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ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

ISOLATION AND MOLECULAR CHARACTERIZATION OF CAMELPOX VIRUS FROM OUTBREAK CASES IN BORENA, ETHIOPIA

MSC THESIS

BY
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JUNE, 2025

BISHOFTU, ETHIOPIA

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MSC THESIS

Submitted To The College Of Veterinary Medicine And Agriculture, Addis Ababa University In Partial Fulfilment Of The Requirement For The Degree Of Master Of Science In Veterinary Microbiology

BY

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DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH MANAGEMENT

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JUNE, 2025

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

As MSc research advisors, I certify that I have read and evaluated the thesis prepared under my guidance by Abdurezak Abrar entitled: ‘’Isolation and molecular characterization of camelpox virus from outbreak cases in Borena, Ethiopia’’ I recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Microbiology.

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

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: Abdurezak Abrar entitled ''Isolation and molecular characterization of camelpox virus from outbreak cases in Borena, Ethiopia'' and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Science in Veterinary Microbiology.

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ACRONYMS AND ABRREVATION

ATIP	A-type Inclusion Protein
CAM	Chorioallantoic Membrane
CMLV	Camelpox Virus
CPE	Cytopathic Effects
CPXV	Cowpox Virus
DNA	Deoxyribonucleic Acid
EEV	Extracellular Vesicle
ELISA	Enzyme Linked Immunosorbent Assay
ICTV	International Committee on Taxonomy of Viruses
IMV	Intracellular Mature Virus
MM	Maintenance Medium
MPXV	Monkey Pox Virus
OPXV	*Orthopoxvirus*
PCR	Polymerase Chain Reaction
PPV	Parapoxvirus
RT-PCR	Real-time Polymerase Chain Reaction
TEM	Transmission Electron Microscopy
VACV	Vaccinia Virus
VNT	Virus Neutralization Test

ABSTRACT

An outbreak investigation was conducted from October-2024 to May-2025 in Borena Zone, Ethiopia focussing on isolation and molecular characterization of camelpox virus from recent outbreak cases. The study included clinical evaluation, isolation and molecular characterization using PCR, RT-PCR, sequencing and phylogenetic analysis. During clinical investigation camels manifesting typical pox-like lesions, fever and swollen lymph nodes were observed. The outbreak had 33.8% morbidity rate, i.e 24 out of 71 camels were clinically sick, and the case fatality rate was 4.2%, 1 camel out of 24 clinically sick camels died. The study showed successful isolation of the virus using Vero cell line, with typical cytopathic effects such as rounding of cells, syncytia, giant cell formation, aggregation and detachment of cell sheet. The conventional PCR result showed skin scab and nasal swab samples yielded the amplification products of the expected 881bp size, which corresponds to the partial fragment of A-type inclusion protein (ATIP) gene. Specifically, 26.3% of skin scab and 26.3% of nasal swab samples tested positive for camelpox virus genome. The RT-PCR employing HRM assay detected CMLV DNA in ten out of 19 samples (52.6%), with a specific melting temperature of 73.00±0.20 °C for CMLV, and no amplific -ation was observed *Capripoxvirus* and *Parapoxvirus.* The molecular characterization and the phylogenetic analysis of the five sequenced isolates of the ATIP gene showed 100% nucleotide similarity with comparable reference CMLV strains of CMLV M-96, CMLV CMS, strain 0408151v and CMLV genome (NC_003391). Despite the overall high similarity, a single nucleotide variations were noted when compared to the previously reported Ethiopian isolates (KU705085-KU705110) and Israeli isolates (MK910851 and MZ300856) at position 448 (A:G). Furthermore, two nucleotides mismatches were observed at positions 6 and 448 (A:G) when aligned with Sudanese isolates (KT931624 and KT931625). The phylogenetic analysis showed that the current isolates of this study clustered with CMLV strains of CMLV M-96, CMLV CMS, strain 0408151v and other, but distinct from previously reported Ethiopian isolates, indicating genetically related but evolutionarily unique viral isolate. This successful isolation and molecular characterization of camelpox virus in Ethiopia provides significant insights on early diagnosis, vaccine development and control strategies.

Key Words: *Camelpox, Camelus dromedary, Ethiopia, Isolation, Molecular characterization.*

1. INTRODUCTION

The one-humped camel (*Camelus dromedarius*) is an essential livestock species, with a global population estimated over 42 million, of which 35.6 million in Africa and 6.76 million in Asia (https://www.fao.org/faostat/en/#data/QCL). The one-humped camel accounts for about 95% of the overall Old-World camel population (Sazmand et al., 2019). Africa is home to more than 80% of the world camel population, with the Horn of Africa accounting for 60% of these species. Somalia, Sudan, Ethiopia, Kenya, Djibouti, and Eritrea harbour the highest percentage of one-humped camels in the world (Othieno et al., 2022). Ethiopia ranks sixth in Africa in terms of camel population, with an estimated 1.75 million camels, predominantly in the arid and semi-arid lowlands of the Somali, Afar, and Southern Oromia regional states (CSA (Central Statistic Authority), 2018), where nomadic herders make up the majority of the population (Kena, 2022).

Camels provide meat, milk, wool and transport, playing crucial roles in the livelihoods of pastoral communities. With their special anatomical, physiological and behavioural features that enable them to cope extremely well in the severe environments of aridness, heat and cold. This remarkable adaptability positions them as a key species in addressing the challenges posed by global climate change, offering a sustainable source of nutrition and resources in regions where other livestock may struggle to survive (Ahmad et al., 2010).

Despite their resilience, camels are susceptible to camelpox, a highly contagious viral disease of camels with the exception of dromedary camel in Australia and tylopods (llamas and related species) in South America (Mosadeghhesari et al., 2014). The causative agent of camelpox is classified taxonomically within the genus *Orthopoxvirus* which belongs to the family *Poxviridae*. Family *Poxviridae* is divided into two subfamilies: *Chordopoxviridae*, which infects vertebrates and *Entomopoxviridae* (ICTV, 2024), which are found in insects. Phylogenetic analyses of CMLV shows the virus is closely related to the Variola virus, the aetiological agent of smallpox (Delhon, 2022). Camelpox occurs in practically every country where camel husbandry is practised, with the exception of the introduced dromedary camel in Australia and tylopods (llamas and related species) in South America. Outbreaks have been reported throughout the world, including the Middle

East (Bahrain, Iran, Iraq, Oman, Saudi Arabia, the United Arab Emirates and Yemen), Asia (Afghanistan and Pakistan), Africa (Algeria, Egypt, Ethiopia, Kenya, Mauritania, Morocco, Niger, Somalia and Sudan) (Wernery et al., 1997), southern parts of Russia and India (Sharawi et al., 2011). Camelpox is endemic in these countries.

The incubation period lasts from nine to thirteen days. The clinical manifestations of camelpox range from mild and inapparent skin-restricted local infections to moderate and severe systemic infections, most likely due to differences in camelpox strains or immunological status of the animals (Wernery et al., 2014). Fever, enlarged lymph nodes and skin lesions, face oedema, lachrymation, pendulous lips and pox lesions are the disease characteristics (Khalafalla et al., 2015). One to three days following the onset of fever, skin lesions appear. They start off as erythematous macules, and then develop to papules and vesicles and ultimately transform into pustules. It is on the ruptured pustules that the crusts form. The head, eyelids, nose and the ear margins are where these lesions initially appear. The entire head may enlarge in severe cases. Skin lesions might later spread to the genitalia, perineum, mammary glands, neck and limbs. In its generalised form pox lesions can spread throughout the body. It might take up four to six weeks for skin lesions to heal. The systemic form of the disease can cause lesions in the respiratory tract and oral mucous membranes and pox lesions can be observed in these areas (Kriz, 1982; Wernery et al., 2014).

Direct contact with an infected animal or indirect contact with a contaminated environment are the two ways that camelpox is transmitted. Direct transmission occurs when infected animal, with skin lesions, comes into contact with vulnerable animals. Additionally, potential vectors like mosquitoes and biting flies can also cause mechanical transfer (Wernery & Kaaden, 2002). Infected camels may shed the virus through scab materials and secretions like milk, saliva, ocular, and nasal discharges into the environment, including water, where they potentially infect vulnerable animals (Wernery & Kaaden, 2002; Khalafalla & Ali, 2007). In dry scabs, the virus can survive for up to 4 months. Depending on the virus strain and the animal's immune status, camelpox is a highly infectious and extremely transmissible disease of skin that affects camels and can cause mild form of skin lesions or severe systemic illnesses (Dahiya et al., 2016; Wernery & Kaaden, 2002). The prevalence of the disease is socioeconomically significant as it causes significant losses in terms of morbidity and mortality, weight loss, abortion and reduced milk production.

Outbreaks of camelpox cause substantial economic damage and necessitate quarantine and containment efforts to prevent the disease from spreading (Aregawi et al., 2018; Tefera & Gebreah, 2001).

Other animal species such as cattle, sheep and goats are not infected by camelpox virus because it is very host specific. However, given the small number of human camelpox cases that have been reported, it appears that the disease is significant for public-health (Kriz, 1982; Bera et al., 2011; Khalafalla & Abdelazim, 2017). Bera et al. (2011) recently reported CMLV zoonosis based on clinical and epidemiological evidence, as well as serological and molecular characterization of the causative agent in three human cases. This was the first incidence of camelpox zoonosis in India as well as in the entire world to be confirmed by laboratory. Khalafalla & Abdelazim (2017) provided further evidence CMLV zoonotic nature through cases in eastern Sudan. Because of the significance of camelpox and scarcity of a comprehensive study, the following study was designed to elucidate the molecular pattern of camelpox virus.

Here, we report an outbreak investigation of camelpox in a dromedary herd in the Borana, Yabello districts of Oromia Regional State that occurred in October 2024. The investigation included culturing of the virus and the virus isolates were molecularly characterized by sequencing and compared by phylogenetic analyses.

1.1 Statement of the Problem

Camelpox is acknowledged as an economically significant disease that affects camel populations, particularly in arid and semi-arid regions of Ethiopia. The disease leads to severe economic losses due to high morbidity and mortality rates, mostly in young camels, as well as reduced productivity in infected herds (Ayelet et al., 2013). Despite the increasing frequency of camelpox outbreak, comprehensive studies focusing on isolation and molecular characterization of CMLV in Ethiopia remain scarce.

Our present limited knowledge of CMLV give rises to a serious of crucial questions that require further investigations. It is believed that different strains of CMLV having different degree of virulence might exist, potentially explaining the two forms of camelpox: moderate and generalized lethal. However, this assumption has not been conclusively demonstrated. Additionally, the apparent restriction of the disease to arid and semi-arid areas might indicate a potential co-evolution of the virus along with the host, which raises the question of whether the virus developed unique adaptations that allows the virus to survive in hot climates.

The recurrent nature of camelpox outbreaks in Ethiopia underscores the urgency of identifying the genetic variations in local strains. Understanding the phylogenetic relationships among CMLV isolates is important for the assessment of the current vaccines effectiveness. There are growing concerns that vaccine failure may contribute to continued outbreaks, highlighting the need for in-depth genetic and phylogenetic analyses to support the development of more effective vaccines (Arog et al., 2024).

Given these knowledge gaps, this study aims to isolate and molecularly characterize the CMLV from recent outbreak cases in observed Borena, Oromia Regional State of Ethiopia that occurred in 2024. This research will provide critical insights into the genetic diversity of the virus and its implications for vaccine development and disease control strategies.

1.2 Significance of the Study

This study on the isolation, molecular characterization of camelpox virus from outbreak studies from Borena, holds significant on understanding of the virus's genetic variations by focusing on molecular characterization of CMLV isolates. From epidemiological perspective, the study will provide essential data on the frequency and distribution of camelpox outbreak, helping veterinary authorities to implement targeted control measures. Identifying genetic variations among Ethiopian CMLV isolates will also shed light on the virus evolutionary patterns and its potential resilience to environmental factors such as high temperature. Furthermore the study will be invaluable for vaccine development, if genetic variations among local strains are going to be observed. This research will offer guidance on designing more effective vaccines tailored to Ethiopian isolates while taking into account the genetic variation, if any.

1.3 Study Objectives

1.3.1 General objective

- The main aim of the present study was to isolate and characterize CMLV from outbreak cases in affected pastoral associations of Yabello, Borena, Ethiopia in October 2024.

1.3.2 Specific objectives

- To isolate circulating CMLV strains from outbreak cases in affected pastoral associations in Yabello, Borena, Ethiopia.
- To perform partial ATIP gene analysis of the CMLV isolates and to assess variability at nucleotide levels.
- To perform phylogenetic analysis of the identified CMLV isolates and understands their evolutionary relationships.

2. LITERATURE REVIEW

2.1 Etiological Agent

Camelpox is caused by CMLV; that is linear double stranded DNA virus which belongs to the genus Orthopoxvirus (OPV), subfamily *Chordopoxvirinae* of *Poxviridae* family (ICTV, 2024). Initially it was considered to be a smallpox-like disease causing agent during smallpox virus eradication (Baxby, 1972). It was later isolated, identified, and characterized as CMLV. Until recently, CMLV is one of the least studied members of OPVs. Based on size, shape, structure, physico-chemical properties and replication mechanisms of the virus, it is quite difficult to distinguish CMLV from the prototypic VACV (Ali et al., 2009). However, CMLV shows clear differences from VARV and VACV due to its genome structure and phylogenetic analysis of the DNA sequences for all open reading frames (ORFs). It has been suggested that CMLV originated from a CPXV-like virus ancestor (Afonso et al., 2002). The sequence of CMLV is most closely related to VARV (Gubser & Smith, 2002). The typically average size of the viron is between 265–295 nm. Orthopoxviruses are brick-shaped, enveloped viruses; having an exterior membrane made up of tubular proteins which are arranged irregularly. The virion also consists of two lateral bodies and a core. Its double-stranded DNA genome is approximately 206 kbp in size (OIE, 2021).

