks associated with IBR in dairy cattle herds can occur more at older ages.

The statistically significant association between IBR seropositivity and history of reproductive problem observed in this study was in agreement with the report of Wedajo *et al.* (2021) who reported a strong association between IBR seropositivity and history of abortion (100 %) and retained placenta (59.8 %). Sibhat *et al.* (2018) also reported that 79.69% of animals with history of abortion were seropositive for IBR. Moreover, the current finding revealed that cattle with respiratory problems were about 0.54 times less at risk than cattle that did not have respiratory problems. This might be due to the presence of different transboundary animal diseases that cause respiratory problems due to multiple stressor factors during animal movement and introduction of cattle which result in an overall immunosuppression that allows the respiratory tract to be invaded by numerous opportunistic pathogens which inhibits the multiplication and development of the BHV 1 into infection. In contrary to these findings, Zewde *et al.* (2021) obtained that cattle showed respiratory problem had 2.2 times more likely to be positive for antibody against BoHV-1 than cattle's which did not manifest the sign.

The present study also showed that the movement pattern of cattle was significantly associated with the seropositivity of IBR. The highest seroprevalences were found in animals crossing parks and sanctuaries (94.62%) and crossing regional boundaries (93.51%). This might be due to the frequent contact of cattle with wild animals which could increase disease jumping into domestic livestock. The highest prevalence related to animal movement in the current study is in agreement with the finding of Gay and Barnouin (2009) who reported that increased animal movement into a herd and close proximity to neighbouring farms increased the risk of IBR spread through contact between naïve herd and infected animals. The long-distance movement of animals in search of pasture and water during the drought season could reactivate the virus from latency. This could also be aggravated by changes in herds' composition as a result of introduction of new animals, calving and coexisting diseases. (Muylkens *et al.*, 2007; Clinton, 2019).

The results of the multivariate logistic regression analysis of this study revealed that some selected risk factors such as herd size, limited movement within district, movement across

national border, contact with a wild animals, retained placenta, and purchased or gifted cattle were found statistically associated with seropostivity of IBR.

In this study, larger and medium herd sizes were observed to be more seropositive compared to smaller herd size, and found statistically significant with IBR seropositivity which is consistent with a study done by Raaperi *et al*. (2010) in Estonian dairy herds, Martinez-Ibeas *et al.* (2015) in Irish, Williams and Van Winde (2014) in the United Kingdom, Segura-Correa *et al.* (2016) in Mexico, and Callaby *et al.* (2016) in Kenya. This higher IBR prevalence recorded in larger herd sizes of cattle could be associated with a high level of contact between individual animals within a herd. In addition, reactivation of latent infections is also more likely to occur in larger herds due to the added stress having more in populated animals (Singh and Sinha 2006; Raaperi *et al.*, 2014; Sayers, 2017). However, these findings were not in agreement with O'Grady *et al.* (2008) who reported herd size in Irish beef herds did not find a significant association with IBR seropositivity.

The present study also revealed that contact with wild animals was found to be associated with IBR seropositivity. This showed wild animals in the two national parks (Mago and Omo), as well as the wild animal sanctuary (Stephanie Wildlife Sanctuary) could maintain and transmit the IBR virus circulating in the area. Similar study conducted by Mollema *et al.* (2005) in Netherlands revealed that red deer may come into contact with cattle and potentially can become infected with BoHV-1. Similarly, other finding reported by Boelaert *et al.* (2000) was also consistent with the current findings who reported that viruses antigenically related to BHV-1 have been isolated from a number of ruminant species, including red deer, reindeer, mule deer, pronghorn antelope, and wildebeest. Buffalo and wildlife may play an important role in the maintenance of the infection. In addition, studies conducted by Thiry *et al.* (2006); Intisar *et al.* (2009); Scicluna *et al.* (2010) revealed that the virus that mostly affects cattle can also affect other species of domestic and wild animals including deer and this wild animal may play a crucial role in the epidemiology and maintenance of BHV-1 in the environment.

In this study, IBR seropositivity was also found significantly associated with animal origin. Introduction of new cattle into a herd by purchase, gift, and cattle rustling was observed a risk factor for IBR. Several studies reported that replacement of cattle in farms or any other livestock production systems through purchasing from auction market without getting the new animal tested could increase IBR seropositivity (Boelaert *et al.*, 2005; Williams and

Van Winden, 2014; Martinez-Ibeas *et al.*, 2015). However, Van Schaik *et al.* (2001), Solis-Calderon *et al.* (2003), Segura-Correa *et al.* (2016), did not find an association between the introduction of new cattle and BoHV-1 infection. In the current study area, the introduction of new animals to the herd is highly related to cattle rustling and gift in marriage ceremonies. Cattle rustling is one cause of death, spread of disease, and insecurity among the pastoral communities occupying the rangelands. Such experience in the study area was in line with Mganda (2013) who stated that traditionally cattle rustling/raiding for the restocking of animals lost through drought or disease which often results in violence is sanctioned and controlled by elders. For pastoralists, it was used as a coping mechanism to restock their herds after natural disasters such as drought and diseases or to address shortfalls during traditional and/or marriage ceremonies. In line with this, during rustling when infected cattle are introduced into such herds, reactivation of the latent infection may occur due to stress and this might cause higher IBR seropositivity.

