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OUTBREAK INVESTIGATION OF LUMPY SKIN DISEASE; ISOLATION AND MOLECULAR CHARACTERIZATION OF THE VIRUS IN SOUTH WOLLO ZONE, NORTHERN ETHIOPIA

MVSc Thesis

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OUTBREAK INVESTIGATION OF LUMPY SKIN DISEASE; ISOLATION AND MOLECULAR CHARACTERIZATION OF THE VIRUS IN SOUTH WOLLO ZONE, NORTHERN ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in veterinary Microbiology

By

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Bishoftu, Ethiopia

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As member of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Outbreak investigation of lumpy skin disease; isolation and molecular characterization of the virus in South Wollo zone, Northern Ethiopia** and recommended that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in Veterinary Microbiology.

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College of Veterinary Medicine and Agriculture, Debre Zeit

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LIST OF ABBREVIATIONS

AGID	Agar gel immuno diffusion
CaPV	Capripox virus
Cm	Centimeter
CO2	Carbon di oxide
CPE	Cytopathic effect
°C	Degree Celsius
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
ETB	Ethiopian birr
GDP	Gross domestic product
GMEM	Glasgow Minimum Essential Media
GTP	Goat pox
GTPV	Goat poxvirus
IFAT	Indirect fluorescent antibody test
KSGPV	Kenyan Sheep and Goat Pox Virus
LSD	Lumpy skin disease
LSDV	Lumpy skin disease virus
Mm	Millimeter
NAHDIC	National animal health diagnostic and investigation center
NVI	National Veterinary Institute
OA3.Ts	Lamb testis cell line
OIE	Office International des Epizooties
PBS	Phosphate-buffered saline

PCR	Polymerase Chain Reaction
rpm	Revolution per minute
SNNPR	Southern nations nationalities and people region
SPP	Sheep pox
SPPV	Sheep poxvirus
USD	United states Dollar

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ABSTRACT

Lumpy skin disease, caused by Lumpy skin disease virus is one of the most economically important cattle diseases in Ethiopia. The current study was conducted from October 2017 to May 2018 with the objective of outbreak investigation and isolation and molecular characterization of the virus from the outbreaks occurred in South Wollo zone. All ages and breeds of cattle from reported disease outbreaks were the study subjects. Cattle were examined for the presence of skin lesions and tissue samples were collected aseptically and transported to National Animal Health Diagnostic and Investigation Centre laboratory. Virus isolation was done by growing on Vero cells and molecular characterization was carried out by extracting DNA of the virus and the extract was subjected to real time polymerase chain reaction assay. Based on clinical examination of cattle at risk (350 cattle), 55 cattle were affected and 10 were died. The morbidity, mortality and case fatality rates were 15.71%, 2.86% and 18.18%, respectively. The highest morbidity (21.42%) was in Kalu/07 PA and lowest (11.76%) in Tehuledere/023 PA. Mortality and case fatality rates were higher in male than in female cattle (3.29 and 28.57%; 2.38 and 11.76% respectively). Morbidity was also higher in crossbred cattle with lower mortality and case fatality rates than local ones. Age wise higher morbidity was observed in younger (16.36%) than adult cattle (15.41%) and high morbidity rates regardless of vaccination status of the animals. Out of 20 samples10 were grown on Vero cells and all of them showed cytopathic effect due to virus infection and 17 samples were positive by Polymerase chain reaction. In conclusion, the disease caused high morbidity rates that could cause great loss to the farmers. Isolation and characterization of the virus from these outbreak cases shows the occurrence of the disease. Observation of the disease in vaccinated cattle shows concern on the efficacy of the vaccine. While prevention of movement of the diseased animals along with vector control and regular annual vaccinations play great role in disease control, further studies on the efficacy of the currently used vaccines and awareness creation for cattle owners are highly recommended.

Key Words: *Lumpy skin disease virus, virus isolation, Out-break investigation, Polymerase chain reaction, South Wollo*

1. INTRODUCTION

In Ethiopia, with the total of about 59.5 million cattle population, livestock production constitutes a vital part of the agricultural system and it accounts about 40% of the agricultural gross domestic product (GDP) (CSA, 2017; Gebreegziabhare, 2010)

Livestock diseases are the major production constraints in Ethiopia in addition to poor nutrition, low genetic potential of indigenous livestock, lack of marketing infrastructure and water shortages (Gebre Egziabhare, 2010). Lumpy skin disease (LSD) is one of the most economically important viral diseases listed as notifiable trans-boundary animal diseases by the World Organization for Animal Health (OIE) and the second significantly important cattle disease in Ethiopia (Gelaye *et al*., 2015; OIE, 2017).

Lumpy skin disease (LSD) which was occurred as new skin disease, referred as 'pseudo urticaria', of cattle was first reported in 1929 in Northern Rhodesia (now Zambia) from where the disease spreads to other southern African countries by the 1940s (Abdulqa *et al*., 2016). In Ethiopia, LSD was first reported in 1981 in the northwestern part of the country near lake Tana (Mebratu *et al*., 1984; Tuppurainen and Oura, 2011).

LSD is caused by LSD virus which is a member of Capri pox viruses (CaPVs) that are large double-stranded DNA viruses belonging to the family *Poxviridae*. The genus includes Sheep pox virus (SPPV), Goat pox virus (GTPV) and Lumpy skin disease virus (LSDV) (Facquet *et al*., 2005; Murphy, 2012). These viruses have genome of approximately 150 kb and share a high degree of sequence homology, with 97% identity between LSDV and both GTPV and SPPV genomes (Tulman *et al*., 2001).

CaPV infections are generally host specific and not reported on CaPV infecting all three species: sheep, goats and cattle (Bhanuprakash *et al*., 2010; Tuppurainen *et al*., 2014). They have also specific geographic distributions in which diseases of GTP and SPP are prevalent in Africa above the equator, Asia, the Middle East, and occasional outbreaks occur in regions of Europe surrounding the Middle East. In contrast, LSD is endemic in

Africa and outbreaks have been occurred in the Middle East countries surrounding Egypt and in some parts of Europe like Greece (Bhanuprakash *et al*., 2011; Tuppurainen *et al*., 2015; Babuik *et al*., 2008).

The mode of transmission of LSD has not been described fully but the biting flies and some tick species are probably the most important method of transmission of LSD and therefore, quarantine and movement control is usually not very effective (Abdulqa *et al*., 2016). The disease is usually more prevalent during wet summer and autumn months, particularly in low-land and mid land areas and around water courses, but outbreaks may also occur during the dry season and winter months (Coezer and Tuppurainen, 2004; Gari *et al*., 2010).