Similar to other OPVs, CMLV reacts differently to physical and chemical agents. In general, CMLV is chloroform sensitive and ether resistant. CMLV is sensitive to both acidic (pH 3-5) and alkaline (pH 8.5-10) conditions (Davies et al., 1975). Like other poxviruses, CMLV is susceptible to various disinfectants including 1% sodium hypochlorite, 1% sodium hydroxide, 1% per acetic acid, formaldehyde, 0.5-1% formalin and 0.5% quaternary ammonium compounds. The virus can be destroyed either by autoclaving or boiling for 10 min and ultraviolet rays (245 nm wave length) in a few minutes (Coetzer & Tustin, 2004). CMLV (H 520 strain of Kenya) hemagglutinates red blood cells (RBCs) of cockerel (Davies et al., 1975), on the other hand, the CMLV Etha 78 strain agglutinates chicken RBCs at room temperature in a pH range of 6–8 (Khalafalla et al., 1998).

2.2 Viral Genome

Camelpox disease in camels is caused by an epitheliotropic DNA virus (Salem et al., 2008). It belongs to the genus Orthopoxvirus (OPV) under subfamily *Chordopoxvirinae* and the family *Poxviridae* (Essbauer et al., 2010). The outer membrane of the brick shaped virion is studded with tubular proteins that are arranged irregularly. A large number of viral encoded enzymes are carried by CMLV, which replicates in the cytoplasm and associated within the virion (Moss, 2007). The CMLV genome is a single linear 205,719 bp double-stranded DNA molecule that is AT-rich (66.9 %) and has 211 putative genes. The two DNA strands in the genome are joined by cross-links at both ends. At both ends of each DNA strands, there are long inverted tandem repeats around 7 kbp in length that cannot completely be base-paired and instead fold into hairpin-like loops. These loops links the double stranded ends to the central region of the genome where genes that are highly conserved among all the sequenced OPXVs. Additionally, CMLV contains unique 3kbp region that encodes for three ORFs (CMLV185, CMLV186, CMLV187) which are not found in other OPVs (Afonso et al., 2002). Nevertheless, towards either terminal are variable regions, which encodes proteins involved with host tropism, virulence or immunomodulation (Gubser & Smith, 2002).

Compared to other OPXVs, CMLV shows genomic difference that occur in terminal regions where small and large nucleotide insertions, deletions and translocations. This leads to decrease in ORF collinearity and the average amino acid identity, which is about 82% similarity with VACV. Notably, CMLV genome lacks a 14.5-kb region which is present in the left end of the CPXV genome, in contrast it contains a 2.9-kb insertion region (position 172,582–175,508), which is absent in VACV and CPXV. Although the overall genome structure and composition are similar to the other OPVs, it is clearly distinct from VARV and VACV as it lacks homologues of sever genes present in VARV (C1L, E7L, A26L, A27L, A39L, A42R, B2L, B3L and B4L), and VACV (K6L, A25L, A40R, A52R and A53R) (Bhanuprakash et al., 2010).

CMLV has 27 ORFs which are not found in VARV, some of which include homologues of *N*-methyl-d-apartate (NMDA) receptor-like protein, tumor necrosis factor receptor (TNFR) II crmE fragment, ankyrin repeat proteins, Schlafen-like protein, M-T4-like virulence

protein, kelch-like proteins, IL-1 receptor (IL-R) fragments, VACV K1L-like host range fragment, and additional copies of CMLV001-003 in the left inverted tandem repeat of CMLV, and 17 ORFs of unknown function. The genome structure and phylogenetic analyses all CMLV ORFs show that CMLV is clearly distinct from VARV and VACV. CMLV and VARV are believed to have originated from a CPXV-like ancestor (Afonso et al., 2002).

2.3 Historical Background

2.3.1 Global scenario

Camelpox became widely known in the early 1970s, although being initially reported in India in 1909 (Leese, 1909). Since then the disease has been reported from many countries. For a long time, the disease was considered to be a generalized pox disease of camels, and the causative virus CMLV was first isolated in chick embryos in 1970 (Sadykov, 1970). In 1972, Ramyar & Hessami isolated the virus in tissue culture (Ramyar & Hessami, 1972).

In the late 1970s, CMLV was considered a "smallpox-like" member of the genus *Orthopoxvirus*, due to its similarities to VARV in terms of culture characteristics, narrow host range, and even serological cross-reactivity (Baxby, 1972; Baxby et al., 1975; Davies et al., 1975). The speculation of similarities between CMLV and VARV was further supported by findings from an in vivo experiment in which camels infected with VARV strain EA8 were protected against challenge with an infective dose of CMLV (Baxby et al., 1975). Those participating in the global smallpox eradication campaign were extremely concerned about this. But twenty years later, genome characterization investigations using *HindIII* enzyme and restriction fragment length polymorphism analysis confirmed that CMLV was a distinct member of the OPV genus (Pfeffer et al., 1996; Renner-Miiller et al., 1995). Afterward, when complete-genome sequencing was conducted CMLV strains showed that the virus is closest to VARV sharing several genes which are involved with replication and host-related functions, and most likely they may share common ancestor (Afonso et al., 2002; Gubser et al., 2007a, b). Outbreaks of CMLV were reported in many countries including (Bahrain, Iran, Iraq, Oman, Saudi Arabia, the United Arab Emirates and Yemen), Asia (Afghanistan and Pakistan), Africa (Algeria, Egypt, Ethiopia, Kenya, Mauritania, Morocco, Niger, Somalia and Sudan) (Wernery et al., 1997), and southern

parts of Russia and India (Sharawi et al., 2011), where the disease is enzootic (Figure 1). A recent isolate of CMLV from Israel was found to be genetically distinct from the currently annotated camelpox isolates; nevertheless, whole genome sequencing is required for definitive conclusion (Erster et al., 2018).

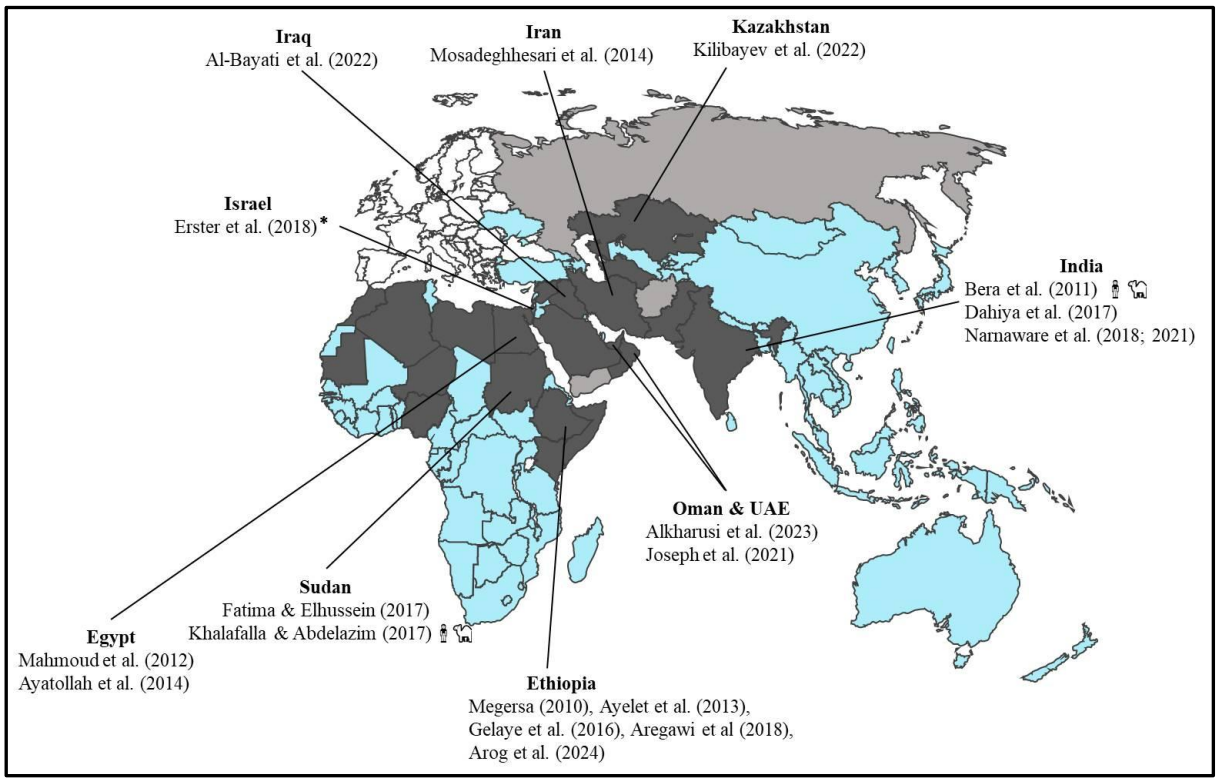

Figure 1: Major camelpox outbreaks since 2010
Countries with specific references are shown in dark grey. Those with outbreaks reported by Wernery & Kaaden (2002) are colored in light grey, new cases are marked with the symbol*, and human cases of CMLV are indicated using the human and camel icons.

2.3.2 Camelpox in Ethiopia

Camelpox is recognized as economically significant viral disease that affects camels in Ethiopia, particularly in pastoral regions. An epidemiological study in the Borena region examined the overall impact of camelpox and other camel diseases (Megersa, 2010). This study confirmed that camelpox is a recurrent problem, causing economic losses due to decreased milk production, weight loss and mortality. The study highlighted the lack of veterinary services and awareness among the pastoralists, which hampers effective detection and effective disease management. It also suggested that the nomadic lifestyle

contributes to the disease's spread across different regions, emphasizing importance of targeted interventions (Megersa, 2010).

Ayelet et al. (2013) reported on epidemiology and genetic features of camelpox in Afar and Jigjiga regions. This work has revealed that the disease is widespread in these areas, especially in rainy seasons, with infection rates of 4.5% in Afar and 3.0% in Jigjiga. This study has also revealed that Ethiopian CMLV isolates share genetic similarities with strains from Kazakhstan and Iran suggesting transboundry transmission. This was the first successful isolation of CMLV in Ethiopia, providing essential data for early diagnosis and control measures.

Genetic characterization of Poxviruses in Camelus dromedarius in Ethiopia (2011-2014), explored the genetic diversity of poxviruses affecting Ethiopian camels by comparing large samples using HA, ATIP and B2L genes. This study identified both CMLV and camel contagious ecthyma (CCEV) in Afar, Oromia and Somali regions. The genetic analysis confirmed that Ethiopian CMLV strains are closely related to those found in Somalia and Syria, while CCEV exhibited more genetic variation. Importantly, this study reported the first case of CCEV in Ethiopia, indicating that some cases previously identified as camelpox were actually CCEV, emphasising the need for improved differential diagnosis between camelpox and other poxviruses (Gelaye et al., 2016).

A seroprevalence study examined the prevalence of camelpox in the PAs of Amibara and Awash Fentale districts by Aregawi et al (2018) found an overall seroprevalence of 19.3% that was detected in 81% of herds, confirming the endemic nature of the disease. The study has also identified seasonal pattern of camelpox outbreaks peaking during the rainy season. Its participatory epidemiology investigation revealed that pastoralist recognized camelpox as one of the top five camel diseases in these regions, linking its spread to seasonal migrations and increased herd commingling. The study emphasized the need of vaccination campaigns before rainy seasons to reduce outbreaks (Aregawi et al, 2018).

Another seroprevalence study was conducted in the Jarar Zone of Somali region in 2023 that assessed the prevalence and associated risk factors for camelpox. This study found an overall seroprevalence of 16%, with female camels 3.2 times more likely to be infected than males. Young camels were also at higher risk being 2.3 times more susceptible than

adults (Arog et al., 2024). This study also emphasised the importance of vaccination programs as a preventive measures and suggested that seasonal outbreaks could be linked to changes in environmental conditions.

2.4 Host

The camel pox virus belongs to the genus Orthopoxvirus of the family Poxviridae (Afonso et al., 2002). Unlike other OPV members such as VACV, CPXV or monkey pox virus (MPXV), CMLV has narrow host range, primarily affecting camels. VARV and CMLV are restricted to a single host, humans for VARV and camels for CMLV, where they cause a severe disease. However other OPVs can infect a wide range of hosts, including rodents, zoo animals, monkeys and humans. Interestingly, camels that have received the VARV vaccine are immune to subsequent CMLV infection (Baxby et al., 1975). Although New World camelids like guanacos may be experimentally infected, Old World (Dromedary and Bactrian) camelids have been recognized as the reservoir hosts of CMLV. Additionally, arthropod vectors involved in the disease transmission may get infected (Wernery & Zachariah, 1999). Intradermal inoculation of the virus into mice, rats, guinea pigs, hamsters, rabbits, sheep and goats were not successful (Ramyar & Hessami, 1972; Bhanuprakash et al., 2010). The hypothesis that CMLV is mostly host specific is supported by the fact that sheep and cows in direct contact with infected camels remained healthy even in a natural infection (Al-Zi et al., 2007). However, experimental infections have been successful in monkeys and infant mice (Baxby, 1972). Additionally an isolate of CMLV (CP/Nw/92/2) from Sudan has been reported to induce local pox lesions in chicken (Khalfalla & Mohamed, 1998). Despite its limited host range, the virus is nonetheless significant due to its impact on the camel populations and strong genetic relationship with VARV. Given the virus's unique host specificity and potential transmission pathways, more research is required to fully understand the epidemiology and possible zoonotic implications.

2.5 Host-Virus Interaction

Poxviruses, such as camelpox employ several strategies to evade the host immunological response, such as interfering with interferon, complement system and pro-inflammatory cytokines (IL-1 , IL-18, Tumor Necrosis Factors) (Duraffour et al., 2011). To facilitate

viral replication and spread throughout the host, the majority of proteins encoded by poxviruses that interfere with the host immune response target the innate immune system.

CMLV has been shown to encode numerous immunomodulatory proteins that help evade the host immune response. One such example is the vaccinia virus B18R gene homolog, which encodes a soluble receptor protein that binds to IFN- α/β at the cell surface with high affinity, and protects both infected and non-infected cells from antiviral state (Alcami et al., 2000). Additionally, the soluble IFN-γ receptors secreted by CMLV binds to and blocks IFN- γ interaction with cellular receptors and thus interfering with its antiviral effect (Alcamí & Smith, 1995). The virus also disrupts mouse splenocytes' ability to produce IFN induced by IL-12.