Highlighting the role and significance of cattle rustling in the transmission of IBR was one of the focus of this study. However, apart from the disease aspect, previous studies done by Hendrickson *et al.* (2009) revealed that livestock rustling indisputably has led to persistent violence's in pastoral areas in Africa with detrimental social and economic effects. A majority of studies have emphasized the social and economic implications of cattle rustling while underestimating the disease aspects.

In this study, isolation of BHV-1 was conducted on MDBK cell line and 25% (6 of 24) showed cytopathic effects. The cultivation of BHV-1 in MDBK cell line is a very useful method for further processing of the virus for various other techniques like PCR, ELISA, or viral purification. In this study finding of CPE of BHV-1 for the first time from cattle with history of respiratory and reproductive problem looking cattle in pastoral setting. The finding of CPE of BHV-1 from the study site would help to better understand the epidemiology of IBR and design feasible control strategies.

This study has the limitation of having a cross-sectional design, which makes it difficult to confirm the temporality of the risk factors for IBR. Furthermore, because a BHV-1-specific antibody ELISA testing kit had been used in the study, it is impossible to determine whether an animal's test-positive result is due to virus retention or adaptive immunity from a previous infection. Security problems related to traditional ethnic conflict within and porous international borders restricted the number of districts surveyed. As the study was carried

out during the drought season in the South Omo Zone and surrounding zones, some pastoralists refused to allow their herds to be sampled contending that requested blood samples from their animals might hamper productivity. In addition, majority of respondents are men, however, in the zones, females typically have a connection with animals, which makes it challenging for females to complete the questionnaires in the presence of their husbands culturally. In line with this, it is also challenging to complete the questionnaire because the majority of respondents do not attend basic school. Furthermore, more than 14 various languages are spoken across the study's four districts, which makes it a greater challenge.

6. CONCLUSION AND RECOMMENDATIONS

The current serological findings revealed higher individual animal and herd level seroprevalence of IBR. The BHV-1 on MDBK cell line and showed cytopathic effects and high serological evidence during this study confirms that the virus is circulating in the study area. This study also provided important insight into the shortage of information on risk factors associated with IBR occurrences in cattle in pastoral and agro-pastoral areas of Ethiopia. But this cannot lead to draw the conclusion that IBR seropositivity can be linked only to the risk factors identified in this study. Factors such as herd size, movement across a national border, contact with wild animals, retained placenta and purchased, gifted or cattle rustling were found statistically associated with IBR seropositivity in multivariable analysis. However, abortion, dystocia, still birth, history of respiratory and ocular problem, sex, age, crossing Park and sanctuary boundary, were not found statistically significant. In line with the current high seroprevalence finding, the latent and subclinical infections might have been undervalued. Since the diseases has the ocular, respiratory, and reproductive forms and thus may lead into significant socio-economic impact in the pastoral and agro-pastoral part of the country. Therefore, based on the findings, the following key recommendations are forwarded:

- Large-scale studies, establishment of surveillance, and the development of effective control measures to prevent the spread of IBR infection is of paramount importance.
- There should be sensitization and trainings on the epidemiology and risk factors of IBR to professionals, community-based animal health workers and the community to reduce the economic loss of the disease
- Strengthen cooperation and collaboration for cross-border animal health and sanitary measures between the bordering countries; based on the signed Memorandum of understanding to prevent disease like IBR
- To minimize IBR transmissions across the border, works should be done to reduce ethnic conflicts and stop cattle rusting through supporting civil society actors to promote change in the values that encourage cattle rustling
- BHV1 isolation and detection studies with greater geographical coverage and sample size need to be conducted, and the current cytopathic effect growth on MDBK cell line has to be exposed to other confirmatory tests such as ELISA antigen detection and PCR tests

- The role of Wildlife in Ethiopia on the maintenance and transmission of IBR need to studied for appropriate prevention and control strategies of the diseases
- Further studies in both production systems need to be conducted to determine the magnitude of the disease in the country and to identify the circulating genotype of BoHV-1 which help design appropriate prevention and control strategies