LSD is an acute, sub-acute or in apparent viral disease of cattle, characterized by fever, lacrimation and the sudden appearance of firm circumscribed skin nodules which undergo necrosis. Similar lesions may be present in the skeletal muscles and the mucosa of the digestive and respiratory tracts. Animals affected by capripox viruses (CaPVs) will eventually clear the infection and do not become carriers (Rao and Bandyopadhyay, 2000; Babuik *et al*., 2008; Gari *et al*., 2015).

LSD as a member of CaPVs, it has a single serotype, do not cause persistent infection, have a limited host range and vaccines are available that may provide long term immunity. These attributes increase the prospect of successfully implementing regional control programs, leading to the elimination of the virus and conceivably global eradication. Control and prevention of LSD in endemic countries like Ethiopia is mainly by vaccination. In Africa and the Middle East countries several live attenuated CaPV vaccine strains are currently used for cattle and small ruminants. These include LSDV Neethling strain, Kenyan sheep and goat pox virus (KSGPV) O-240 and O-180 strains, Yugoslavian RM65 SPP strain, Romanian SPP and Gorgan GTP strains (Gari *et al*., 2015; Babuik *et al*., 2008). In Ethiopia there are problems related to lack of vaccine efficacy and continuous outbreaks in vaccinated animals (Tilahun *et al*., 2014).

The disease is now the problem of almost all the regions and agro ecological zones of Ethiopia. A major outbreaks of LSD have been occurred in different regions of Ethiopia like Amhara and W/ Oromia Regions in 2000/2001, Oromia and Southern nations nationalities and people (SNNP) regions in 2003/2004 and Tigray, Amhara and Benishangul regions in 2006/2007 (Ayelet *et al*., 2013). LSD is an OIE listed disease because of considerable financial losses and in Ethiopia due to the endemic nature of LSD; the country is facing serious difficulties in exporting live cattle and their products. In addition, this situation contributes a negative impact on the national economic growth through the loss of meat and milk production and poor quality of skin and hides (Gelaye *et al*., 2015). Consequently continuous surveillance on the status of the disease and genetic information on circulating field viruses is mandatory in order to take effective measures for the control and there by eradication of the disease in the country (Body *et al*., 2011).Therefore the objectives of the current research were:

- To investigate LSD outbreaks in the three districts of South Wollo Zone
- To isolate the field virus responsible for the disease
- To characterize the virus by polymerase chain reaction

2. LITERATURE REVIEW

2.1. History

Lumpy skin disease was first described in Zambia in 1929 and it was considered to be the result of poisoning or a hypersensitivity to insect bites due to its clinical appearance. The disease was then spread to other African countries like Botswana (Bechuanaland), Zimbabwe (Southern Rhodesia) and the Republic of South Africa in the years between 1943 and 1945. During the following decades, LSD spreads slowly northwards and is currently present throughout the entire continent of Africa, including Madagascar but with some exceptional free countries like Libya, Algeria, Morocco and Tunisia (Abdulqa *et al*., 2016; Tuppurainen and Oura, 2011).

In East Africa LSD was identified in Kenya in 1957and Sudan in 1972 Sudan (Ali and Obeid 1977) and West Africa in 1974 while, it was spreading into Somalia in 1983. The first LSD outbreak to occur in Egypt was reported in May 1988. In Ethiopia, the disease was observed in North Western, Western and central regions between the years 1981 to 1983 with considerable morbidity and mortality rates (Mebratu *et al*., 1984).

Lumpy skin disease was limited to African continent until 1989 but later it moved outside Africa to Madagascar and the Middle East and caused a serious economic loss to the livestock production. Prior to 2012, only sporadic LSDV outbreaks were reported in the Middle East region (Tuppurainen and Oura, 2011).

2.2. Lumpy Skin Disease Virus

Lumpy skin disease is caused by Lumpy skin disease virus, one of the members of Capri pox viruses which are enveloped, brick shaped with complex symmetry, measuring 300x270x200 nm in size (Shakya, 2001). Mature *Capripoxvirions* have a more oval profile and larger lateral bodies than *Orthopoxvirions* (Abdulqa *et al*., 2016). These viruses are generally resistant to drying, survive freezing and thawing, and remain viable for months in the lyophilized state. Sensitivity to heat differs among strains (Rao and Bandyopadhyay, 2000).

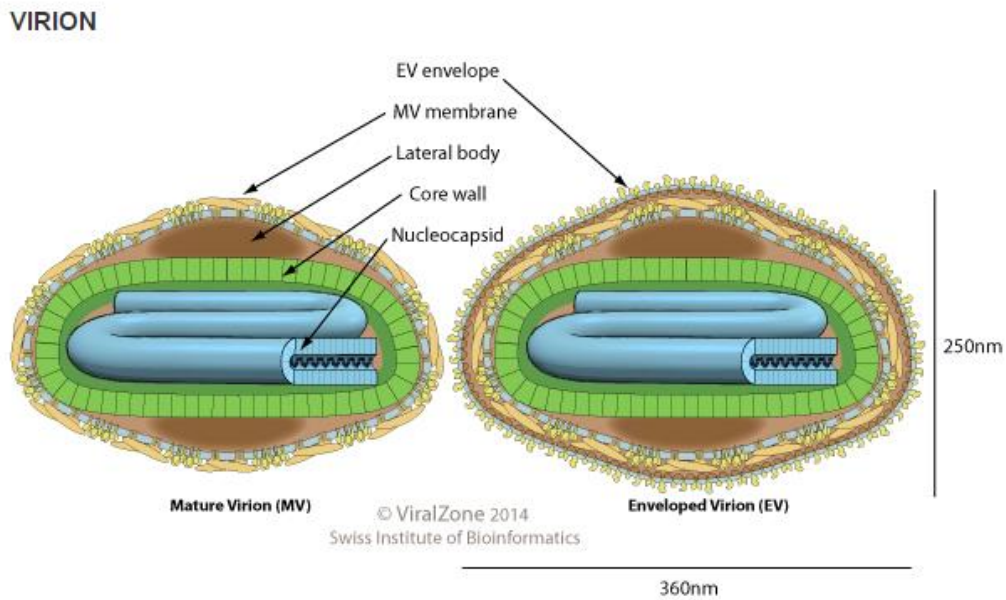

Figure 1: Capri pox virus structure (**Source**: viral zone, 2014)

CaPVs are double-stranded DNA viruses with genomes approximately 150 kbp in size. LSDV shares a close genetic relationship with SPPV and GTPV (Gelaye *et al*., 2015) but has an additional nine genes that are non-functional in SPP and GTP viruses, some of which are likely responsible for their ability to infect cattle (Tulman *et al*., 2001). CaPV isolates are extremely conserved with genome identities of at least 96% between SPPV, GTPV and LSDV (Tulman *et al*., 2001; Babuik *et al*., 2008).