It has been demonstrated that CMLV expresses a 35-kDa chemokine binding protein (vCKBP) in vitro, that binds CC but not CXC or C, preventing the migration of chemokine based immune cells to the sites of infection and inflammation (Alcamí et al., 1998). Furthermore, the Golgi apparatus contains v-GAAP protein, encoded by CMLV, and has independent effects on migration, cell adhesion and apoptosis (Carrara et al., 2015). A schlafen-like protein 176R-(v-slfn-57 kDa) (Gubser et al., 2007a; Gubser et al., 2007b), which is expressed both early and late phase of infection and contributes to the modulation of the innate and adaptive immune system against pathogens (Geserick et al., 2004).

CMLV encodes homologues of pox viral proteins of vaccinia, myxoma virus and rabbit fibroma virus, which has been shown to affect virulence or host range. Proteins encoded by open reading frames (ORF) 31L, 188R and 200R have similarity to serpins that have anti-fusion or anti-apoptotic activity and are involved in the inflammation (McFadden, 1995), while proteins encoded by ORFs 32L and 55L are similar to VAVC proteins K3L and E3L that mediate resistance to IFN-induced antiviral response (Smith et al., 1998).

The CMLV enters most commonly through skin. However, the oro-nasal infection has also been reported. Following local replication and development of a primary skin lesion, the virus spreads to local lymph nodes, resulting in a leukocyte-associated viremia that may be associated with pyrexia. During this period, viruses can be isolated from various tissues, including the skin, turbinates, lungs and lymphoid organs. Widespread secondary skin

lesions appear a few days after the onset of viremia, and new lesions continue to appear for 2–3 days, at that time the viremia subsides (Bhanuprakash et al., 2010).

Although these mechanisms have been described in vitro, they may reflect the in vivo situation and explain the pathogenicity of CMLV in its host, the camel. As explained above, CMLV may utilize several ways to alter or shut down the host immune response. Understanding these mechanisms is crucial for developing effective control measures against CMLV and related *orthopoxvirus* infections.

2.6 Clinical Signs

Camelpox has a 9-13 days incubation period, beginning with an initial rise in temperature, followed by enlargement of lymph nodes, skin lesions and prostration. Camelpox can cause mild local to severe systemic illness, depending on the CMLV strains involved in the infection (Wernery & Kaaden, 2002). The typical skin lesion/rash will pass through all the stages of pox lesions progression including papules on labia, macules, papules, pustules, vesicles and scabs (Duraffour et al., 2011; Wernery et al., 1997a; Wernery et al, 1997b).

One to three days after the onset of fever, skin lesions appear, progressing as erythematous macules and develop into papules and vesicles, and pustules, eventually forming crusts as the pustules ruptures. The lesion usually takes 4-6 weeks to heal. The eruptions are mainly localized, often affecting the head, nostrils, margins of the ears and eyelids, as well as on the mucous membranes of the lips, nose and oral cavity, but in severe cases, lesions may extend to the neck, limbs, genitalia, mammary glands and perineum or scrotum (Duraffour et al., 2011).

The lesion is usually localized in skin but occasionally, however it can progress to generalized form. The later form is frequently seen in young animals aged 2-3 years in a herd associated with weaning and poor nutrition, and can be fatal due to secondary bacterial infections like *Staphylococcus aureus*. This infection can lead to septicaemia and the affected animals may show salivation, anorexia, lacrimation, mucopurulent nasal discharge and diarrhoea. Pregnant animals may abort as well (Nothelfer et al., 1995; Wernery & Kaaden, 2002).

In most cases, CMLV infection has been reported to be relatively benign, especially in adult camels, however, severe infections with a high CFR and blindness are common in young camels, while abortions, still birth, weight loss and reduced milk yield may be seen in adult animals (Kriz, 1982; Ramyar & Hessami, 1972; Higgins et al., 1992). Unlike smallpox, which mainly causes pustules on the skin and the squamous epithelium of the oropharynx, severely affected camels also develop proliferative poxviral lesions in the bronchi and lungs (Kinne et al., 1998).

The histopathology of skin lesions shows characteristic cytoplasmic swelling, vacuolation and ballooning of the keratinocytes of the outer stratum spinosum of the epidermis, leading to vesicle formation and localized oedema (Duraffour et al., 2011; Duraffour et al., 2009). The lung lesions are typically characterized by hydropic degeneration, proliferation of bronchial epithelial cells associated with proliferative alveolitis and bronchiolitis with macrophage infiltration, necrosis and fibrosis, which leads to obliteration of normal architecture (Pfeffer et al., 1998; Pfeffer et al., 1998b).

2.7 Transmission

Camelpox is transmitted through direct contacts with sick animals via skin abrasions or aerosols (Wernery & Kaaden, 2002). Scab materials, saliva and secretions from infected camels may shed virus into the environment, such as in water which then serve as a source of infection (Khalafalla & Ali, 2007). Dried scabs shed from the pox lesions may carry live virus particles for up to 4 months and contaminate the environment (OIE, 2021). Numerous studies have revealed that the incidence of camelpox outbreaks increased during rainy seasons, with the appearance of more severe forms of the disease, whereas during the dry season milder forms are seen (Wernery et al., 1997a, Wernery et al., 1997b; Khalafalla & Ali, 2007). It is hypothesized that CMLV strains of different virulence may explain the differences in pathogenicity seen between dry and rainy seasons; however this has never been assessed.

Another theory is the involvement of arthropod populations, which are abundant during rainy seasons and may exert a greater virus pressure on camel populations. This hypothesis is confirmed by the isolation of CMLV from *Hyalomma dromedarii* ticks (Wernery et al., 1997a; Wernery et al., 1997b). During the 1995–1996 camelpox outbreak in the United

Arab Emirates, twenty ticks were collected from five camels infected with generalized camelpox (Wernery et al., 1997), processed for electron microscopy and cell culture analyses, were found to have CMLV (Wernery & Kaaden, 2002). However, it is unclear whether ticks might transmit CMLV mechanically or whether they might be a true reservoir of the virus. In the last case, camelpox would be maintained and spread through transstadial transmission (the pathogen is maintained in the vector from one developmental stage to subsequent stages) or transovarial transmission (the female vector passes the infectious agent to the next generation via her eggs).

Hyalomma dromedarii have been found to be the predominant (90%) tick species infesting camels. Ticks seasonality or periods with the highest infestations have been seen in Egypt (March-November), Sudan (March–October), and Saudi Arabia (May, August, October and December) (AlKhalifa et al., 2007b; Elghali et al., 2009). However, these exhaustive reports did not reveal any correlations between tick's infestations with temperature, relative humidity or rainfall (Wernery et al., 1997; Elghali et al., 2009). Further research is required to confirm the role of arthropods in the transmission of CMLV, but if proven, it would be the first OPV transmitted via arthropods. Identified risk factors associated with higher incidence of camelpox include the average age of the animals (less than four years old), the rainy season of the year, the introduction of new camels into a herd and the common watering points (Khalafalla & Ali, 2007).

2.8 Public Health Significance

Camelpox is the most common viral infectious disease of skin that primarily affects Old-World camelids such as Dromedary and Bactrian camels. With the exception for Australia, it is endemic in almost all countries where camel husbandry is practiced (Khalafalla & Hussein, 2021). In Somalia, where a 40-year-old man, unimmunized against smallpox and who had come into contact with diseased camels, developed a rash on his arms that progressed through vesicular, pustular and scab stages. This was the first documented case of human camelpox. The presence of *Orthopoxvirus* antibodies was confirmed through a passive hemagglutination inhibition test. Furthermore, electron microscopy of samples taken from sick animals in the patient's group tested positive for *Orthopoxvirus* and the camelpox virus was successfully isolated (Kriz, 1982).

The first strong evidence of human zoonotic transmission of CMLV infection was reported in 2009 by Bera et al (2011), who described camelpox outbreaks in dromedary camels in northwest India. Clinical and epidemiological findings, serological testing and molecular characterization of the virus were used to confirm three human cases. The lesions appeared only on their hands and fingers of the camel handlers, who were in direct contact with infected camels, which progressed through all stages of pock lesions until the development of scabs (Bera et al., 2011). Interestingly, none of the suspected patients had received the vaccine against smallpox. These three suspected patients' serum samples showed neutralizing antibodies against CMLV. Conventional PCR was employed to detect the amplification of a CMLV-specific gene (*C18L*) in one of the three human cases, and further amplification and sequencing of other genes whose sequences confirmed to correspond with CMLV.

(Khalafalla & Abdelazim (2017) provided further evidence of the zoonotic potential of the camelpox virus by describing cases that occurred between September and December 2014 in the Showak region of eastern Sudan involving three camel herders and dromedary camels. The herders developed erythema, vesicles and pustules on the arms, hands, legs, back and abdomen, which disappeared after 2 months without being human-to-human transmission. PCR, virus isolation in cell culture and partial genome sequencing were used to confirm the diagnosis.

During the smallpox eradication campaign, camelpox was once thought to be a potential non-human reservoir of VARV (Variola virus) as the two viruses showed no detectible differences under particular laboratory circumstances (Duraffour et al., 2011). The disease is largely restricted to camels although there have been occasional reports of camelpox infections in humans. However, there are concerns; the virus could be pathogenic to human, similar to cowpox and monkeypox (Marennikova et al., 1975), especially in immunocompromised individuals. However, because there are no immunological surveys for specific camelpox antibodies among unvaccinated herds, no systematic epidemiological research have been undertaken on human cases (Azwai et al., 1996). Despite these concerns, CMLV is considered of limited public health importance due to its mild nature of the virus in humans and the limited ability of the virus to spread among people. However, its potential as a bio-warfare agent remains a subject of concern, given its

genetic similarity to variola virus the causative agent of smallpox (Balamurugan et al., 2008).

2.9 Diagnosis

Clinical signs can help to make to a presumptive diagnosis of camelpox infection. However, it is important to differentiate mild cases and early clinical stages of camel infections from contagious ecthyma (Orf), which is caused by insect bites, papillomavirus infections, and parapoxvirus (PPV). Thus clinical signs alone are not sufficient to differentiate camelpox from other diseases. Although there are several diagnostic methods available for the diagnosis of camelpox, it is preferred to use more than one test for confirmatory diagnosis (OIE, 2021).

Few complementary techniques namely transmission electron microscopy (TEM), virus isolation using cell culture, standard PCR assays, immunohistochemistry and demonstration of neutralizing antibodies, might be recommended for the diagnosis of camelpox (Balamurugan et al., 2013). However, the identity of the causative agent as CMLV must be confirmed by TEM, PCR and/or sequencing (OIE, 2021).

Camelpox can be differentiated from other OPXV and PPV infections by the use of restriction enzyme analysis (REA) (Murphy, 1999). Recently, C18L gene based species specific PCRs (in conventional and real-time formats) have been developed to differentiate CMLV from BPXV (a regional isolate of VACV) and other OPXVs (Balamurugan et al., 2009).

2.9.1 Transmission Electron Microscope

One rapid and accurate method to demonstrate OPXV in scabs or tissue samples is by transmission electron microscopy. This technique enables the differentiation between OPVs (brick-shaped) and PPVs (ovoid-shaped) which is the etiological agent of camel contagious ecthyma (Orf) (Knipe & Howley, 2007). However, a positive diagnosis necessitates a high viral concentration in the sample (Duraffour et al., 2011). TEM is now the most effective technique for differentiating clinical cases of camelpox and Orf caused by camelpox and PPVs, respectively, despite the fact that it can’t differentiate CMLV from

other OPXV species. Although serological and PCR assays can distinguish between the viruses (Dinter & Morein, 1990).

2.9.2 Immunohistochemistry

Camel pox can be confirmed by immunohistochemical demonstration of the camel pox antigen in scabs and pox lesions in tissues. It is a fast method and can be used in lieu of TEM to establish a tentative diagnosis (Nothelfer et al., 1995). Additionally, paraffin-embedded samples can be kept for a long period, enabling future epidemiological and retrospective studies (Wernery & Kaaden, 2002). This technique works with both monoclonal and polyclonal antibodies. However, due to greater degree of similarity between VACV and CMLV, almost any polyclonal antibody against VACV is likely to produce results in this test (Nothelfer et al., 1995).

2.9.3 Virus isolation

Buchnev and Sadykov isolated the Camel pox virus for the first time in 1969 in Russia (Buchnev & Sadykov, 1969). Isolation of the virus can be employed using embryonated eggs and various cell lines. Embryonated chicken eggs specifically, those aged 11 to 13-day old through chorioallantoic membrane route (CAM) (OIE, 2021). The eggs should be incubated at 37°C for 5 days, following which characteristic dense, greyish white pock lesions appear on the CAM. The ideal temperature for the formation of pock lesions is 38.5°C. If incubated at 34.5°C, the pocks are flatter and a hemorrhagic center may develop (Tantawi et al., 1974). On the fifth day, opaque white proliferative pock lesions of approximately 0.5-0.6 mm in diameter are demonstrated on the CAM without any hemorrhagic lesions but with stunted growth. Vero cell-adapted virus produce longer, opaque, white proliferative (tigroid) pock lesions on CAM (Marodam et al., 2006).

Various cell lines such as HeLa, GMK-AH1, WISH, Vero (Baxby, 1972), MA-104 and BHK21 cells, in addition to primary cell cultures like lamb testis and kidney, camel kidney, calf kidney and chicken embryo fibroblast (Davies et al., 1975) can be used for isolation study. CMLV shows typical cytopathic effects in infected cell cultures, including rounding of cells, vacuolization of cytoplasm, and formation of multinucleated giant cell with syncytia and cytolysis changes, especially in vero cells (Marodam et al., 2006). It is

noteworthy that although CMLV is restricted to camels, it has also been shown that CMLV can infect human keratinocytes. CMLV has also been shown to infect human embryonic lung fibroblasts, unlike VACV or CPXV, which have wider host specificity, (Duraffour et al., 2011). Intra-cytoplasmic eosinophilic inclusion bodies, characteristic of pox virus infection, can be demonstrated in infected cells using hematoxylin and eosin staining (Duraffour et al., 2011).

2.9.4 Serological tests

Antibodies against poxvirus can be easily detected in animal's serum than virus isolation. All OPXV viruses are serologically cross-reactive. PPVs and CMLV do not cross-react; hence infections of camelpox and camel Orf can be distinguished serologically.