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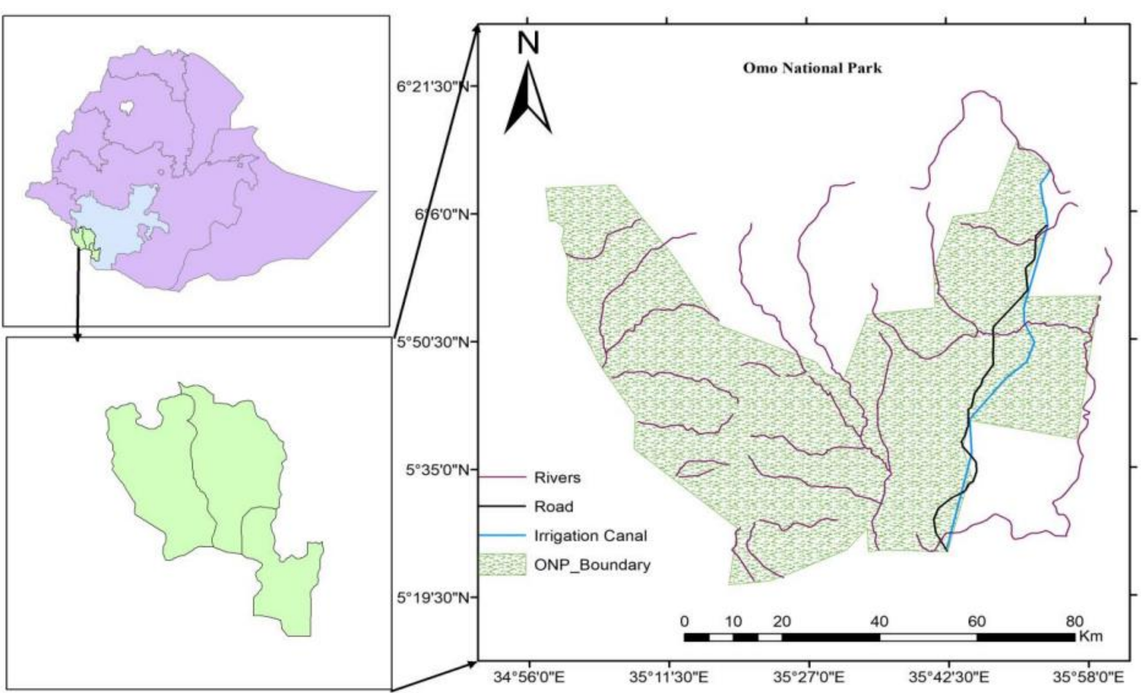
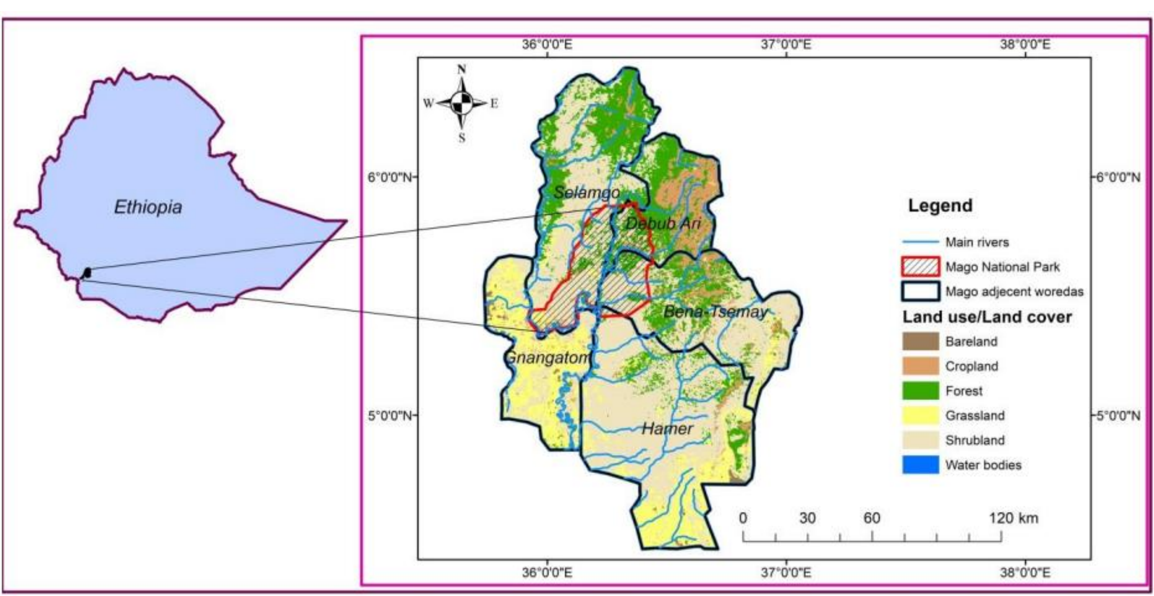
8. ANNEXES