LSDV genome consists of a central coding region which is bounded by identical 2.4 kbp-inverted terminal repeats and contains 156 putative genes. LSDV genes share a high degree of colinearity and amino acid identity (average of 65%) of its genomic region with genes of other known mammalian poxviruses like suipoxvirus, yatapoxvirus, and leporipoxviruses (Madhavan *et al*., 2016; Tulman *et al*., 2001).

Even if CaPVs share high nucleotide sequence identity, they are phylogenetically distinct. Phylogenetic analysis showed that members of the genus could be delineated into three distinct clusters of GTPV, SPPV and LSDV based on theP32genomic sequence. There is an additional aspartic acid at 55th position of P32 present in sheep poxvirus which is absent in GTP and LSD viruses (Hosamani *et al*., 2004).

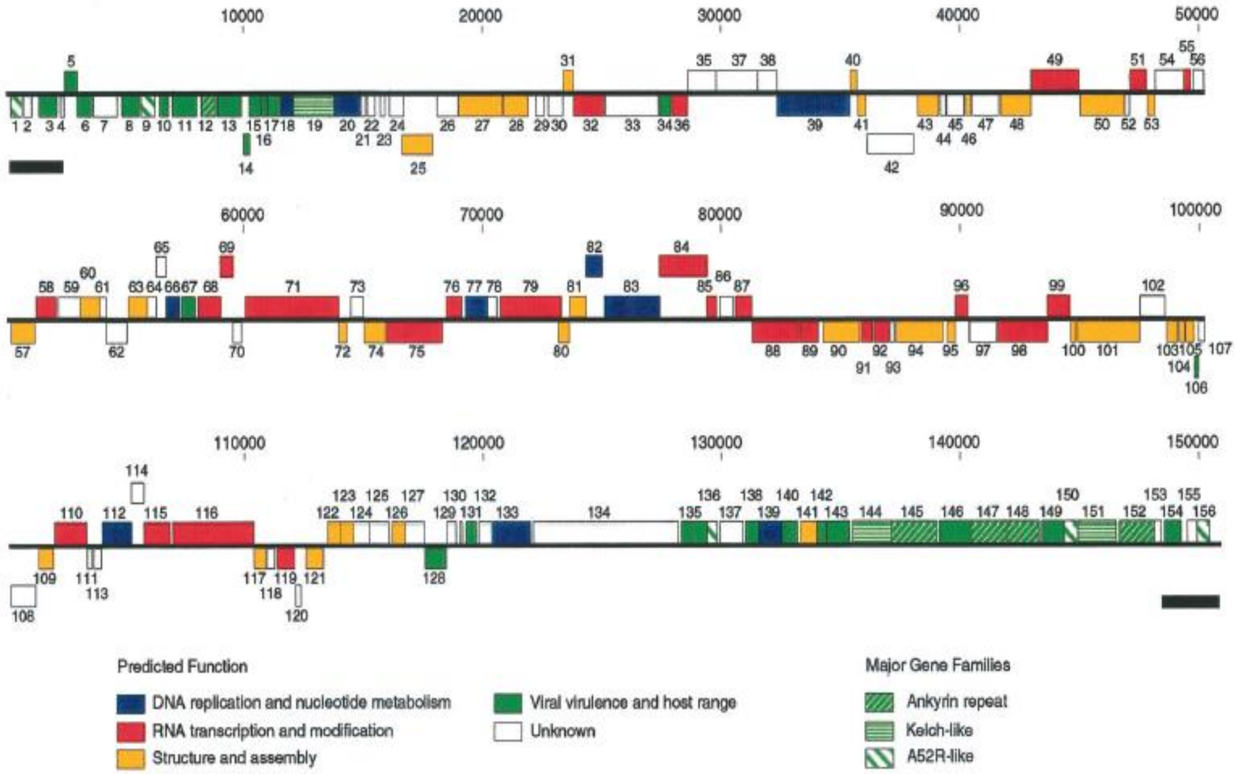

Figure 2: Linear map of the LSDV genome (**Source:** Tulman *et al*., 2001)

2.3. Epidemiology of LSD

2.3.1. Occurrence of the disease

LSD is an endemic disease of most African countries particularly in those of the sub-Saharan region. After 2012 it has spread rapidly through the Middle East, south-east Europe, the Balkans, Caucasus, Russia and Kazakhstan (OIE, 2017; Coezer and Tuppurainen, 2004). Mostly, field outbreaks can be severe and generalized infection with high morbidity and mortality rates, while in others there may be few affected animals and few or no deaths recorded but in general outbreaks are more severe with the initial introduction of the infection to a region and then will decrease, probably associated with the development of widespread immunity. Morbidity rates reach 80% during epizootics, but are nearer 20% in endemic areas (Radostits *et al*., 2006).

2.3.2. Hosts and susceptibility

Domestic cattle and Asian water buffalo are the animals affected by LSDV naturally during field outbreaks (El-Nahas *et al*., 2011; Al-Salihi, 2014). Some strains may replicate in sheep and goats but to date no epidemiological studies have evidenced small ruminants as reservoirs for the virus (Tuppurainen, 2017).Very little is known about the susceptibility of wild ruminants to LSDV. The susceptibility of host animals mostly depends on immune status, age and breed rather than the virulence of the virus. European cattle breeds are generally more susceptible than indigenous African and Asian breeds (Tageldin *et al*., 2014).

2.3.3. Sources of the virus

Capripox viruses are highly resistant viruses to physical and chemical action. They can survive in scab or tissue fragments for very long periods of time (Davies, 1991). It can be recovered from skin nodules kept at –80 °C for ten years and from infected tissue culture fluid stored at 4 °C for about six months (Coezer and Tuppurainen, 2004). LSDV can be isolated for up to 35 days or longer from skin nodules, scabs and crusts which are known to contain relatively high amounts of virus. It can also be isolated from blood, saliva, ocular and nasal discharges (Weiss, 1968), and semen (Irons et al., 2005) of infected animals. LSDV is found in the blood intermittently from approximately 7 to 21 days post-infection at lower levels than present in skin nodules. Viral shedding in semen can be prolonged and it has been isolated from the semen of an experimentally infected bull after 42 days (OIE, 2017).