A wide range of serological assays are available to identify camelpox. Conventional serological tests such as hemagglutination (HA), HA inhibition (HI), virus neutralization test (VNT) , Enzyme linked immunosorbent assay (ELISA), Western blot analysis, complement fixation (CFT) and fluorescent antibody tests (FAT) have been employed to detect CMLV antibodies (Balamurugan et al., 2013; Bhanuprakash et al., 2010). VNT and ELISA are the two tests recommended by OIE (OIE 2025).

The VNT test relies on the virus and specific antibody in the test serum reacting. Before inoculating the virus into cell culture, virus is mixed with products containing a neutralizing antibody under appropriate conditions. The presence of neutralized virus was detected by the absence of plaque formation (cytopathic effect). In this method the test sera are titrated against a constant titre of camelpox virus (100 TCID50 [50% tissue culture infectious dose]) on Vero cells (OIE, 2021).

ELISA and Western blotting have recently been developed by Azwai et al. (1996) to detect IgG and IgM antibodies to CMLV in camel sera, and OPXVs can be differentiated from PPV infections in camels. Because ELISA is simple, it can be applied successfully for both retrospective and epidemiological investigations. For the detection of antibodies to VACV and monkeypox virus (MPXV), the test is more sensitive than VNT, HA inhibition, peripheral hemagglutination PHA and indirect fluorescent antibody tests (Azwai et al., 1996; Marennikova et al., 1981). Although VNT is frequently used to detect OPXV

infection, it does not measure antibodies to envelope antigens, which are the key player in CMLV pathogenesis. OPXVs and PPVs are known to cause similar pox-like lesions in camels, the specificity of a test is of crucial to differentiate in such cases (Munz et al., 1990; Munz et al., 1986). Notably, studies have shown 95% prevalence of camelpox antibodies in camels from Kenya, Somalia and Sudan has been reported using ELISA (Pfahler et al., 1986), in which semi-purified CMLV antigen was employed. This was employed to increase the specificity of ELISA for CMLV, which resulted in low background signal with the negative control camel sera (Azwai et al., 1996).

2.9.5 Western blot analysis

Western blotting is applied for the confirmation of the specificity of the ELISA. When comparing the blotting pattern of MPXV, VACV, BPXV, CPXV and ectromelia viruses (Arita et al., 1977; Novembre et al., 1989), three characteristic bands of MW 23, 31 and 35 kDa for CMLV were observed. These bands enable differentiation of CMLV from the above listed OPXVs. CMLV can be distinguished from the VACV group by the absence of a 23 kDa protein band and is distinguished from all others by possessing 31 and 35 kDa protein bands. MPXV (human and monkey isolates) lacks 23 kDa CMLV band, while it has been found in other OPXVs. 31 and 35 kDa proteins are present in all CMLV-positive camel sera (Azwai et al., 1996).

2.9.6 Restriction enzyme profiling

Restriction enzyme (RE) analysis of viral genomic DNA enables the comparison of CMLV isolates. The genome of CMLV is completely sequenced as the virus is closest to VARV (Afonso et al., 2002; Gubse et al., 2007). Examination of the genome organization of CMLV has revealed a distinct pattern after *Hind III* digestion (Fenner et al., 1990). Comparative RE studies of genomic DNA of five isolates of camels from different geographical locations of Africa and Asia using *Hind III* and *Xho I*, demonstrated minor differences between them (Renner-Müller et al., 1995; Pfeffer et al., 1996). This finding was similar to the conclusions reported for CMLV isolates from Dubai, however, it was contrary to the RE profile of VACV strains (Pfeffer et al., 1996). Nonetheless, no major differences have been found between these isolates and the vaccine strain so far (Wernery et al., 1997).

2.9.7 Polymarase Chain Reaction

PCR is a fast and sensitive method for the detection of OPXV DNA, which can allow the detection of even a few copies of viral DNA from the clinical samples (Nagarajan et al., 2011). PCR assays developed targeting various genes, including A-type inclusion protein (ATIP) gene (yields 881 bp CMLV-specific amplicon), HA (Hemagglutinin) gene (yields a 1100 bp CMLV-specific amplicon) have been developed for genus-specific detection and differentiation of OPXV species by providing distinct amplicon sizes (Meyer et al., 1994). The amplification of genes could be achieved by using template DNA derived from homogenized skin biopsy and from infected Vero tissue cultures (Balamurugan et al., 2009). A PCR RFLP based on the variability of the A36R gene is also available to differentiate amongst OPXVs (Lapa et al., 2002).

Species-specific (CMLV) single PCR assays targeting tumor necrosis factor binding protein receptor-II (TNFR-II) gene were employed to confirm CMLV. The TNFR-II gene based PCR assay specific to CMLV (Lapa et al., 2002; Marodam et al., 2006). Furthermore, PCR assay based on the C18L gene (encoding ankyrin repeat protein) and DNA pol gene (DNA polymerase) have been developed, which yields a specific amplicon of 243 bp of the C18L and 96-bp products of DNA pol genes, respectively, in CMLV suspected cases (Balamurugan et al., 2009).

2.9.8 Loop Mediated Isothermal Amplification (LAMP) Assay

LAMP is a rapid, simple, highly sensitive and cost-effective novel nucleic acid amplification technique that operates under isothermal conditions (60–65 °C). This assay standardized for amplification of CMLV DNA in 2012 (Venkatesan et al., 2012). As reported by the original developer of the test, it has great potential application in developing countries for diagnosis without requiring sophisticated equipment and skilled personnel (Notomi et al., 2000). Since 2000, the LAMP has been used widely for the diagnosis of various diseases (Dhama et al., 2014). LAMP assay based on the highly conserved region of ankyrin repeat protein gene (*C18L*), which is specific only for CMLV, has been developed for the diagnosis of CMLV and evaluated using field clinical samples. The amplicon size of the LAMP product is 198 bp.

2.10 Antiviral therapy

While no specific post-exposure therapeutic approaches for camelpox infection has been established. However, application of antibiotics and administration of supplements may be useful to reduce the severity of the disease (Wernery & Kaaden, 2002).

Among the antiviral druges reported for treatment of diseases due to poxviruses, Cidofovir, CMX001 and ST-246 are strong inhibitors of CMLV replication in vitro. In mouse models of camelpox infection, cidofovir either formulated as cream or for systemic use protected animals from disease development and/or death. Additionally, CMX001 and ST-246 have the advantage of being orally available which may render them more attractive for veterinary applications.

Certain novel antiviral drugs are effective orally against pox viruses including CMLV. They target cellular enzymes [IMP dehydrogenase inhibitors, such as ribavirin, as well as the tyrosine kinase inhibitor (STI-571), also known as imatinib mesylate, or Gleevec] and viral enzymes including inhibitors of viral morphogenesis (TTP-6171) may also offer therapeutic benefit. Other ANPs (Smee et al., 2002) that show promise for treating poxvirus infections include HPMP-5-azaC, cHPMP-5-azaC and HDE-cHPMP-5-azaC (Duraffour et al., 2007). Despite these advancements, cidofovir-resistant forms of CMLV, CPXV, MPXV and VACV have been developed by prolonged passage of these viruses in Vero 76 cells in the presence of drug. Cidofovir resistance develops due to the mutation in the *E9L* gene (encodes for DNA polymerase) of VACV, highlighting the need for continuous research into alternative anti-viral strategies (Andrei et al., 2008).

2.11 Prevention and Control

Camel pox can be controlled or prevented by vaccination. Currently there are two types of vaccines, live attenuated and inactivated camel pox vaccines. Both vaccines are commercially available. Vaccination with live attenuated vaccine provides at least 6 years of protection; however a booster vaccination is recommended for young animals vaccinated before the age of 6-9 months. Inactivated vaccine offers 12 months protection, however the animals must receive a vaccination every year (OIE, 2021; Moss, 2007).

Currently, four camelpox vaccines are available worldwide (Duraffour et al., 2011). Recently, Punjab (India), the former USSR and the Arabian Bedouin have employed lactotherapy (the scarification of a mixture of milk and camelpox infected crusts) to control camelpox. When administered intradermally or subcutaneously to camels, a Saudi isolate of CMLV attenuated (Jouf-78 strain) in camel kidney cell cultures (CKCC) at passage level 78 was both safe and potent (at 103 TCID50) (Hafez et al., 1992). The Jordanian Vaccine Company (JOVAC) produces this Jouf-78 strain commercially as the OrthovacR vaccine, which is being in use currently in numerous countries. Russia, Egypt and Morocco have all used a similar kind of vaccine (apps.cfsph.iastate.edu). Additionally, Onderstepoort Biological Products (OBP) developed the Vero cell attenuated camelpox vaccine (Dubai camelpox attenuated vaccine) in the UAE using an isolate of UAE (strain CaPV298-2) (Azwai et al., 1996; Pfeffer et al., 1996) this vaccine is known as DucapoxR and produced by Highveld Biologicals, Republic of South Africa (RSA) (Wernery & Zachariah, 1999). Two animals received immunity from this vaccine for up to 6 years after vaccination. However, for young animals vaccinated before 6 months, a booster shot is recommended. Additionally, Mauritania has evaluated the attenuated CMLV strain VD47/25, which has been passaged 80 times in cell culture, as a vaccine against camelpox (Guerre & Medecine, 1996).

Similarly, to this, Morocco offers formalin inactivated aluminium hydroxide adjuvanted camelpox vaccine (CMLV strain T8-1984), which is said to provide protection for just a year (OIE, 2021). This vaccine is developed and distributed by Biopharma, is safe for both young and adult camels and has been demonstrated to produce CMLV neutralizing antibodies (El Harrak & Loutfi, 2000). However, for effective protection booster vaccination on annual basis is still necessary. Pregnant camels were proven to be safe for both the ‘‘DucapoxR’’ and inactivated camelpox vaccines (Khalafalla & And, 2003). However, these poxviral vaccines must be thermostable in order to be used in hot, arid regions where the disease frequently occurs.

CMLV is susceptible to a number of common disinfectants. Additionally, autoclaving, short term exposure to Ultra Violet light and boiling for at least 10 minutes can all destroy it. Similar to smallpox in humans, camel herders may employ these methods to reduce the risk of environmental contamination (Bray & Babiuk, 2011). To achieve effective camelpox control biosecurity measures such as strict quarantine procedures, movement

restrictions and proper disinfection must be implemented, particularly in endemic regions. The ‘’Ring Vaccination’’ strategy, which involves vaccinating camels in and around outbreak zones, has been proposed as an effective containment method (Moss, 2007).

3. METHODOLOGY

3.1. Study Area

The study was conducted from October 2024 to May 2025 in selected camel herds in the Borana lowlands of Southern Ethiopia, specifically in villages of the Borana zone. Borana is located in the lowlands of the Southern part of Oromia, Ethiopia, and is characterized by a semi-arid to arid climate with bimodal rainfall distribution. The long rainy season occurs from March to May, while the short rainy season is from September to November. However, rainfall has become increasingly erratic, leading to frequent droughts and variability in livestock production (Mulugeta, 2023).

The study area, which is the town of Yabello (which lies at a latitude of 4°53'N and longitude of 38°5'E, 565 km south of Addis Ababa at an elevation of 1857 meters above sea level), is representative of typical pastoral settings where livestock, particularly cattle, goats, sheep and camels, are raised together (Hunduma et al., 2024). Pastoralists in the area heavily rely on livestock for their livelihood, with about 70% of the population practicing pastoralism. In response to the increasing aridity, many Borana pastoralists have diversified their herds by raising more drought-resilient animals such as camels, goats and sheep (Megersa et al., 2014).

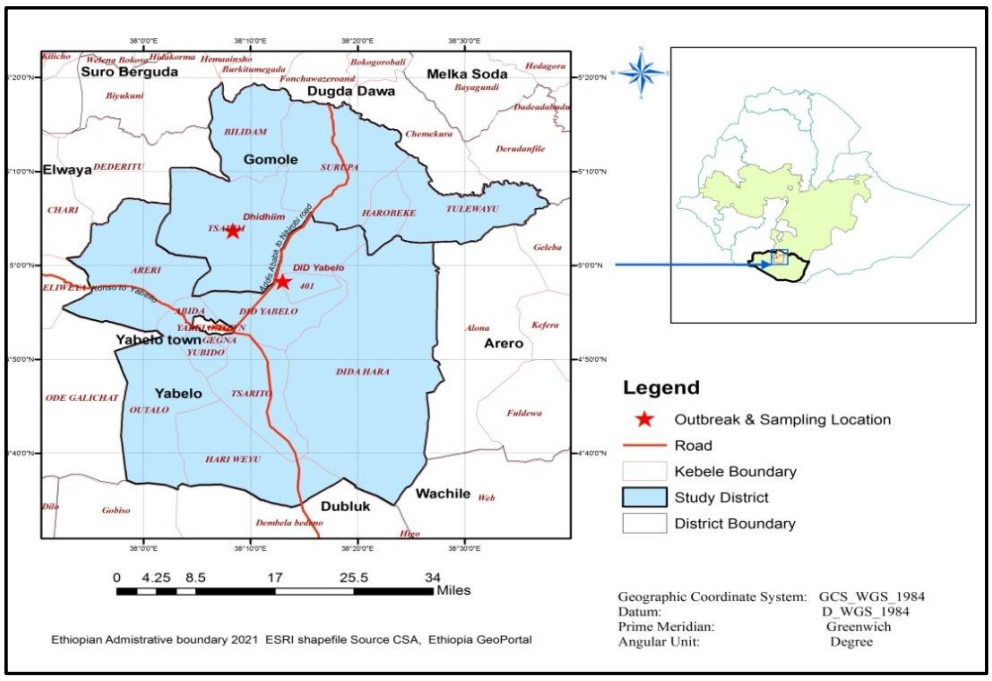

Figure 2: Map showing location of camelpox outbreaks and sampled area

The Borana community includes both pastoralists and agro-pastoralists, which primarily rely on combination of livestock rearing and crop cultivation to sustain their livelihood. The region experiences frequent cross-border interactions with neighbouring Kenya and Somalia through grazing, livestock trade and smuggling of animal and human drugs. Animal husbandry in the region follows an extensive system, with seasonal mobility and herd management strategies that may involve moving herds to better forage or water points, or splitting herds by keeping lactating and young animals near homesteads while the rest move to distant areas for grazing (Mulugeta, 2023).