Annex 1: The location and key features of the Omo national park and its surrounding regions



Annex 2: The location and key features of the Mago national park and its surrounding regions



Annex 3: Cross-sectional Questionnaires Survey

A. General Questions

Sampling Date_________

1. Region ________Zone ________District ________PA ________PA (specific name) _______Herd No. _______Coordinate X (E) ________Y (N) _______
2. Name of the owner __________Age________ Phone No.__________

   2.1 Educational status: A) Illiterate B) Basic writing & reading C) Primary School (Grades 1 to 8) E) Secondary school and above
3. Livestock population in the district

   A) Cattle ______B) Sheep ______C) Goat _____D) Equine ________Camel_______
4. Who is responsible for managing cattle in the household?

   A) Household Head B) Spouse of Household Head C) Son/Daughter D) Sibling E. Nephew/niece F. Grandson/daughter G. Herdsman/woman H) Other(Specify above)

B. Questions related to risk factors for IBR in the herd

5. Have you ever seen respiratory problems in your cattle/herd? A) Yes B) No
6. If "yes", list the name of the diseases that cause such problem (in the local name) and rank them in order of severity

   A) __________B) _________ C) ________ D) _________ E) ________
7. Have you ever seen ocular signs in your cattle/herd? A) Yes B) No
8. . If "yes", list the name of the diseases that cause such problem (use local name) and rank them in order of severity.\

   A) ________B) _________ C) __________ D) _________ E) ________
9. Have you ever seen reproductive problems in your herd/cattle? A) Yes B) No
10. If "yes" list the name of the diseases that cause such problems (use local name) and rank them in order of severity

    A) _________ B) _________ C) __________ D) ___________ E) _________
11. What type of breeding services do you use when animals are on heat? A. AI B. Bull C) Both
12. If you buy a new animal, do you examine the animal? A. Yes B. No C. If yes, how?
13. Do you know of any disease that causes abortions/stillbirth/retained placenta/ dystocia?

    A) Yes B) No
14. If "yes" list the name of the diseases (use local name)

A) _________ B) _________ C) __________ D) _________ E) ________

15. Have you observed abortion/stillbirth/ retained placenta/ dystocia in your herd? A. Yes B) No
16. How many abortions/stillbirths/retained placenta/dystocia have you encountered during the last five years?

    A. No. of abortion ________B. No. of stillbirth ________C. No. of retained placenta___________ D) No. Dystocia _______
17. At what stage of pregnancy do you face abortion? A. 1st trimester B. 2nd trimester C. 3rd trimester.
18. In which stage of parity is abortion observed?
19. What do you do with your animals that have encountered abortions/stillbirth/retain placenta?

    A. Home/Ceremony slaughter B. Sell to another farmer C. Give as a gift D. Other (specify)
20. Do your animals share pasture and watering points with wild animals? A. Yes B. No
21. If yes, which wild animals (Please list wild animals w/h share grazing and watering points with domestic animals
22. Have you ever found evidence of wildlife abortion in your grazing areas? A) Yes, B) No. If yes, which wild animals?
23. Which country does this district border?
24. Is there any movement of animals across the border? ________Yes/No. If yes, from where to Where?
25. What is the name of the tribe at the border where it neighbors with this district?

C. Specific information

IBR Blood sample collection format:

1. Region ____Zone______ District _______PA ____Longitude_______ Latitude______
2. Animal ID______ Species ________Breed________ Herd ID_______ Herd Size_______
3. Sex________ Age ________
4. Clinical condition Yes______ No______
5. History abortion Yes______ No________
6. History of stillbirth Yes______ No _______

7. History of retained placenta Yes______ No_______

8. History of ocular problem Yes______ No_______

9. History of respiratory problem Yes_____ No ______

10. Contact with wild animal Yes _______ No_______

11. Limited movement in the district Yes_______ No ______

12. Cross district border Yes_______ No_______

13. Cross Park & Sanctuaries Yes_______ No_______

14. Cross regional boundary Yes_______ No________

15. Cross-national border Yes_______ No ______

16. Origin Home cattle _______ Purchased, gift or cattle rustling __________

Annex 4: Pictures of different herds

Annex 5: Procedures of blood and nasal swaps collection

A. Blood Sampling Procedure

- Restrain animal with head elevated and jugular vein exposed
- Swipe with antiseptic gauze to remove superficial dirt and debris. This may also assist in visualizing raised vein.
- Occlude jugular vein by applying pressure at the base of the jugular groove and visualized the raised vein.
- With bevel up, insert needle firmly into skin and into vein 20 0 angle
- If using vacutainer, once needle inserted, stabilize needle and push the vacutainer tube into hub. If you have hit the vein, blood will be flow freely into tube (Note: Don't pull needle out of vein with vacutainer tube still attached as this will release vacuum in vacutainer).
- If you have missed the vein, carefully reposition needle, with vacutainer attached, until vessel penetrated. Vessel is fairly deep and may roll away from needle. Typically no more than two to three attempts should be made at a time to minimize distress to the animal and potential damage to the vein.
- Once collection complete, remove vacutainer tube, and then, applying pressure over injection site, remove needle.
- Dispose of needle in approved sharps container.
- In order to ensure adequate haemostasis, apply pressure with gauze for 30 to 60 seconds.