2.3.4. Transmission

Studies have shown that the main route of transmission for LSD is through vectors whereas transmission ways like direct contact are not effective (Magori-Cohen *et al*., 2012). Stomoxys, Musca confiscate and *Aedes egypti* mosquitos and the three common African hard tick species, namely, *Rhipicephalus appendiculatus*, *Amblyomma hebraeum* and the African blue tick *Rhipicephalus (Boophilus) decoloratus*, were reported to have a great role in the transmission of LSD (Chihota *et al*., 2001; Chihota *et al*., 2003; Yeruham *et al*., 1995).

Studies have shown that it is possible to transmit LSDV by Aeidesa egypti to susceptible animals without the subsequent development of clinical disease in the animals (Chihota *et al*., 2001). Transstadial and transovarial transmission of LSDV byBoophilus decoloratus ticks and mechanical or intrastadial transmissionby Rhipicephalus appendiculatus and Amblyomma hebraeum ticks has been shown (Tuppurainen *et al*., 2011). Studies also showed that the disease can also transmit when common drinking troughs are used, thus confirming the suspicion that infected saliva might contribute towards the spread of the disease. The disease is transmissible to young calves through infected milk (Coezer and Tuppurainen, 2004).

2.4. Pathogenesis and Clinical Signs

The actual incubation period of LSD under field conditions has not been reported, but following experimental inoculation of the virus is 6–9 days until the onset of fever. LSDV replicates inside the host cells such as macrophages, fibroblasts, pericytes and endothelial cells in the lymphatics and blood vessels walls leads to vasculitis and lymphangitis, in severe cases thrombosis and infarction may also develop (Al Salhi, 2014).

In the acutely infected animal, there is initial pyrexia, which may exceed 41°C and can persist for 1 week. The superficial lymph nodes become enlarged and lesions may develop over the body, particularly on the head, neck, udder, scrotum, vulva and perineum between 7 and 19 days and the first ones usually appearing in the perineum. In lactating cattle there is a marked reduction in milk yield (OIE, 2017; Radostits *et al*., 2006). Lesions of LSD are round and firm, 1 to 4 cm in diameter, and are flattened and the hair on them stands on end. They vary in number from a few to hundreds; they are intradermal and, mostly confined to the skin area. Lacrimation, nasal discharge, salivation, and lameness can also be observed in association with the pyrexia. Lesions in the nostrils and on the turbinates, causing mucopurulent nasal discharge, respiratory obstruction and snoring; plaques and ulcers in the mouth causing salivation, nodules on the conjunctiva, causing severe lacrimation can be observed in severe cases. Lymph nodes draining the affected area become enlarged and cause local edema (Radostits *et al*., 2006; Maclanchilan and Dubovi, 2011).

In experimental studies, the intravenous route develops severe generalized infection, while in the intradermal inoculation only 40-50% of animals may develop localized lesions or no apparent disease at all. A localized swelling at the site of inoculation after four to seven days and enlargement of the regional lymph nodes, develop after subcutaneous or intradermal inoculation of cattle with LSDV (Al-Salihi, 2014; Abdulqa *et al*., 2016).

2.5. Diagnosis of LSD

There are no available commercial diagnostic test kits for LSD virus detection. Thus, the tentative diagnosis of LSD is usually based on the characteristic clinical signs, differential diagnosis, and confirmation is done by laboratory tests using molecular techniques of conventional or real time polymerase chain reaction (PCR) and cell culturing. LSD should be suspected clinically when there are characteristic skin nodules, fever and enlargement of superficial lymph nodes (Abdulqa *et al*., 2016;Tuppurainen, 2017a; OIE, 2017).

2.5.1. Virus isolation

Confirmation of LSD in a new area requires virus isolation and identification. Virus isolation is the method used to investigate the viability of the virus in the samples (Tuppurainen, 2017a). LSDV will grow in tissue culture of bovine, ovine or caprine origin, although maximum yield is obtained using lamb testis or bovine dermis cells. In cell culture, LSDV causes a characteristic cytopathic effect (CPE) and intracytoplasmic inclusion bodies that is distinct from infection with Bovine herpesvirus 2, which causes pseudo-lumpy skin disease and produces syncytia and intranuclear inclusion bodies in cell culture (Abdulqa *et al*., 2016; OIE, 2017).

2.5.2. Molecular detection methods

Laboratory confirmation of LSD virus can be done very rapidly using a PCR method specific for Capri poxviruses or by the demonstration of typical Capri pox virions in biopsy material or desiccated crusts using the transmission electron microscopy (TEM). Genome detection using Capri pox virus-specific primers for the attachment protein and fusion protein gene has been reported, and several conventional and real-time PCR methods have been established to be used on blood, tissue and semen specimens (Abdulqa *et al*., 2016; OIE, 2017; Abera *et al*., 2015).

Recently, a capripoxvirus real-time PCR method using primers and a probe has been validated (Bouden *et al*., 2009; Tuppurainen and Oura, 2011). Molecular tests using loop-mediated isothermal amplification to detect capripoxvirus genomes are also reported to provide sensitivity and specificity similar to real-time PCR with a simpler method and lower cost (Das *et al*., 2012; Murray *et al*., 2013).

2.5.3. Serological tests

Serological tests that can be used for LSDV include an indirect fluorescent antibody test (IFAT), virus neutralization, enzyme-linked immunosorbent assays (ELISA) and immune blotting (Western blotting) (Abera *et al*., 2015). The virus neutralization test (VNT) is the only validated serological test available. The agar gel immune diffusion test (AGID) and IFAT are less specific than the VNT due to cross-reactions with antibody to other poxviruses. Western blotting using the reaction between the P32 antigens of LSDV with test sera is both sensitive and specific, but is difficult and expensive to carry out. Some antibody-detecting ELISAs have been described but none is sufficiently validated to be recommended for use (OIE, 2017; Babuik *et al*., 2008).

2.5.4. Differential diagnosis

The main differential diagnosis is pseudo-LSD caused by bovine herpesvirus 2 (BoHV-2). This is usually a milder clinical condition, characterized by superficial nodules, resembling only the early stage of LSD. Intra-nuclear inclusion bodies and viral syncytia are histopathological characteristics of BoHV-2 infection not seen in LSD(OIE, 2017; Radostits *et al*., 2006). Other differential diagnoses (for integumentary lesions) include: dermatophilosis, dermatophytosis, bovine farcy, photosensitisation, actinomycosis, actinobacilosis, urticaria, insect bites, besnoitiosis, nocardiasis, demodicosis, onchocerciasis, pseudo-cowpox, and cowpox. Differential diagnoses for mucosal lesions include: foot and mouth disease, bluetongue, bovine viral diarrhoea, malignant catarrhal fever, infectious bovine rhinotracheitis, and bovine popular stomatitis (OIE, 2017; Abera *et al*., 2015).