3.2 Study Design and Study Population

The objective of the study was to investigate camelpox outbreaks in camels that occurred in October 2024, in a particular area Yabello, Borena: namely Dhadiim and Dida Yabello districts. According to outbreaks reports from the district animal health services office, the study population consisted of specifically selected herds with camels. Camels were examined for clinical symptoms of CMLV, such as fever, skin nodules, swollen lymph nodes and lacrimation, and camels manifesting these signs considered positive for CMLV. The age, sex and vaccination history of the animals were also documented. An outbreak investigation approach was used in the study to assess the disease incidence and fatalities in the affected camel herds. Data were gathered through collection techniques observation and interviews with camel owners and experts in animal health. Camels suspected with CMLV were purposively sampled. Descriptive statistics were used to assess the collected data in order to determine the disease's incidence, mortality and case fatality rates in the affected population.

3.3 Clinical Evaluation of Diseased Animals

Clinical data were collected with the help of district animal health workers that are based in Yabello. Camelpox outbreaks were reported in 12 PAs. 46 diseased camels and 13 deaths were reported to National Veterinary Institute in October 2024. While young camels are severely affected, adults have also been observed to suffer from the disease. Cases of abortion were also reported in some PAs. During clinical examination camels with typical pox lesions; macules, pustules and scabs involving most of the body with more lesions around the eyes, lips, nares, the thighs and the upper neck region were encountered. All

affected camels were thoroughly inspected. Samples were taken from skin nodules and nasal swabs and tested for CMLV using routine diagnostic methods such as both conventional polymerase chain reaction (PCR), RT-PCR, and virus isolation. More detailed data of the affected herds were summarized in (Table 2).

3.4 Sample Collection

A total of 19 samples: 5 scabs from skin lesions and 14 swab samples were collected from infected camels in two villages from 2 PAs in Yabello, Borena Zone, Oromia Region, specifically from Dhadiim and Dida Yabello, as part of a study on camelpox outbreaks. These villages were selected due to frequent reports of camelpox disease from 12 PAs to the National Veterinary Institute, with herds showing a history of camelpox incidents. Camels exhibiting clinical signs of camelpox, such as fever and skin lesions, these lesions first appear on the head, eyelids, nostrils and the margins of the ears. In severe cases, the whole head may be swollen. In addition to skin scabs, nasal swab samples were collected aseptically from infected animals. Skin scalp samples were collected by incising the nodule using a sterile surgical scalpel blade by holding the tissue with forceps. Swab samples were directly collected from nasal cavity using sterile swab. All collected samples were labelled with relevant details, including location, date and clinical signs. The specimens were sent to the National Veterinary Institute in Bishoftu using viral transport medium (VNT) supplemented with 10% antibiotics in universal and special tubes on ice in a cool box and stored at -20°C before testing or at -70°C if longer storage was required. All samples were collected in accordance with the procedures approved by the Institutional Animal Care and Use Committee of AAU-CVMA (Approval No. VM/ERC/04/6617/2025) while adhering to established animal welfare guidelines.

3.5 Virus Isolation

3.5.1 Sample preparation

Skin scrapping samples were washed three times in sterile PBS containing antibiotics and antifungal. 1 gm of sample was grounded using sterile pestle and mortar with addition of 10 ml of PBS supplemented with 1% penicillin-streptomycin, and 0.3% Amphotericin B. The resulting 10% (w/v) tissue homogenate was freeze-thawed 2-3 times to enhance the viral release from the cells. The mixture was centrifuged at 1000×g for 10 minutes and the

supernatant was carefully collected. To minimize bacterial contamination, the supernatant was filtered through 0.45μm syringe filter (Millipore, USA). The swab samples were clarified by centrifugation at 1000 ×g for 10 minutes and processed in similar manner as the skin scraping. All samples were handled following WOAH standard operating procedures (OIE, 2021).

3.5.2 Virus isolation using Vero cell

The supernatant of pathological tissue and swab homogenates were collected, and 1 mL was inoculated onto cells in 25 cm^2 tissue culture flasks, with one well maintained uninoculated as cell control per plate, which were subsequently supplemented with growth media (Glasgow Minimum Essential Medium with 2% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 0.3% Amphotericin B). The cultures were incubated at 37°C, 5% CO_2, and monitored daily for the appearance of cytopathic effects (CPE) for 5-7 days (OIE, 2021). The samples were frozen upon observing CPE, and samples that did not show CPE after three blind passages were considered negative.

3.6 Polymerase Chain Reaction

The pathological tissue and swab homogenates (10% w/v in PBS) were centrifuged at 1000 g for 10 min at +4 ^{O}C. DNA was extracted directly from the supernatant of clinical specimens using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. The eluted DNA was labeled and stored at 20 ^{O}C until further testing

PCR Amplification of the CAMPOX-Spe gene DNA extraction was performed using a Qiagen extraction kit according to the manufacturer's instruction. 200μl of camelpox infected skin scrap cell suspension was added to a clean tube, followed by addition of 20 μl of proteinase K and 200μl of AL buffer. The mixture was vortexed for 15 seconds and incubated at 60℃ for 10 minutes to lyse the cells and release viral DNA. After incubation, 200 μl of 96-100% ethanol was added, and the solution was homogenized. Then, the suspension was transferred to mini spin column, and centrifuged at 8,000 rpm for 1 minute, discarding the flow-through. Next 500 μl washing buffer (AW1) was added to the column and centrifuged at 8,000 rpm for1 minute. The flow through was discarded. This followed

by the addition of 500 µl AW2 washing buffer, with subsequent centrifugation at 14000 rpm for 3 minutes to thoroughly wash the DNA. The mini spin column was placed in the 2 ml collection tube and centrifuged again for 1 minute at 14000 rpm to dry the column matrix. Finally, DNA was eluted by adding 50µl elution buffer and centrifuging at 8000 rpm for 1 minute. The eluted DNA was stored at -20℃ until further processing.

PCR assay was done as described by (Meyer et al., 1994; WOAH, 2021) allows the detection and differentiation of species of the genus orthopoxvirus because of the size differences of the amplicons. Using the primer pair: CAMPOX-Spe-Fow-5'-AAT-ACA-AGG-AGG-ATC-T-3' and CAMPOX-Spe-Rev-5'-CTT-AAC-TTT-TTC-TTT-CTC-3' the gene sequence encoding the ATIP was amplified. The size of the PCR product, specific for the CMLV, is 881 bp.

DNA amplification was carried out using a Qiagen PCR kit that contained a PCR premix microtube. The PCR reaction was carried out in a final volume of 20 µl containing 2 µl of each primer, 3 µl of DNA template and an appropriate volume of nuclease-free water. The samples were incubated in a thermal cycler using the following conditions: 5 minutes at 95 ^{o}C (initial denaturation step), followed by 35 cycles of 1 minute at 95 ^{o}C, 1 minute at 55 ^{o}C and 1.5 minutes at 72 ^{o}C, and a final elongation step of 7 minutes at 72 ^{o}C. The temperature was then held at 4 ^{o}C until analysis.

Ten microliters of the PCR samples products were mixed with a 6X loading buffer and loaded onto 1.5% agarose in TBE (Tris/Borate/EDTA) buffer stained with GelRed. A 100 bp DNA molecular marker ladder was loaded into the first lane for size comparison. After that, the gel was subjected to electrophoresis at 100 volts for approximately 60 minutes. Gel documentation using (UV transilluminator, UVI TEC, UK) was used to visualize the DNA bands after electrophoresis, enabling the estimation of PCR product sizes through comparison with the bands of the molecular marker (OIE, 2021).

3.7 RT-PCR

quantitative PCR (qPCR) which is HRM multiplex real-time assays was utilized to differentiate between poxviruses based on high-resolution melting curve analysis of PCR amplicons produced using genus specific primer pairs and double stranded DNA binding dye and discrimination is based on the differences in fragment size and GC content. The method generates three well separated melting regions for each genus (*Orthopoxvirus, Capripoxvirus,* and *Parapoxvirus)* and provides additional genotyping of the viruses within each of the three genera (cowpox virus and camelpox virus in the *Orthopoxvirus* genus; GTPV, SPPV, and LSDV in the *Capripoxvirus* genus; ORFV, PCPV, and BPSV in the *Parapoxvirus* genus) described by (Gelaye et al., 2017). The reaction mix was prepared as follows: 2 µL of DNA template, 10 µL of 2×SsoFast™ EvaGreen® Supermix (Bio-Rad), and 0.4 µM each of primer and ddH2O, to a final reaction volume of 20 µL. Positive control plasmids representing orthopoxviruses (CMLV- Hadow/01/2012), and a negative control composed of nuclease-free water were included in each run.

The PCR reactions and melting curve analysis were performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories), following the condition previously described by (Gelaye et al., 2017) with a slight modifications. Briefly, the initial denaturation step at 95 °C for 4 minutes was followed by 40 cycles of 1 s at 95 °C, 2 s at 59 °C and 2 s at 72 °C. The PCR products were then denatured at 95 °C for 30 s, cooled at 60 °C for 60 s, and melted from 65 °C to 85 °C with a temperature increment of 0.2 °C every ten seconds and a continuous data recording. The data was analysed using the CFX Maestro Software (Bio-Rad) and the Precision Melt Analysis Software (Bio-Rad).

Table 1: Primers used in this study for the HRM assay

Method	Primer name	Primer sequence	Amplicon size	Target and references
HRM	CaPV-HRM-For	TCCTGGCATTTTAAGTAATGGT	100	Capripoxviruses (Gelaye et al., 2017)
	CaPV-HRM-Rev	GTCAGATATAAACCCGGCAAGTG		
HRM	PPV-HRM-For	TCGAAGATCTTGTCCAGGAAG	112	Parapoxviruses (Gelaye et al., 2017)
	PPV-HRM-Rev	CCGAGAAGATCAACGAGGTC		
HRM	OPV-HRM-For	AGGACTAGCCGCGGTAACTTT	56	Orthopoxviruses (Gelaye et al., 2017)
	OPV-HRM-Rev	ACAAGATAGAAGCGATGGATACTT		

3.8 Sequencing and Phylogenetic Analysis

All PCR products were separated by electrophoresis on a 1.5% agarose gel at 100 V for 1 h. The positive PCR products were purified using the Wizard SV Gel and PCR clean-up system kit (Promega) according to the manufacturer's instructions (Appendix 6), the purified products were quantified spectrophotometrically using the NanoDrop 2000 spectrophotometer (Thermo Scientific) then sequenced commercially by LGC Genomics (Germany).

The obtained nucleotide sequences were properly trimmed with Finch Tv (version 1.4.0). The resulting sequences were compared with the CMLV reference virus sequence (Accession No.: MK910851) through NCBI BLAST. Multiple sequence alignments were performed on BioEdit Software (version 7.2.5) using the ClustalW. The phylogenetic tree was constructed on MEGA Software (version 12) and inferred through the Maximum Likelihood method using Hasegawa-Kishino-Yano model program. The reliability of the tree was tested by performing 1,000 bootstrap replicates (Kumar et al., 2024).

3.9 Data Analysis

Clinical findings, disease incidence, mortality and case fatality rates among affected camel herds were summarized using descriptive statistics to analyze the collected data; the results were clearly presented in tables and figures for ease of understanding. Molecular diagnostic results such as PCR and RT-PCR outputs were interpreted based on expected amplicon sizes. Bioinformatics tools were used to align and compare sequenced data, and the phylogenetic analysis was determined through the construction of a maximum likelihood method tree using MEGA software. This analysis provided the genetic similarity between local isolates and reference CMLV strains from GenBank.

3.10 Ethical Statement

Ethical approval for the study was obtained from the Institutional Review Board of Addis Ababa University College of Veterinary Medicine and Agriculture (Reference No.: VM/ERC/04/6617/2025). We were committed to adhering to the institute's guidelines for ethical animal treatment as well as all relevant ISO and OIE regulations involving animals. Throughout the study, all the methodologies will align with these ethical and regulatory frameworks to ensure the highest levels of animal welfare and ethical conduct.

4. RESULTS

4.1 Clinical Observations

One-humped camels (Camelus dromedarius) reared in the two districts in Yabello, Ethiopia where camels made up 4-6% of the total herd on composition handled by the local pastoral communities, were found to have pox-like skin lesions. All age and sex groups had the same skin lesions. However, young camels of less than two years old had more severe lesions. High fever (39-40 ^{o}C) and nodular skin lesions, where the lesions spread to various body parts like head, neck, limbs, genitalia and perineum and inguinal regions were observed mostly in adult camels, which were the major camelpox clinical signs recorded (Figure 4). In general, the morbidity rate was 33.8% in the visited outbreak areas. Among 71 camels in Yabello, 24 (33.8%) cases were clinically sick of camelpox, with 2 severe cases and 21 recoveries (91.7%). Only one fatality (CFR = 4.2%) was recorded, despite all herds being vaccinated. The percentage of infected animals varied between herd ranging from 11.5%-60%.

Table 2: Information on the samples from which specimens were collected for this study

Owners name	**No of Animals**	**No of Diseased**	**% of Cases**	**Severe Cases**	**Recovered Cases**	**No of Deaths**	**CFR %**	**No of Samples**	**Vaccination History**
Herd 1	19	7	36.8	1	6	0	0.0	5	Vaccinated
Herd 2	15	9	60.0	0	8	0	0.0	8	Vaccinated
Herd 3	11	5	45.5	1	4	1	20	3	Vaccinated
Herd 4	26	3	11.5	0	3	0	0.0	3	Vaccinated
Total	**71**	**24**	**33.8**	**2**	**21**	**1**	**4.17**	**19**	**-**

4.2 Propagation Isolated Virus in African Green Monkey Cells (Vero Cell Lines)

All of PCR confirmed CMLV were inoculated on Vero cell line, the isolated CPV was produce the cytopathic effect CPE of CPV after the second passage 4-7 days post inoculation the CPE was characterized by cell rounding, syncytia, giant cell formation, aggregation and detachment of the cell sheet, nonetheless the control remained unchanged, as shown in (Fig. 3).