Nasal swap Procedure

Materials needed for the collection of samples

- Leak-proof sterile tube swab with 0.5 ml
- Viral transport media
- Scissors and marker pen
- Lab submission form

Collection procedure

Restrain the animal’s head as animal head movement may cause the swab to break off in the nose or pharynx

1. Use a fresh, disposable cloth to clean the exterior nares.
2. Estimate the distance between the nostril and the eye's medial canthus.
3. Pull in the swab approximately 3-5cm.
4. Push the inner swab sheath approximately 3-5cm through the end of the outer clear tube.
5. Vigorously rotate the swab against the pharyngeal mucosa for 30-45 seconds.
6. Retract the swab back into the swab sheath.
7. Remove the entire sterile swab from the animal’s nose.
8. Using clean scissors, cut the tip end of the swab roughly 15cm from the tip.
9. Do not cut the tip too short, as they can be difficult to remove from the transport tube.
10. Label the transport tube with the animal’s identification and data. Place the swab in the sterile transport medium tube (VTM)
11. Pack properly and refrigerator the sample until shipment
12. Transport to AHI
13. Submit to viral isolation lab
14. Proceed culture procedure as per protocol

Annex 6: Pictures during blood collection

Annex 7: ELISA test procedures

General Information

The diagnostic kit is designed to detect antibodies against the gB glycoprotein of the BHV-1 virus by competitive ELISA. It used with serum.

Description and principles

Microwells are coated with purified BHV-1 lysat. Samples to be tested and controls are added to the wells. Anti-gB antibodies. If present, forms an antibody antigen complex which masks the gB epitopes. After elimination of the sera by washing an anti-gB horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the remaining free gB epitopes forming an antigen-conjugate-HRP complex. After elimination of the excess conjugates by washing, the substrate solution (TMB) is added. The resulting coloration depends on the quality of specific antibodies present in the specimen to be tested.

- In the absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.
- In presence of antibodies no coloration appears

The micro plate is read at 450 nm.

Kit components

Reagents
Micro plate coated with BHV-1 purified lysate (12 x 8 well strips)
Ready- to- use conjugate
Positive control
Negative control
Dilution Buffer 1g
Wash Concentrate(20X)
Substrate solution
Stop Solution(0.5M)

1. The conjugate, the control, and the substrates solution must be stored at 5 °c (±3 °c).

2. Other reagents can be stored between $+2^{0}$c and $+25\ ^{0}$c.

3. Components bearing the same name (wash solution, dilution buffers) can be used for the entire IDvet product range.

Materials required

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µl, 100 µl and 200 µl.
2. Disposable tips
3. 96-well microplate reader
4. Distilled or deionized water
5. Manual or automatic wash system

Sample preparation

In order to avoid differences in incubation times between specimens; it is possible to prepare a 96–well plate contacting the test and controlled specimens before transferring them into an ELISA microplate using a multichannel pipette.

Wash solution preparation

If necessary bring the wash concentrate (20X) to room temperature and mix thoroughly to ensure that the wash concentrate (20X) is completely solubilized. Prepared wash solution (1X) by diluting the wash concentrate (20X) to 1/20 in distilled/deionized water**.**

Testing Producer

Allow all reagents to come to room temperature (21°c + 5°c) before use. Homogenize all reagents by inversion or Vortex.

Serum samples (short and overnight incubation)

1. Add
    - 50 µl of the dilution buffer 19 to each well
    - 50 µl of the positive control to wells A1 and B1
    - 50 µl of the negative controls to wells C1 and D1
    - 50 µl of each sample to be tested to the remaining well
2. Incubate 2h + 5 min at 37 0c (+2 °c) or 16-20 hours at 4°c (+2°c)
3. Empty the wells. Wash each well 3 times with approximately 300 µl of the wash solution. Avoid drying of the wells between washings.

4. Add 100 µl of the ready-to-use conjugate to each well
5. Incubate 30 min + 3 min at 37 °c (+ 2 °c) 6. Empty the wells. Wash each well 3 times with approximately 300 µl of the wash solution. Avoid drying of the wells between washings.
6. Add 100 µl of the substrate solution to each well.
7. Incubate 15 min + 2 min at 21 °c (+ 5°c) in the dark.
8. Add 100 µl of the stop solution to each well in order to stop the reaction.
9. Read and record the O.D at 450 nm.