2.6. Economic Importance

LSD is an economically important disease of cattle, serious economic losses from outbreaks that have a high morbidity and can produce a chronic debility in infected cattle. There is a great loss of milk production since the disease is more severe in cows in the peak of lactation and causes a sharp drop in milk yield because of high fever caused by the viral infection itself and secondary bacterial mastitis predisposed by the development of lesions on the teats (Abera *et al*., 2015; Radostits *et al*., 2006).

Even though the mortality rates of LSD are usually low, it is an economically important disease of cattle in Africa because of the prolonged loss of productivity of dairy and beef cattle, use of the animals for traction, decrease in body weight, mastitis, severe orchitis, which may result in temporary infertility and sometimes permanent sterility (Abera *et al*., 2015; OIE, 2017; Gari *et al*., 2011). A study done in Ethiopia has shown that the annual financial cost calculated as the sum of the average production losses due to morbidity and mortality arising from milk loss, beef loss, traction power loss, and treatment and vaccination costs at the herd level was estimated to be USD 6.43 (5.12–8) per head for local zebu and USD 58 (42–73) per head for HF/crossbred cattle (Gari *et al*., 2011). An other study also showed that the average cost of a single ox dying from LSD was calculated as 9,000 Ethiopian birr (ETB), equivalent to US$477.7 (USD1 = 18.84 ETB) (Ayelet *et al*., 2014).

In addition to quality degradation of skin and hides skin LSD induces associated economic losses due to reduction of wool quality, meat, losses as a result of culling and mortalities and related with cost of treatment and prevention of the diseases. Even though there are no specific antiviral treatments for LSD-infected cattle, there will be treatment cost for secondary bacterial infection. Treatment cost represents the expenses incurred by farmers for medication at the local public veterinary clinics when farmers bring their clinically sick animals for treatment (Abera *et al*., 2015b). Emaciation and a long convalescence period can also significantly decrease the growth rate in beef cattle (Tuppurainen *et al*., 2015).

LSD have been identified as one of the major impediments for genetic improvement of cattle populations and, consequently, for the development of intensive production units in Africa. It is well known that high producing dairy cattle, such as Holstein-Friesian (HF) and Jersey are more susceptible to CaPV infection than indigenous African and Asian cattle breeds (Bhanuprakash *et al*., 2011; Tuppurainen and Oura, 2011). The susceptibility of European cattle breeds and challenges facing dairy-genetics improvement in LSDV-endemic settings in Ethiopia was recently highlighted (Gari *et al*., 2011).

Currently live cattle export from Ethiopia is largely feedlot based. The introductions of LSD into feedlots certainly affect access to specific markets. For longer time, Middle

East markets are the traditional destination of Ethiopian live bulls. However, the current health status of Borena bulls in market chain unquestionably becomes a challenge for the country's future live cattle export opportunities to those countries (Alemayehu *et al*., 2012). Costly control and eradication measures such as vaccination campaigns as well as the indirect costs because of the compulsory limitations in animal movements also cause significant financial losses on national level (Tuppurainen and Oura, 2011; Gari *et al*., 2011; Abera *et al*., 2015b).

2.7. Control and Prevention

For lumpy skin disease, control measures with the exception of vaccination are usually not effective. Vaccination will greatly reduce the morbidity and epizootics but may not completely limit the extension. In endemic countries, vaccination is considered the only economically feasible way to control the spread of LSD and improve cattle productivity (OIE, 2017; Abera *et al*., 2015). Numerous live attenuated vaccines have been developed and used worldwide, while inactivated vaccines are considered less effective (Boumart *et al*., 2016). In addition, live attenuated vaccines are currently available which are cheap and provide good protection if sufficient herd immunity (over 80%) is maintained by carrying out annual vaccinations (Tuppurainen *et al*., 2015).

Live vaccines can help to control losses from lumpy skin disease in endemic areas. Four live attenuated strains of CaPVs have been used as vaccines specifically for the control of LSD (OIE, 2017; Brenner *et al*., 2009; Carn, 1993). These are: a strain of Kenyan sheep and goat pox virus, Yugoslavian RM 65 sheep pox strain, Romanian sheep pox strain and lumpy skin disease virus strain from South Africa (Al-Salihi, 2014). In endemic regions vaccine failure is a great problem for the effective control of LSD (Gari *et al*., 2015). It was also reported that CaPV vaccine strains produce a large local reaction at the site of inoculation in *Bos taurus* breeds (Davies, 1991) which some stock owners find unacceptable. This has discouraged the use of vaccine, even though the consequences of an outbreak of LSD are usually more severe (OIE, 2017b).

Outbreaks can also be controlled by strict quarantines to avoid introduction of infected animals into safe herds, isolation and prohibition of animal movements, slaughtering of all sick and infected animals (Depopulation of infected and exposed animals), proper disposal of carcasses (Incineration), cleaning and disinfection of the premises and insect control (Abera *et al*., 2015; Tuppurainen and Oura, 2011).

2.8. Status in Ethiopia

In Ethiopia LSD is the one of the most economically important livestock diseases. After the first occurrence in 1981 it has spread widely throughout the country and now it is the problem of almost all the regions and agro ecological zones (Gari *et al*., 2010; Mebratu *et al*., 1984). Its spread was mainly enhanced by cattle movements, communal grazing and watering, and pastoralist ways of life (Tuppurainen and Oura, 2011; Gari *et al*., 2012) .

In Ethiopia from 2007-2011 total of 1352 disease outbreaks of LSD have been reported and highest frequency was documented in Oromia region and the least in Afar region (Gumbe, 2018). Another study also showed that a total 3811 LSD outbreaks reported in Ethiopia between 2000 and 2015. Most of these outbreaks were from Oromia (54.5%), Amhara (27.9%), SNNP (10.1%) and Tigray regional states (3.6%) No out breaks were reported from Harari and Dire dawa. It also shows that LSD affects districts for one or two years and then spreads to other nearby areas with a susceptible cattle population with a trend of LSD outbreaks increased over time (Molla *et al*., 2017a).