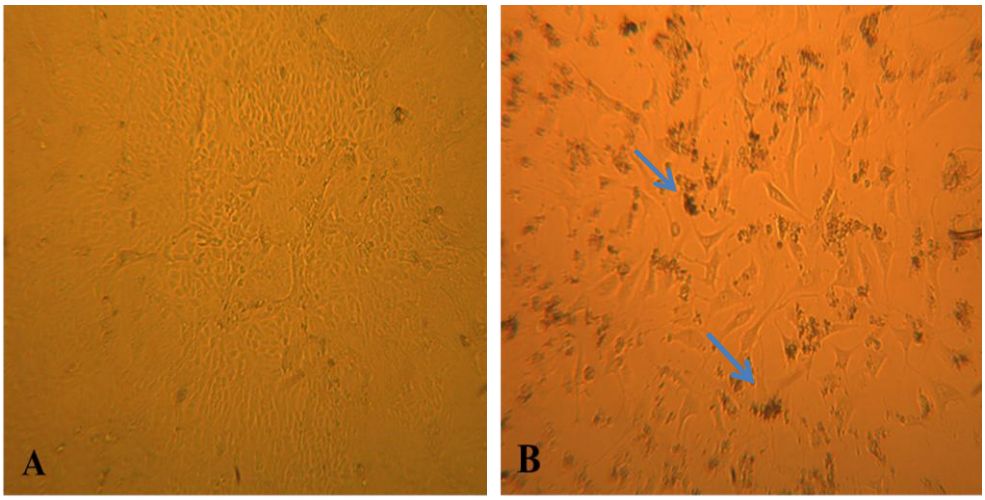

Figure 3: Virus isolation on Vero cell
Cytopathic effect was seen four days after inoculation (**B**), while uninfected cells maintained normal cell morphology (**A**).

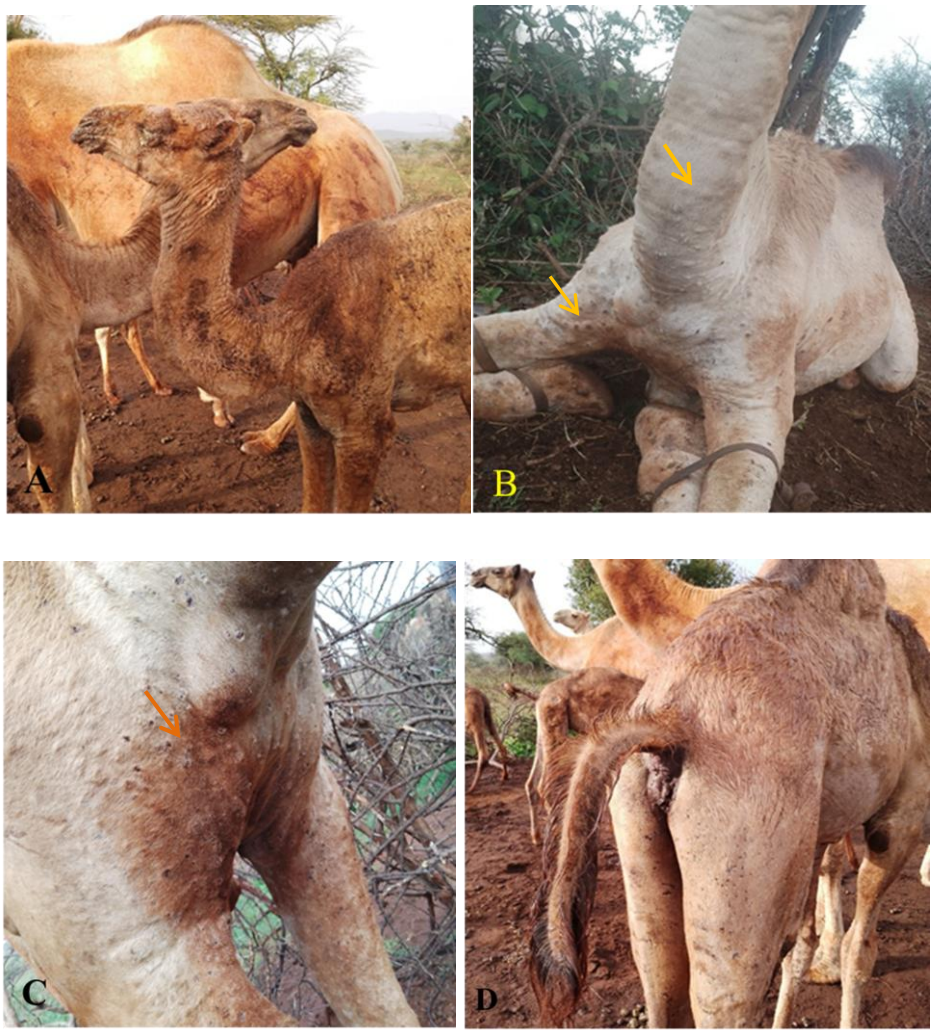

Figure 4: Clinical observations of camelpox specific lesions

4.3 Conventional PCR

Conventional PCR testing of skin scab and nasal swab from camels suspected of CMLV disease yielded amplification products of the expected size (881bp) corresponding to partial fragments of ATIP gene. Of the 19 representative clinical samples selected from a total of 71 suspected camelpox cases across two districts of Yabello, 5 skin scabs samples (26.3%) and 5 nasal swabs (26.3%) tested positive for camelpox virus genome. The 881bp PCR fragments were successfully amplified in 10 CMLV positive samples.

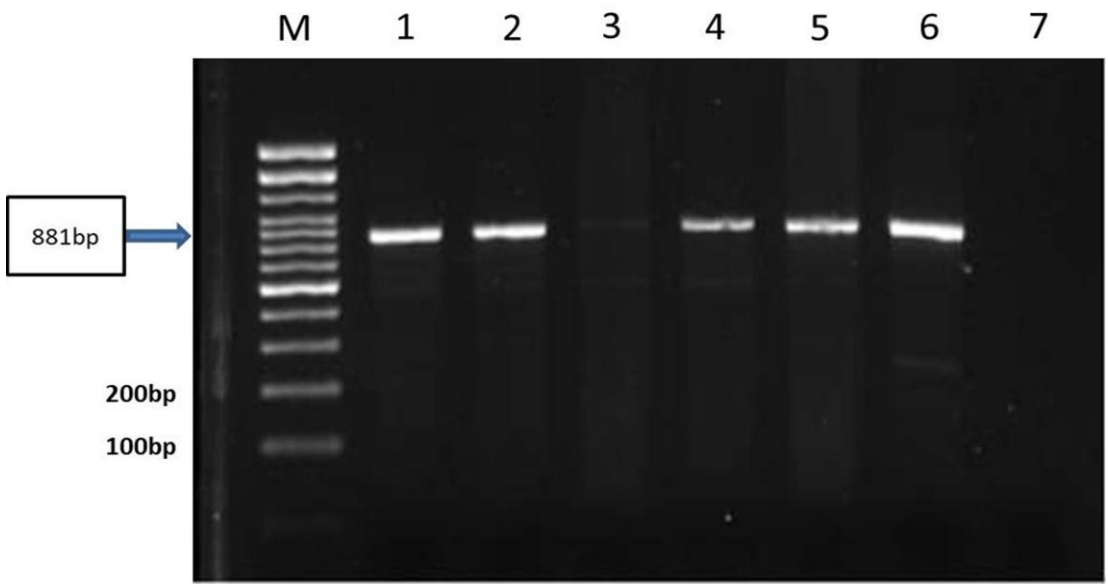

Figure 5: Results of PCR products of ATIP gene:

Lane M: DNA molecular marker, Lane 1-5 are skin nodules and swab samples from field, Lane 6: Positive control and Lane 7: Negative control

4.4 RT-PCR

We detected CMLV DNA in ten out of nineteen samples, with (52.6%) of the samples tested positives using the HRM Assay, of which five were tissue and five were swab samples. The melting temperature of the samples was recorded: CMLV *Tm* (73.00±0.20 °C). Figure 6 shows the amplification curves corresponding to CMLV in Borena, Yabello samples, there was no amplification corresponding to *Capripoxvirus* and *Parapoxvirus*.

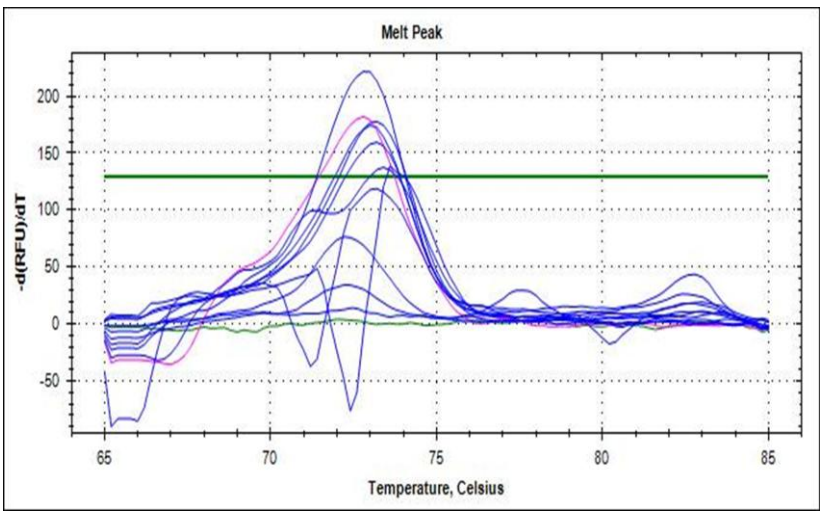

Figure 6: HRM detection of camelpox virus diseases

Skin and nasal swab samples of camels tested by HRM assay for ten samples. Red indicates positive control for CMLV displaying a unique melting peak; ten samples from Yabello, clustering with the control are shown in blue.

4.5 Molecular characterization and phylogenetic analysis

Of the ten positive samples, only five samples yielded sufficient amount of DNA for further sequencing. The sequences were analyzed and deposited in the GeneBank with the following (GeneBank accession numbers: PV737715-PV737719). These isolates revealed no nucleotide variation among themselves, showing that they are highly identical. The partial sequence of this gene showed 100 % nucleotide sequence similarity with the reference sequences of CMLV strains found in Mangystau oblast in Kazakhstan (GenBank accession number: AF438165), Iran (GenBank accession number: AY009089), CMLV strain 0408151v (GenBank accession number: KP768318) and CMLV genome (GenBank accession number: NC_003391).

Multiple nucleotide sequence alignment of the partial ATIP gene sequences of these five Ethiopian CMLV isolates, alongside homologous CMLV sequences retrieved from GenBank (Appendix 7) revealed a single nucleotide variation at position 448. Specifically (A:G) variation was observed at position 448 when compared to CMLV isolates from Israel (MK910851 and MZ300856), and previously reported Ethiopian CMLV isolates (KU705085-KU705110). Additionally, alignment with Sudanese isolates (KT931624 and KT931625) revealed two nucleotide mismatches A:G at nucleotide 6 and 448.

At amino acid level, all five isolates showed 100% identity with A-type inclusion protein partial genes of CMLV (GenBank accession numbers: AOC59220, AMR98480, AXO77476, AMR98476, AMR98478, QCW07459, NP_570534, Q05482) while minimum identity of 93.46% with MPXV gp137 protein of Monkey poxvirus (GenBank accession number: WNN25674).

A total of 58, ATIP gene sequences submitted to GenBank were used for phylogenetic analysis of CMLV isolated from Borena (Figure 7). The phylogenetic tree was constructed by maximum likelihood method using Hasegawa-Kishino-Yano in Mega 12 program including 1000 replica of bootstrap. The phylogenetic analysis revealed that all five isolates from Yabello, Borena clustered with CMLV strains 0408151v, M-96 and CMS than to other Orthopoxviruses with high bootstrap value.

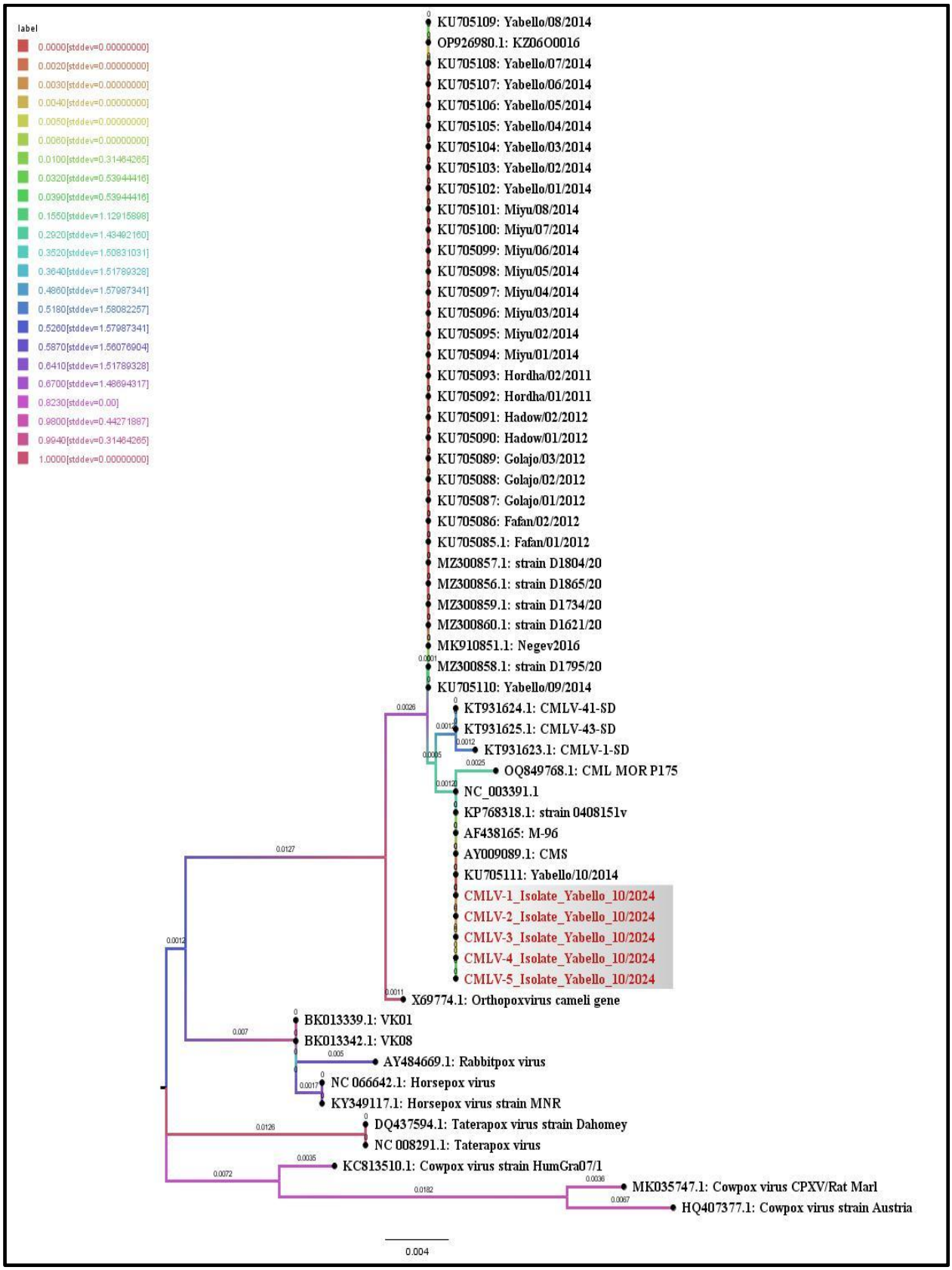

Figure 7: Phylogenetic analysis of camelpox isolates from Borena using ATIP gene sequences.