Validation

The test is validated at:

- The mean value of the negative control OD (ODnc) is greater than 0.7.

  $$ODnc > 0.7$$

- The mean value of the positive (ODpc) is less than 30% of the OD.

  $ODpc/ODc < 0.3$

Interpretation

For each sample, calculate the composition percentage (S/N %)

$$S/N\ \% = OD\ sample/OD\ nc\ x100$$

For sample with a S/N %:

- ✓ Less than or equal to 45 % considered positive
- ✓ greater than 45% and less than 55% are considered doubtful
- ✓ greater than or equal to 55% are considered negative

Serum and milk samples	
S/N % ≤ 45%	Positive
45% < S/N % < 55%	Doubtful
S/N % ≥ 55%	Negative

Annex 8: Pictures of laboratory process and ELISA result

Annex 9: Virus isolation procedures

Procedure

Preparation of clinical specimens and inoculation of cell lines

A. **Reagents**

- ✓ A monolayer cell (>70% confluence)
- ✓ PBS with antibiotics and antimycotic
- ✓ 70% ethanol
- ✓ 1-2% virkon's or other disinfectants
- ✓ sterile distilled water
- ✓ Growth media with antibiotic and antimycotic

B. **Materials**

- ✓ Scissors
- ✓ Forceps
- ✓ Vortex mixer
- ✓ Centrifuge
- ✓ Refrigerator
- ✓ Freezer (-20 and -80°c)
- ✓ 15 ml centrifuge tube
- ✓ 5ml tube
- ✓ Bio safety cabinet class II
- ✓ Personal protective equipment
- ✓ 1,2,5 and 10ml pipette with pipette aid
- ✓ Permanent marker
- ✓ Hand sprayer with 70 % ethanol
- ✓ Beaker with 1-2 % virkon's or other disinfectants
- ✓ Sterile sand
- ✓ Pipette cylinder containing water and disinfectant

Sample preparation: - The swab samples are vortexed and macerated well in the transport medium itself used for sample collection. The resulting suspensions are then transferred to a centrifuge tube and centrifuged at 3000 rpm for 10 minutes using a refrigerated centrifuge.

The supernatants are collected, filtered using 0.45um Millipore syringe filters, and ready to be inoculated into the suitable cell lines or stored at – 20 °c until inoculation.

Cell culture preparation: - Madine-Darby Bovine Kidney Epithelial cells (MDBK, passage 84) obtained from Athens Veterinary Diagnostic Laboratory, University of Georgia, USA, were revived from liquid nitrogen and re-cultured in a $25cm^2$ tissue culture flask. The confluent flask was then sub-cultured to multiple $25cm^2$ TC flasks and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2% Glutamax and 1% Antibiotic-Antimycotic solution at 37ºC in a humidified incubator at 5% CO_2. According to the number of samples to be isolated, cells were subcultured to 24-wells tissue culture plates until they become 80-90% confluent.