Since the country has no a well-designed control strategy for this disease it is continuing to be a great problem. Even if the animal health authorities undertake vaccination campaigns when outbreak is reported, researches have shown that the different vaccines used in Ethiopia are not fully effective (Molla *et al*., 2017b; Ayelet *et al*., 2013). There have been repeated concerns reported to NVI on the insufficient protection provided by the vaccine, for cattle against LSDV. In addition to this, lack of genetic information on the circulating isolates in the field and their relation to the vaccine strain in use, which is essential for better vaccine matching, is also a great problem in the country (Gelaye *et al*., 2015).

3. MATERIALS AND METHODS

3.1. Study Area

In this study, four Peasant associations (PAs) from three districts of South Wollo zone were included and these were Tehuledere PA 023, Kalu PA 03 and PA 07 and Dessie Zuria PA 08. South Wollo zone is located in the Northern part of Ethiopia about 375 kms North East of Addis Ababa (CSA, 2008). The area is featured by numerous mountains, plateaus, hilly and sloppy areas, rivers, streams and lakes. The altitude varies from 1500-2600 meters above sea level. The area has a bimodal rainfall, with a mean value varying between 39.63 to 1000mm. The maximum and minimum daily temperature is 23.9 ^{0}C and 11.6 ^{0}C, respectively. The relative humidity of the area ranges between 23 to 79%. In the region there are three main seasons in a year: the dry season ("Belg") that lasts from October to the beginning of January, the rainfall of 100-300mm, and the big rainy season ("Kiremt") from July to the end of September which has got an average rainfall of 200-800mm. There is also a small dry period in May and June (BoARD, 2006). The total cattle population of the area was estimated to be 1,624,582 (South wollo zone livestock development office, 2018).

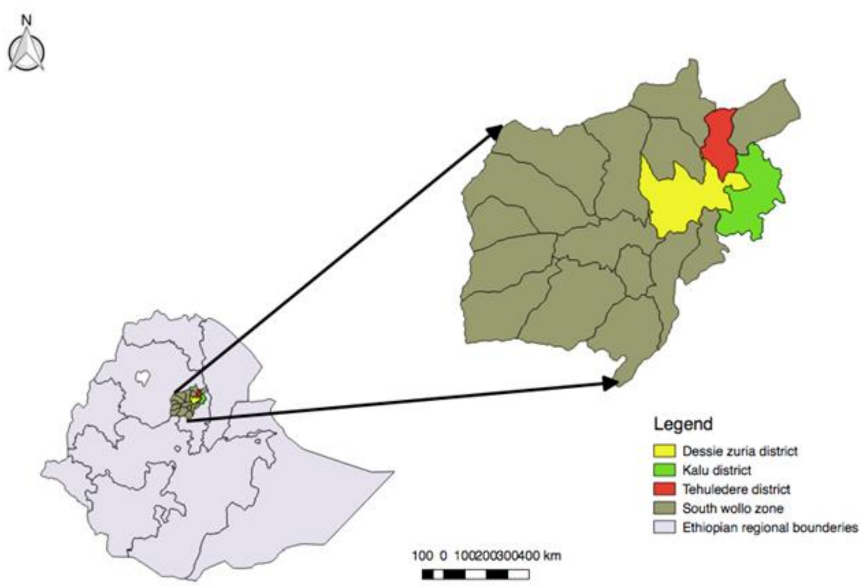

Figure 3: Map of the study areas

3.2. Study population

The study animal population comprised of local and crossbred cattle in the 46 households of the three districts of the study area. The average number of cattle per household varies from 1 to 10. Active disease outbreak investigation was made based on information obtained from District and PA veterinary clinics in the area. The individual herds maintained comprised of cattle of all age groups, which were mostly reared semi intensively. All investigations were in response to LSD outbreaks.

3.3. Study design and sampling

3.3.1. Active disease investigation

The households under investigation were purposively selected on the basis of outbreak reports from the district Animal Health Services Departments. Animals were examined for characteristic clinical signs of LSD, such as visible skin nodules, enlarged lymph nodes, lameness and fever. In Addition, data like vaccination history, age, sex and breeds of animals were collected.

3.3.2. Sample collection

Samples for virus isolation and identification were collected within the 1-4th week of the occurrence of clinical signs up on the outbreak reports from the affected districts. Nodular skin biopsies were collected aseptically from acutely sick cattle. About 2–5 g of samples were collected and placed in a sterile universal bottle with a phosphate buffer saline (PBS) at a pH of 7.2–7.6 with antibiotics (gentamycin),prepared as virus transport medium (VTM) (OIE, 2017; Ayelet *et al*., 2014). Identification number, species, sex, age, breed, date of collection and names of PAs were labeled and recorded, and the samples collected were immediately placed in ice box and transported to the virology laboratory at the National animal health diagnostic and investigation center (NAHDIC) on the next day after the day of collection, and kept at -20°C until processing.

Table 1: List of collected samples from different study areas

Sample no	Kebele	Sample type
S1	023/Tehuledere	Skin nodule
S2	023/Tehuledere	Skin nodule
S3	023/Tehuledere	Skin nodule
S4	023/Tehuledere	Skin nodule
S5	023/Tehuledere	Skin nodule
S6	023/Tehuledere	Skin nodule
S7	023/Tehuledere	Skin nodule
S8	023/Tehuledere	Skin nodule
S9	023/Tehuledere	Skin nodule
S10	08/Dessie zuria	Skin nodule
S11	08/Dessie zuria	Skin nodule
S12	08/Dessie zuria	Skin nodule
S13	08/Dessie zuria	Skin nodule
S14	08/Dessie zuria	Skin nodule
S15	03/Kallu	Skin nodule
S16	03/Kallu	Skin nodule
S17	07/Kallu	Skin nodule
S18	07/Kallu	Skin nodule
S19	07/Kallu	Skin nodule
S20	07/Kallu	Skin nodule

3.3.3. Laboratory diagnosis

Virus isolation

The biopsy samples were thawed at room temperature and washed three times in sterile phosphate-buffered saline (PBS, pH 7.2). Approximately 1 gram washed tissue sample was mixed with 9 ml sterile PBS containing antibiotic (0.1% gentamicin, Sigma-Aldrich, Germany) and ground using a sterile mortar and pestle. The tissue suspension was then centrifuged at 600 x *g* for 15 min and the supernatant filtered through a membrane of pore size 0.45 µm (Millipore, United States of America [USA]). Approximately 1 ml filtered supernatant was inoculated onto a monolayer of Vero cells in 25 cm2 tissue culture flasks, incubated at 37°C for an hour for adsorption, and then 9 ml Glasgow minimum essential medium (GMEM, Sigma-Aldrich), containing 0.1% gentamicin and 2% fetal calf serum (Sigma- Aldrich), was added. The inoculated flasks were incubated at 37°C in a humidified incubator with 5% CO2. Cells were monitored daily for 14 days, using an inverted microscope, for evidence of virus-induced cytopathic effects (CPEs); finally cells were frozen at –80°C (Ayelet *et al*., 2014; OIE, 2017).