5. DISCUSSION

Camelpox virus in camels is caused by a smallpox-like illness known as camelpox disease. With the exception of the introduced dromedary camel in Australia and tylopods the disease is enzootic in almost all regions where camel husbandry is practiced (llamas and related species) in South America (Mosadeghhesari et al., 2014), and is responsible for severe economic losses. Although it is genetically the closest known virus to variola virus, the etiologic agent of smallpox, CMLV remains poorly studied (Duraffour et al., 2011). In the present study camelpox virus was successfully cultured and isolated molecularly detected and the ATIP gene was sequenced and the evolutionary relationship with other poxviruses were determined using samples from outbreak cases in affected pastoral associations in Yabello, Borena.

The present outbreak occurred in mid-October which is a short rainy month in the Borena Zone of Oromia. According to several researches, the incidence of more severe form of camelpox outbreaks increases during the rainy season (Ayelet et al., 2013; Khalafalla et al., 2015). Adult camels were primarily affected with the disease, which manifested skin lesions, typically on hairless areas such as the face, neck, genitalia, mammary glands and perineum, while in several cases, the lesions spread throughout the body. In the current study, the typical clinical symptoms of camelpox such as fever, anorexia, swollen lymph nodes, weakness, lack of appetite, abortion, mortality lacrimation and pustular skin lesions were observed which coincided with several reports by other investigators (Wernery & Kaaden, 2002; Kachhawaha et al., 2014; Motalab & Ahmed, 2014; Mosadeghhesari et al., 2014; Gatie, 2016; Aregawi & Feyissa, 2016).

The morbidity and mortality rate for the entire population were 33.8% and 1.4% respectively, but 4.17% (n = 1 out of 24) of the clinically affected camel died of generalized camelpox. This outbreak investigation result differs from that of Joseph et al. (2021), who reported morbidity and mortality rates of 1.10% and 0.10% respectively, in a large population of over 5000 dromedary camels in United Arab Emirates. The difference could arise from variation in sample size, herd management practice, population immunity or environmental conditions. While the UAE outbreak affected a highly controlled commercial herd with a history of sporadic occurrence, the present study involved small

camel herds in a pastoral system, where variability in immune response and poor pastoral management could have induced the disease. Additionally stress factors, parasites burdens and the timing of the outbreak within the rainy season could have contributed to increased severity and spread of the disease among the affected herds.

The present study's results interestingly showed that our primary attempt to isolate tissue specimens from the suspected camel pox lesions in Vero cells was successful, producing a typical cytopathic effect. The clear CPE formation in the Vero cells in the present study confirmed the report by Mosadeghhesari et al. (2014), who reported that virus from scab samples collected from clinically sick camels grows well in Vero cells. They demonstrated that the CPE produced by CMLV resulted in rounding of the cells, plaque formation, cytoplasmic elongation and multinucleated giant cell formation in all cell culture.

The confirmatory diagnosis of the suspected camelpox virus was made by conventional PCR performed according to the method described by Meyer et al. (1997) where specific 881 bp amplification products was obtained. These findings agree with some research reports (Meyer et al., 1997; Ali et al., 2009 ; Mosadeghhesari et al., 2014; Al-Bayati et al., 2022) in using PCR ATIP position 881 bp were identified for all of their isolates. In contrast Yousif & Al-Naeem (2012) reported the recovery of about 1500 bp from camelpox vaccine strain at the same position, attributing the difference to sequence variation or insertion events within the target region, possibly linked to vaccine attenuation. Similarly, Ayelet et al. (2013) reported comparable variations, reporting that five of their Afar region samples produced a shorter sequence (about 486bp) length, amplified only by reverse primer, this shorter fragment might be due to occurrence of gene deletion or change at the point of forward primer attachment site.

Our findings showed the relevance of molecular methods for differential diagnosis and the management of pox diseases in camels. This robust HRM assay enabled not only the detection and differentiation camelpox virus from other *Orthopoxvirus*; but also other poxviruses i.e GTPV, SPPV, and LSDV in the *Capripoxvirus* genus; ORFV, PCPV, and B PSV in the *Parapoxvirus* genus in a single test that would not have been discovered otherwise.

In the present study, multiple sequence alignment of the partial ATIP gene, we found that there is 100 % similarity between our samples and CMS and M-96, strain 0408151v, KU705111 which is local isolated reported by Gelaye et al. (2016) and one other camelpox with (Genebank accession number: NC_003391), and therefore our finding suggest that there is a close genetic relationship between the Ethiopian CMLV isolates and those from Central Asia and the Middle East. Notably, the high degree of similarity with the isolates shows that the Ethiopian isolates may share common ancestral origin with these geographically distant strains. This aligns with the previous phylogenetic observation reported by (Gelaye et al., 2016), wherein Ethiopian CMLV strains were clustered closely with those from Kazakhstan and Iran, supporting the idea of a relatively conserved global CMLV genome.

Despite the overall similarity, comparative analysis revealed specific nucleotide variations when our isolates were aligned with previously reported local and other global CMLV strains. A single nucleotide variation was observed at position 448: an (A:G) variation compared to previously reported Ethiopian isolates (KU705085-KU705110), and a similar variation was also observed when compared to the Israeli isolates (MK910851 and MZ300856). Furthermore, alignment with Sudanese isolates (KT931624 and KT931625) revealed two mismatches at position 6 (A:G) and 448 (A:G), indicating genetic divergence among CMLV strains.

A phylogenetic tree was constructed to determine the genetic relationships between the current Ethiopian field isolates identified in this study and other isolates retrieved from GenBank. The phylogenetic analysis revealed that the current Ethiopian isolates are distinct from previously reported Ethiopian strains described by Gelaye et al. (2016). Therefore, this highlights the presence of genetically related but evolutionarily unique local viral strains within Ethiopia. Therefore the molecular characterization presented in this study contributes valuable insights into the genetic diversity of CMLV in Ethiopia. These findings provide useful information for designing CMLV vaccine that can match strains of the virus that are circulating in Ethiopia.

6. CONCLUSION AND RECOMMENDATION

The present study confirmed an outbreak of camelpox among dromedary camels in Borena Zone, Ethiopia, through clinical evaluation, virus isolation using Vero cells, conventional PCR, RT-PCR, and molecular characterization of the ATIP gene. According to the genetic analysis of the study there is a high similarity between CMLV strains circulating in the study and those that have been previously reported in Kazakhstan and Iran, indicating a close phylogenetic relationship and possible trans-boundary transmission patterns.

This research highlights camelpox as one of the most significant viral diseases affecting camel herds in the pastoral regions of Ethiopia. This study not only provided the genetic evidence supporting the classification of Ethiopian CMLV isolates but also it has contributed valuable insights into their molecular characteristics. The molecular methods used as rapid, reliable diagnostic tool, helping accurate detections, characterization and diff -ereniation of CMLV from other Orthopoxviruses, Capripoxviruses and Parapoxviruses

The systemic form of the disease observed in the camels and the associated mortality underscore the importance of early diagnosis and intervention. The nomadic lifestyle of camel herd keepers coupled with limited diagnostic facilities and irregular disease reporting, contributes to the silent spread and persistence of the virus in endemic regions. The economic impact of camelpox is enormous which can lead to production losses, mortality, abortions and reduced milk yield.

Based on the above conclusion the following recommendations are forwarded

- Strengthening diagnostic capacity by equipping veterinary diagnostic facilities in camel rearing regions with molecular techniques such as PCR and RT-PCR to allow for early detection and confirmation of camelpox cases.
- To improve vaccination programs, start targeted vaccination campaigns before high risk periods such as the rainy season, prioritize young camels and areas with recurrent outbreaks.

- Training camel owners and pastoralists on biosecurity practices, hygiene and early disease recognition to improve herd health management. Encourage the isolation of healthy and infected animals and improve animal care during outbreaks.
- Encouraging regional collaboration (e.g., Somalia, Kenya, and Sudan) for coordinated disease control and research, particularly among shared pastoralist populations.
- To support research and vaccine development, molecular investigations should focus on whole-genome sequencing of local CMLV isolates to identify genetic diversity and vaccine escape mutations. This can help in the development of more effective, region-specific vaccines.
- To address public health and zoonotic concerns, camelpox is considered host-specific; however occasional human cases reported elsewhere require awareness campaigns and the preventative measures by the camel handlers and veterinarians.

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8. APPENDIX

Appendix 1: CELL CULTURE MEDIA

1.1. Growth media

Formulation

basal GMEM	80%
TPB	10%
fetal calf serum	10%
antibiotic solution (where applicable)	0.2% of the final formulation

Preparation procedure

1) weigh the required amount of GMEM/DMEM and $NaHCO_3$ according to the manufacturer's direction, and dissolve with distilled water
2) agitate thoroughly for about half an hour
3) adjust the final volume with distilled water
4) add the required amount of thawed fetal calf serum, TPB, and antibiotic solution (where applicable)
5) adjust the pH to 7.2 using 1M HCl and/or NaOH at room temperature
6) sterilize by filtration with 0.22 µm filter while dispensing aseptically into aliquots of 1 litre
7) incubate at 37°C for 48 hours to check for bacterial contaminants
8) put at room temperature till the 14th day to check for fungal contaminants
9) store at 4°C until use

Appendix 2: BALANCED SALT SOLUTIONS

2.1. Phosphate-buffered saline (PBS)

Formulation (for 1 litre)

NaCl	8 g
KCl	0.2 g
KH_2PO_4	0.24 g
$Na_2HPO_4.7H_2O$	2.16 g
or $Na_2HPO_4.2H_2O$	1.44 g

or $Na_2HPO_4.12H_2O$	2.76 g
Distilled water	added until final volume reaches 1000 ml

Preparation procedure

1) weigh the required amount of each of the ingredients and dissolve with distilled water
2) agitate thoroughly for about half an hour
3) adjust the final volume with distilled water
4) adjust the pH to 7.2 at room temperature using 1M HCl and/or NaOH
5) dispense into appropriate volumes
6) sterilize by autoclaving at 121°C for 15 minutes
7) store at room temperature until use

Appendix 3: ENZYME SOLUTIONS

3.1. Trypsin 2.5% (w/v) stock solution

Formulation (for 1 litre)

NaCl	8 g
KCl	0.4 g
Na_2HPO_4	0.0475 g
KH_2PO_4	0.06 g
$NaHCO_3$	0.35 g
trypsin (l:250)	25 g
distilled water	added until final volume reaches 1000 ml

Preparation procedure

1) weigh the required amount of each of the ingredients and dissolve with distilled water
2) agitate for about an hour
3) adjust the final volume with distilled water
4) adjust the pH to 7.8 at room temperature using 1M NaOH and/or HCl
5) sterilize by filtration with 0.22 µm filter while dispensing aseptically into appropriate volumes
6) store at -20°C until use

Appendix 4: ANTIBIOTICS

4.1. Penicillin-streptomycin (penstrep) stock solution

Formulation (for 100 ml)

penicillin G	5 vials of 10^6 IU
streptomycin sulfate salt	5 vials of 1 g
PBS	added until final volume reaches 100 ml

Preparation procedure

1) prepare the required amount of each of the constituents and mix them up well
2) sterilize by filtration with 0.22 µm filter while dispensing aseptically into appropriate volumes
3) store at -20°C until use
4) for use, add 1 ml of this solution into each 500 ml of medium/solution

4.2. Gentamicin

Formulation

gentamicin	5 vials of 10^6 IU
PBS	added until final volume reaches 100 ml

Preparation procedure

Follow of section 4.1.

Appendix 5: Culture of Vero cell

Materials

- Frozen Vero cell stock (stored in liquid nitrogen or at -80°C)
- Dulbecco's modification Eagle medium (DMEM)
- supplemented with 10% heatinactivate ,fetal bovine serum (FBS)
- filter sterilized (see recipe)
- 15mL sterile conical tubes
- 25cm2 or 50cm2 sterile tissue culture flasks with vented caps
- Sterile serological pipets
- 70% ethanol solution (for decontamination)
- Water bath set at 37°C

- Humidified incubator set at 37°C with 5%CO2
- Centrifuge
- Laminar flow hood (for sterile work)

Preparation procedure

1. Quickly thaw vial (cryovial) of Vero cells by gently swirling in a 37°C water bath
2. Once thawed, decontaminate the vial by spraying with 70% ethanol
3. Transfer the Vero cell suspension from the cryovial into a 15mL conical tube containing 10mL of DMEM + 10% FBS to dilute and remove the DMSO
4. Centrifuge the tube at 200 × *g* for 5 minutes at room temperature
5. Remove and discard supernatant resuspend the cell pellets
6. Transfer Vero cell suspension to tissue culture flask with vented cap
7. Incubate flasks in 37°C incubator with 5% CO2
8. Monitor cells daily or every other day. Change media every 3-4 days.
9. When cell sreach a >90% confluent monolayer, passage cells into new tissue culture flasks.

Appendix 6: DNA Purification by Centrifugation

Gel Slice and PCR Product Preparation

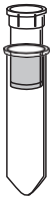
Dissolving the Gel Slice

Prepare gel slice or PCR product.