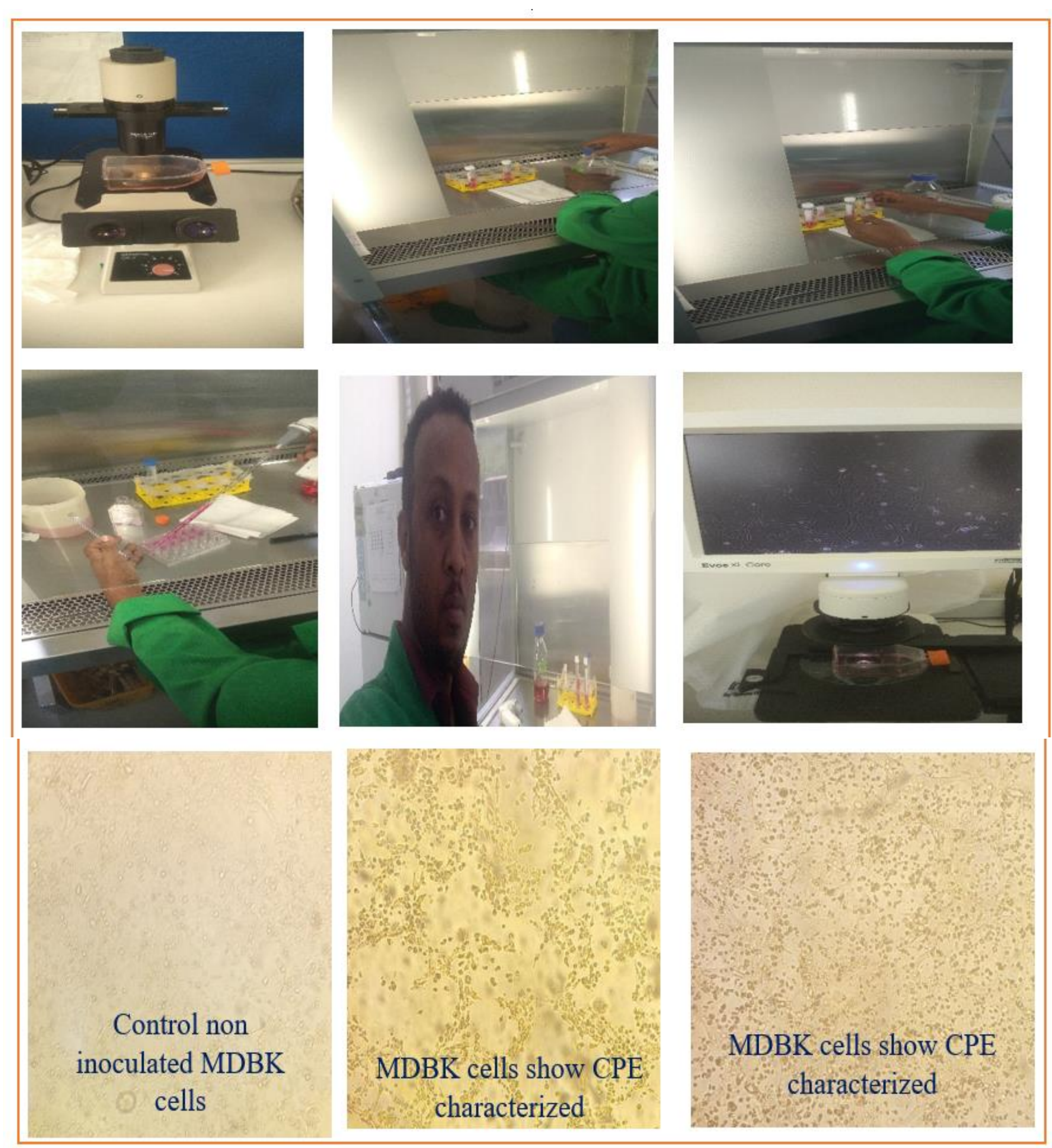
✓ **Inoculation of suitable Madine-Darby Bovine Kidney Epithelial cells (MDBK, passage 84)with the collected supernatants**

- Select an MDBK cell culture with a confluence of >70% and remove the growth medium.
- Wash the culture twice with PBS
- Inoculate the specimen suspension on the test flask and PBS with antibiotics and antimycotic (VTM) on the negative control flask and incubate at 37ºC for 60 minutes to allow the virus to adsorb onto the cell culture. (1ml for $25cm^2$ and 3ml for$75cm^2$tissue culture flask)

- Add maintenance medium (MEM with 2% FCS) and incubate the flask at 37ºC for the appropriate time, 3-7 days. Keep also control flasks without any specimen inoculums.
- Observe for the effect of virus action.

Finally, each material be passed in cell culture at least three times before declaring any specimen negative. The presence of viruses can be detected by observing cytopathic.

Annex 10: Pictures of laboratory process and cell culture result



Annex 11: Lists explanatory variables and variables defined**.**

Explanatory Variable	A variable defined (with operational definitions)
Result	Animal Positive if the test detects antibodies directed against the gB glycoprotein of the BHV-1 virus by c-ELISA
	Animal negative if the test detects antibodies directed against the gB glycoprotein of the BHV-1 virus by c-ELISA
Animal life stage	Young (> 6 month to 2 years), Adult (> 2 years)
Herd size	smallholder (< 20 Cattle), medium (20-50 cattle), and large (> 50 cattle)
Clinical condition	A diseased animal is an impairment of the normal state of an animal that interrupts ocular, respiratory, or reproductive functions
	An apparently healthy animal is an animal that is healthy and is not suffering from ocular, respiratory, or reproductive illness.
History of abortion	Yes (if abortion occurs at least once a time)
	No (if abortion did not occur at least once a time)
	Abortion in cattle is commonly defined as the loss of the fetus between the age of 42 days and approximately 260 days
History stillbirth	Yes (if stillbirth occurs at least once a time)
	No (if stillbirth did not occur at least once a time)
	Stillbirth is the calf that is born dead between 260 days and the full term of pregnancy.
History of Retained Placenta	Yes (if abortion occurs at least once a time)
	No (if retained placenta did not occur at least once a time)
	Retained placenta is failure to expel fetal membrane within 24 hours after parturition
History of Dystocia	Yes (if dystocia occurs at least once a time)
	No (if dystocia did not occur at least once a time)
	Dystocia refers to an abnormal or difficult birth
History ocular problem	Yes (if an ocular problem occurs at least once a time)
	No (if the ocular problem did not occur at least once a time)
	The ocular problem included conjunctivitis, which is either unilateral or bilateral and associated with profuse lacrimation and pink eye. Photophobia and lacrimal discharge.