DNA extraction

DNA was extracted by Qiagen kit, according to the manufacturer's instructions. The tissue sample was cut in to pieces and grinded with sterile sand by adding PBS buffer and after centrifugation at 2000rpm for 2 minutes, the supernatant was collected in to new micro centrifuge tubes after that 200µl of it was taken and 20 µl of proteinase K was added, mixed by vortexing, incubated at 56°C for 10 minutes and it was briefly centrifuged. Then 200µl ethanol (96-100%) was added and mixed thoroughly for 5 sec by vortex mixer and briefly centrifuged. This mixture was applied to the QIAamp mini spin column and centrifuged at 6000 xg (8000rpm) for 1 minute. The spin column was transferred in to 2 ml collection tube and 500 µl buffer AW1was added then centrifuged it at 8000rpm for 1 min. After that 500µl buffer AW2 was added and centrifuged at full speed of 14000 rpm for 3 min. after discarding the filtrate 200 µl of buffer AE was added and incubated at room temperature for1 minute and continued with centrifugation at 8000rpm for 1 min and this step was repeated to get the finale extract.

Polymerase chain reaction

Real time polymerase chain reaction (RT- PCR) assay was used to detect the virus with Capripoxvirus-specific primers used.
Forward: 5'- GGTGTAGTACGTATAAGATTATCGTATAGAAACAAGCCTTTA-3'
Reverse: 5'-AATTTCTTTCTCTGTTCCATTTG-3'

DNA was amplified in a final volume of 20 µl containing the following: 10 µl of eva green super mix, containing 2 µl of forward and reverse primers (4 µl), 4 µl of RNase free water and 2 µl of Template DNA. The following amplification program was applied: initial denaturation at 95°C for 3 min, followed by 45 cycles at 95°C for 15 s, 58°C for 80 s and last cycles of 95°C for 1 min, 40°C for 1 min and 40- 85°C for 5-10sec for melting curve analysis.

After amplification of the DNA template, the positive samples were noted by amplification fluorescence curves, melting curves (at 73 °C), and cycle threshold (Ct) values from the assay which were used to describe the positive samples: Ct values with no or higher than 37 were indicated as negatives suggesting absence of the virus from the tissue specimens.

DNA Sequence Analysis

The isolates were sent for further gene sequencing to Germany and still in progress. The results will be added to the paper when it is ready.

3.4. Data management and analysis

The collected data were recorded on Microsoft Excel spreadsheets. Descriptive statistics like percentages were used to discuss about morbidity, mortality and case fatality rates and chi square analysis (with p value 0.05 and 95% confidence interval) was used for the detection of statistical significance of the risk factors.

4. RESULTS

4.1. Active Outbreak investigation

In this study three active outbreaks were investigated between October, 2017 and May, 2018. The first outbreak was reported from Tehuledere District 023 Peasant association (PA) and the second one was from Dessie Zuria District 08 PA. Similarly, the third one was reported from Kalu District 03 and 07 PAs. During these outbreaks, 55 cattle were affected and 10 animals died. The LSD affected all age groups and both local and cross breeds. Out of 200 suspected cattle with vaccination history, 27 animals were affected by LSD. The most commonly observed clinical signs of LSD were initial fever, skin nodules on different body parts, enlarged peripheral lymph nodes, depression, lameness and lacrimation (Figure 4).

Figure 4: Clinical signs of LSD affected cattle

The highest morbidity rate (Figure 5) was recorded at Kallu/07, followed by Dessie zuria/08, Kallu/03 and Tehuledere/023. Mortality rates were recorded in Dessie zuria/08, Kallu/03, Kallu/07 and Tehuledere /023 in descending order. The case fatality rate was also highest in Dessie Zuria/08.

Table 2: Morbidity, mortality and case fatality rates with respect to PAs of LSD outbreak

Area collected	Number of cattle at risk	Number of affected and Morbidity rate (%)	Number of deaths	Mortality rate (%)	Case fatality rate (%)
Tehuledere/023	136	16 (11.76)	3	2.2	18.75
Dessie Zuria/08	60	11 (18.3)	3	5	27.27
Kallu/ 07	84	18 (21.42)	2	2.38	11.1
Kallu/ 03	70	10 (14.2)	2	2.85	20
Total	350	55 (15.71)	10	2.86	18.18

Morbidity, mortality and case fatality rates were assessed within age groups of <2 and ≥ 2 years old cattle, local and crossbred cattle, female and male cattle and between vaccinated and not vaccinated cattle within 6 months (table 3 and 4).

Table 3: Morbidity rates according to age, sex, breeds and vaccination status of animals

Variables	Number at risk	Number affected	Morbidity rate (%)	X^2	P –value
Sex					
Female	168	34	20.23	4.99	0.025
Male	182	21	11.53		
Age (years)					
<2	110	18	16.36	0.05	0.82
≥ 2	240	37	15.41		
Breed					
Local	320	45	14.06	23.7	0.00
Cross	30	10	33.3		
Vaccination status					
Vaccinated	200	27	13.50	1.48	0.22
Not vaccinated	150	28	18.66		
Total	350	55	15.71		

Table 4: Mortality and case fatality rates of LSD according to age, sex, and breed and vaccination status of animals

Variables	Number at risk	Number affected	Number of dead	Mortality rate (%)	Case fatality rate (%)
Sex					
Female	168	34	4	2.38	11.76
Male	182	21	6	3.29	28.57
Age (years)					
<2	110	18	2	1.82	11.11
≥ 2	240	37	8	3.33	21.62
Breed					
Local	320	45	9	2.81	20.00
Cross	30	10	1	3.33	10.00
Vaccination status					
Vaccinated	200	27	3	1.50	11.11
Not vaccinated	150	28	7	4.66	25.00
Total	350	55	10	2.86	18.18

4.2. Virus Isolation

From the total of 20 samples collected, 10 of them were grown on Vero cells for virus isolation. Cytopathic effects (CPE) were observed in all plates starting from the 3rd day of culture. CPEs were characterized by rounding of single cells, aggregation of dead cells and destruction of monolayer. None of the negative control cultures showed any CPE.