1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
2. Add 10µl of Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

Processing PCR Amplifications

3. Add an equal volume of Membrane Binding Solution to the PCR amplification.

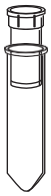


Add dissolved gel Mixture or prepared PCR product to SV Minicolumn assembly.

Binding of DNA

4. Insert SV Minicolumn into Collection Tube.
5. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
6. Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.

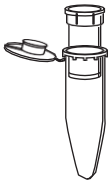
Centrifuge.



Wash, removing solution by centrifugation.

Washing

7. Add 700µl of Membrane Wash Solution (ethanol added). Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
8. Repeat Step 4 with 500µl of Membrane Wash Solution. Centrifuge at 16,000 × *g* for 5 minutes.
9. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.



Elute DNA.

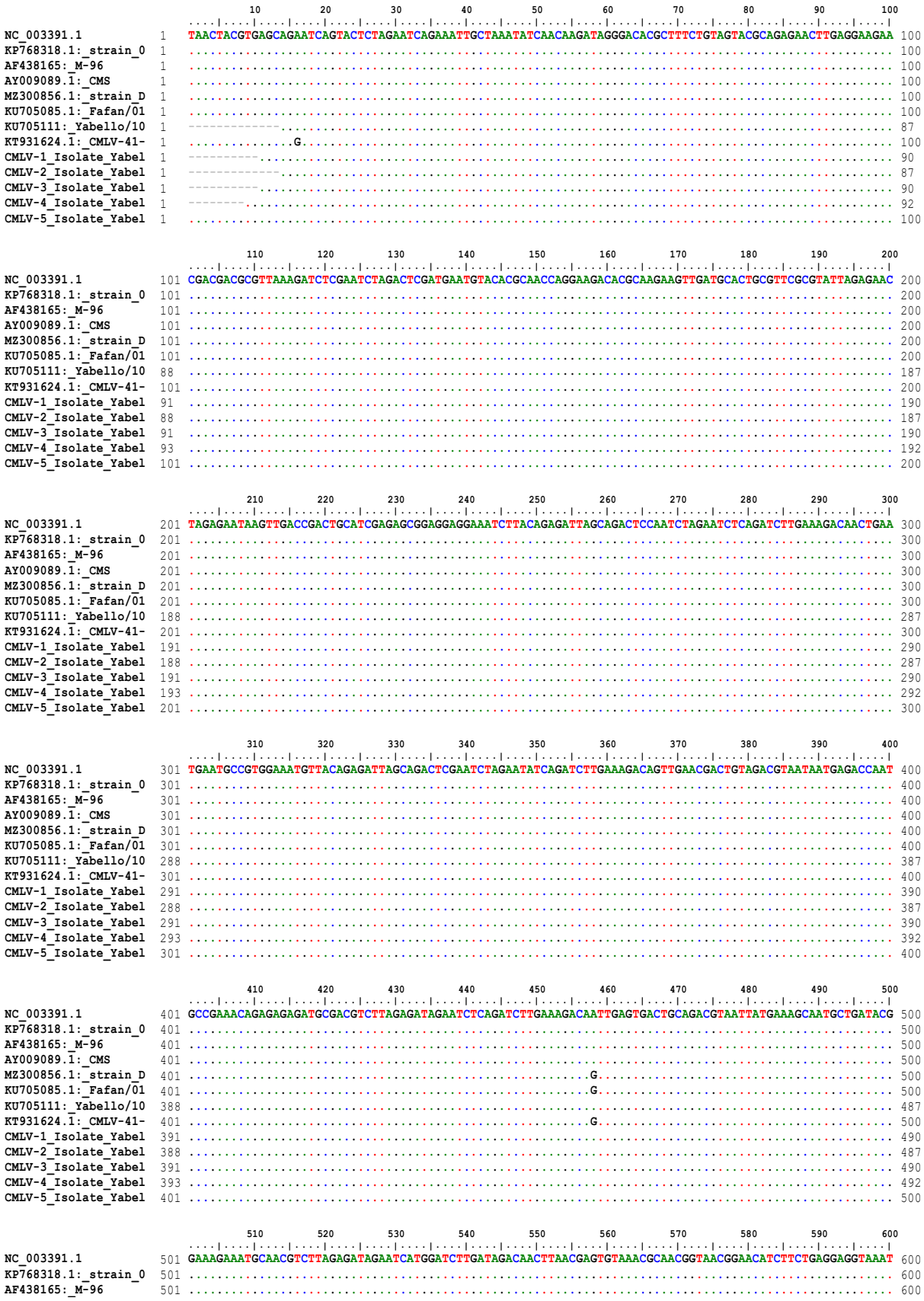
Elution

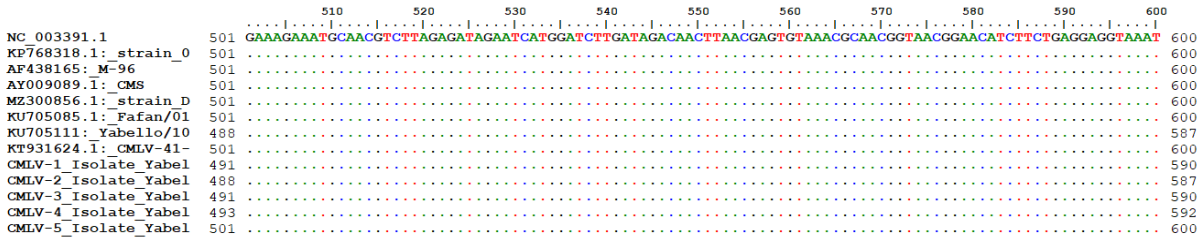
10. Carefully transfer Minicolumn to a new 1.5ml microcentrifuge tube.
11. Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × *g* for 1 minute.
12. Discard Minicolumn and store
13. DNA at 4°C or –20°C.

Appendix 7: List of Poxviruses sequences used for alignments

Organism	Isolate Name	Accession no.	Reference
Camelpox virus		NC_003391.1	(Afonso et al., 2002)
Camelpox virus	strain 0408151v	KP768318.1	
Camelpox virus	M-96	AF438165.1	(Afonso et al., 2002)
Camelpox virus	CMS	AY009089.1	(Gubser & Smith, 2002)
Camelpox virus	Negev2016	MK910851.1	(Israeli et al., 2019)
Camelpox virus	D1795/20	MZ300858.1	(Joseph et al., 2021)
Camelpox virus	D1621/20	MZ300860.1	(Joseph et al., 2021)
Camelpox virus	D1734/20	MZ300859.1	(Joseph et al., 2021)
Camelpox virus	D1865/20	MZ300856.1	(Joseph et al., 2021)
Camelpox virus	D1804/20	MZ300857.1	(Joseph et al., 2021)
Camelpox virus	CMLV-1-SD	KT931623.1	
Camelpox virus	CMLV-41-SD	KT931624.1	
Camelpox virus	CMLV-43-SD	KT931625.1	(Khalafalla & Abdelazim, 2017)
Camelpox virus	CML MOR P175	OQ849768.1	(Bamouh et al., 2022)
Camelpox virus		KU705085.1-KU705111	(Gelaye et al., 2016)
Orthopoxvirus cameli		X69774.1	(Meyer et al., 1994)
Camelpox virus	KZ06O0016	OP926980.1	
Vaccinia virus	VK01	BK013339.1	(Brinkmann et al., 2020)
Camelpox virus	CMLV-1, Yabello/ETH/10-2024	PV737715	This study
Camelpox virus	CMLV-2, Yabello/ETH/10-2024	PV737716	This study
Camelpox virus	CMLV-3, Yabello/ETH/10-2024	PV737717	This study
Camelpox virus	CMLV-4, Yabello/ETH/10-2024	PV737718	This study
Camelpox virus	CMLV-5, Yabello/ETH/10-2024	PV737719	This study
Vaccinia virus	VK08	BK013342.1	(Brinkmann et al., 2020)
Horsepox virus		NC_066642.1	(Tulman et al., 2006)
Horsepox virus	strain_MNR	KY349117.1	(Noyce et al., 2018)
Rabbitpox virus		AY484669.1	(Li et al., 2005)
Cowpox virus	strain_HumGra07/1	KC813510.1	(Dabrowski et al., 2013)
Taterapox virus	strain_Dahomey	DQ437594.1	(Esposito et al., 2006)
Taterapox virus	Taterapox virus	NC_008291.1	(Esposito et al., 2006)
Cowpox virus	CPXV/Rat Marl	MK035747.1	(Antwerpen et al., 2019)
Cowpox virus	strain Austria 1999	HQ407377.1	(Carroll et al., 2011)

Appendix 8: Plot identity of ATIP gene nucleotide sequences (881bp) CMLV of the current isolates in comparison with the previously sequenced CMLVs retrieved from GenBank.

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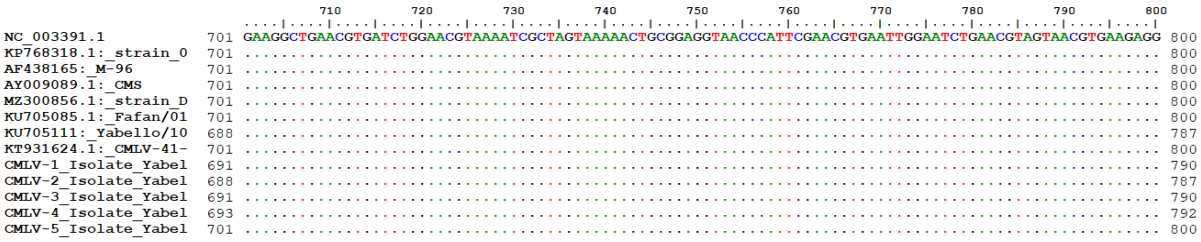
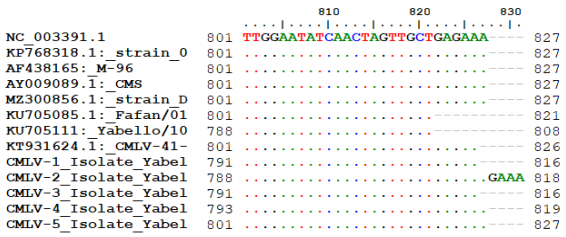
NC_003391.1 501 GAAAGAAATGCAACGTCTTAGAGATAGAATCATGGATCTTGATAGACAACTTAACGAGTGTAAACGCAACGGTAACGGAACATCTTCTGAGGAGGTAAAT 600
KP768318.1:_strain_0 501 600
AF438165:_M-96 501 600
AY009089.1:_CMS 501 600
MZ300856.1:_strain_D 501 600
KU705085.1:_Fafan/01 501 600
KU705111:_Yabello/10 488 587
KT931624.1:_CMLV-41- 501 600
CMLV-1_Isolate_Yabel 491 590
CMLV-2_Isolate_Yabel 488 587
CMLV-3_Isolate_Yabel 491 590
CMLV-4_Isolate_Yabel 493 592
CMLV-5_Isolate_Yabel 501 600


710 720 730 740 750 760 770 780 790 800
NC_003391.1 701 GAAGGCTGAACGTGATCTGGAACGTAAAATCGCTAGTAAAAACTGCGGAGGTAACCCATTCGAACGTGAATTGGAATCTGAACGTAGTAACGTGAAGAGG 800
KP768318.1:_strain_0 701 800
AF438165:_M-96 701 800
AY009089.1:_CMS 701 800
MZ300856.1:_strain_D 701 800
KU705085.1:_Fafan/01 701 800
KU705111:_Yabello/10 688 787
KT931624.1:_CMLV-41- 701 800
CMLV-1_Isolate_Yabel 691 790
CMLV-2_Isolate_Yabel 688 787
CMLV-3_Isolate_Yabel 691 790
CMLV-4_Isolate_Yabel 693 792
CMLV-5_Isolate_Yabel 701 800


810 820 830
NC_003391.1 801 TTGGAATATCAACTAGTTGCTGAGAAA---- 827
KP768318.1:_strain_0 801 ----- 827
AF438165:_M-96 801 ----- 827
AY009089.1:_CMS 801 ----- 827
MZ300856.1:_strain_D 801 ----- 827
KU705085.1:_Fafan/01 801 ----- 821
KU705111:_Yabello/10 788 ----- 808
KT931624.1:_CMLV-41- 801 ----- 826
CMLV-1_Isolate_Yabel 791 ----- 816
CMLV-2_Isolate_Yabel 788 GAAA 818
CMLV-3_Isolate_Yabel 791 ----- 816
CMLV-4_Isolate_Yabel 793 ----- 819
CMLV-5_Isolate_Yabel 801 ----- 827

Appendix 9: Ethical Approval

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

Ethical clearance certificate

Certificate Ref. No: VM/ERC/04/66/17/2025

Name of Applicant: **Abdurezak Abrar Reda** (BSc in Vet. Lab. Tech, MSc student)

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

Title of the project: *Isolation and molecular characterization of camelpox virus from outbreak cases in Borena, Ethiopia*

Date of application:	**December, 2024**
Nature of the project:	**Field investigation**
Target animal species:	**Camels**
Number of animals involved:	**19**
Study area:	**Borena, Ethiopia**

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

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Abdurezak Abrar Reda.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

Addis Ababa University College of Veterinary Medicine and Agriculture

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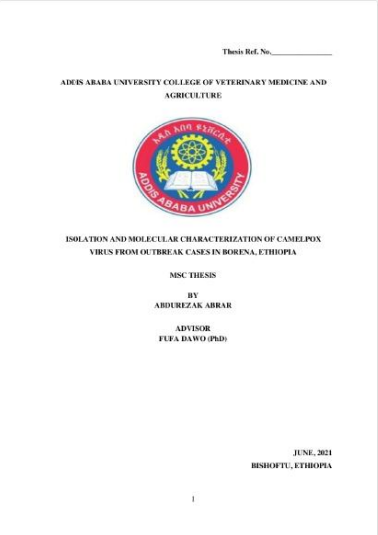

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ADUES ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

ISOLATION AND MOLECULAR CHARACTERIZATION OF CAMELPOX VIRUS FROM OUTBREAK CASES IN BORENA, ETHIOPIA

MSC THESIS

BY
ABDUREZAK ABRAR

ADVISOR
FUFA DAWO (PhD)

JUNE, 2021
BISHOFTU, ETHIOPIA

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