History of respiratory problem	Yes (if a respiratory problem occurs at least once a time)
	No (if a respiratory problem did not occur at least once a time)
	The respiratory problem included serous and mucopurulent nasal discharge; mucosa of the nares becomes reddened and shallow erosions, excessive salivation, and an oral lesion. And also coughing.
Contact with a wild animal	Yes(if the cattle/herd are contacted with wild animals at least once a time)
	No (If the cattle/herd doesn't have contact with wild animals)
Limited movement in the district	Yes (if the cattle/ herd are limited in their movement in the district)
	No (if the cattle /herd didn't limit their movement in the district)
Cross district border	Yes (if the cattle/herd are cross the district border at least once a time)
	No (if the cattle/herd didn't cross the district border at least once a time)
Cross Park & Sanctuaries	Yes (if the cattle/herd cross the parks and sanctuaries)
	No(if the cattle/herd didn't cross the park and sanctuaries)
Cross-regional boundary	Yes (if the cattle/ herd are cross the regional boundary)
	No (if the cattle/ herd doesn't cross the regional boundary)
Cross-national border	Yes (if the cattle/herd crosses the national border of Ethiopia)
	No (if the cattle/herd doesn't cross the national border of Ethiopia)
Origin	The home breed is cattle born in the home.
	Purchased/gift/cattle rustling is if there is an animal introduced from another herd by means of Purchased/gift/cattle rustling
	Cattle rustling is a forceful acquisition of cattle from one community by another using guns or other weapons and in turn, leaving behind the destruction of property and loss of lives

Annex 12: Ethical Clearance Certificate

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

Ethical clearance certificate

Certificate Ref. No: VM/ERC/11/02/15/2023

Name and affiliation of applicant: **Melkamu Tadesse (DVM, MSc student)**
Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project*: Sero-prevalence and risk factors for infectious bovine rhinotracheitis in cattle of selected districts in South Omo Zone, Ethiopia*

Date of application: **December, 2022**
Nature of the project: **Field investigation**
Target animal species: **Cattle**
Number of animals involved: **384**
Study area: **South Omo Zone, Ethiopia**

Minutes No. and date of review: **VM/ERC/02/15/022, 23/12/2022**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of **Melkamu Tadesse.**

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Please quote Our Ref. No. When replying

ፋክስ
Fax 251-11-4339933
ስልክ
Tel. +251 114338450
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P.o.x. Box}34
ቢሾፍቱ፣ ኢትዮጵያ
Bishoftu, Ethiopia

Annex 13: Consent form for Questionnaire

Seroepidemiology of Infectious Bovine Rhinotracheitis and isolation of Bovine Herpes Virus -1 from local breed cattle in selected districts of South Omo Zone, Ethiopia.

Good morning/good afternoon!

I am Melkamu Tadesse, an MSc student at Addis Ababa University's College of Veterinary Medicine and Agriculture. I am researching seroprevalence and risk factors for Infectious Bovine Rhinotracheitis in cattle of selected districts in Borena Zone, Ethiopia. The purpose of this study is to determine the Seroprevalence of IBR in the South Omo Zone and assess the potential risk factors associated with IBR disease and also to undergo virus isolation in the study subjects. I expect that this study will be very useful in determining the seroprevalence of IBR and the contributing factors. It will generate original data that will greatly assist the public sector and other stakeholders in informing future action. In light of this, it is critical that you provide honest answers to all of the questions. There are no benefits or hazards to you personally by participating in this study; it is completely voluntary. I guarantee that your response will be kept confidential and that any information acquired about your personal identity will not be shared with a third party, as is standard in research. During the report or presentation of this study, no one will be able to tell your identity from the questions and answers you provided.

Respondent statement: I have understood the above statements:

1. Yes (Agree to participate on a voluntary basis)

2. No (Not agree to participate, I stop here without doing anything)

Name of respondent: ____

Signature: ____________________

Date: ________________________

Annex 14: Photo Consent Form

I, ______________________ with a mailing address of ________________________ City of ______________________, Phone ______________________ (the "Releasor") grant permission and give my consent to ______________________ (the "Releasee") for the use of research-related personal, institutional, domestic, and wild animal, photograph(s) or electronic media images that captured during field missions below for any presentation of any and all kind whatsoever under any legal use. I understand that I may revoke this authorization at any time by notifying _____________________________ in word/ writing.

Releasor's Signature ______________________ Date ______________

Releasee's Signature ______________________ Date _____________

Name_________________________

Adresse________________________

District_________________________

Kebelle_________________________

Phone_________________________