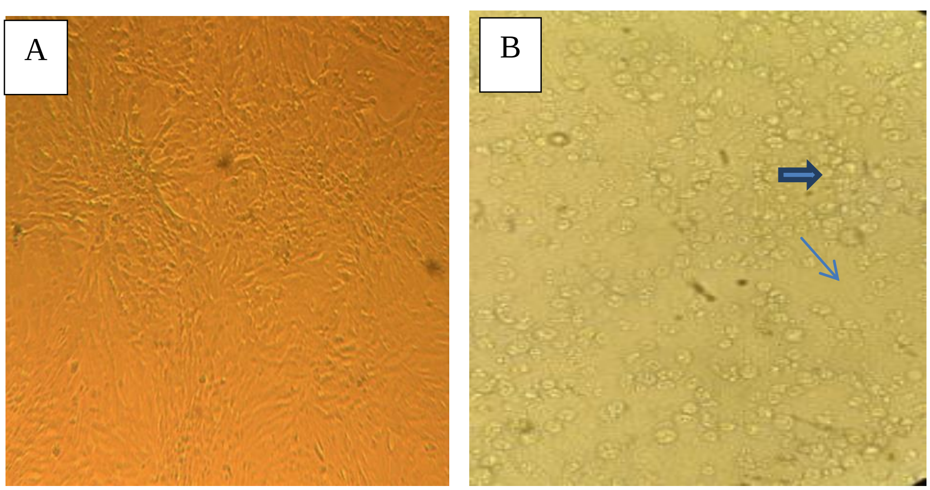

Figure 5: A) Normal cell mono layer B) The arrow shows aggregation and destruction of cell mono layer

4.3. Molecular characterization

4.3.1. Polymerase chain reaction

Out of 20 extracted DNA samples amplified by real time PCR, 17 samples (85%) of them were positive. Below are indicated amplification curves of the real time PCR (Figure 6).

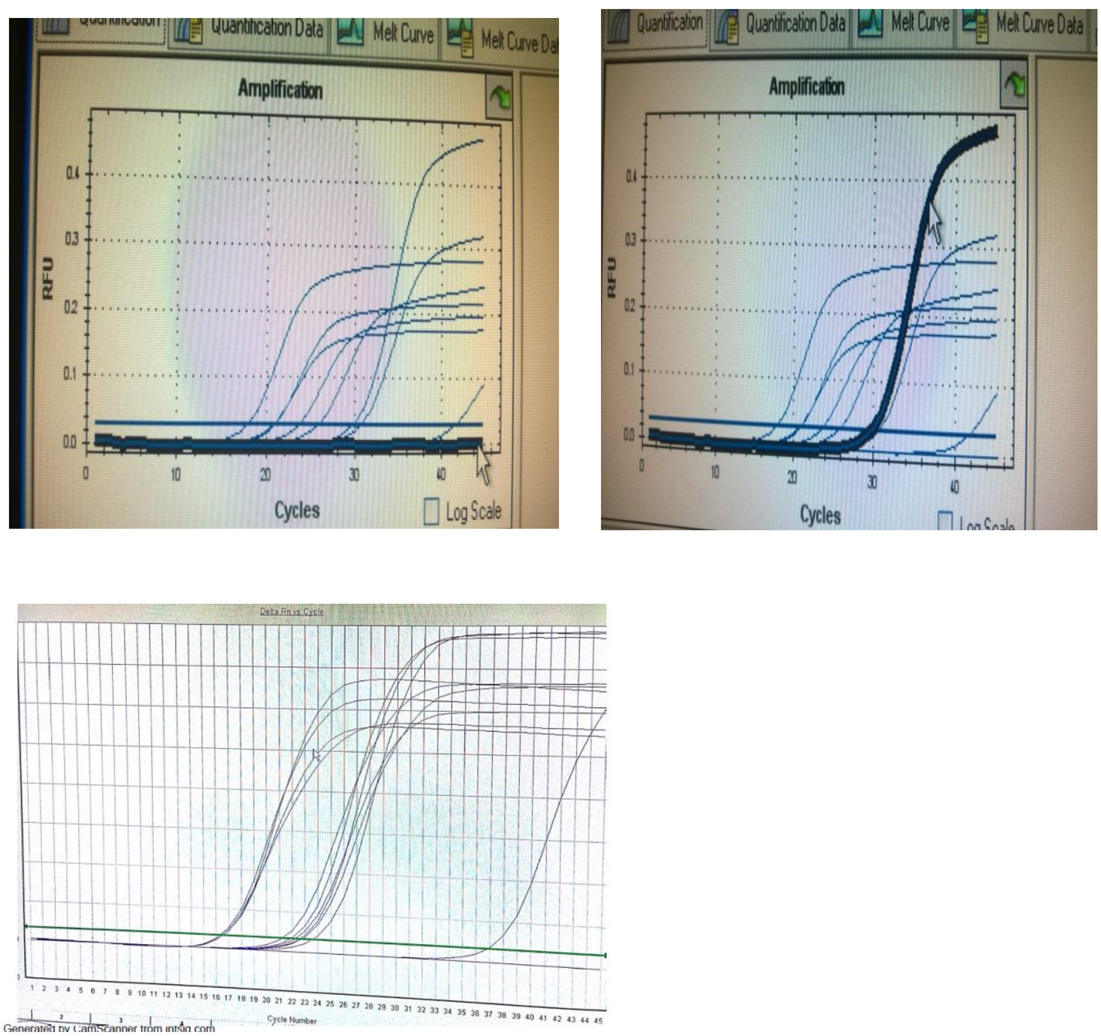

Figure 6: Real time PCR results

As indicated in the following table (Table 5), positive samples have the Ct values lying between16-30. As compared to the positive LSD controls, most of the values are lower, suggesting high concentrations of the virus. The negative specimens were indicated by Undet which indicates undetermined values or very high Ct values (around 41).

Table 5: Real time PCR Ct values

Sample no	Kebele	Ct value
S1	023/Tehuledere	30.60
S2	023/ Tehuledere	23.59
S3	023/Tehuledere	**41.56 (Negative**)
S4	023/Tehuledere	17.79
S5	023/Tehuledere	20.75
S6	023/Tehuledere	20.91
S7	023/Tehuledere	25.79
S8 Positive control	…..	**29.67**
S9	08/Dessie zuria	21.96
S10	08/Dessie zuria	16.60
S11	08/Dessie zuria	22.79
S12	08/Dessie zuria	16.45
S13	08/Dessie zuria	22.26
S14	08/Dessie zuria	16.58
S15	08/Dessie zuria	21.23
S16 LSD Positive control	……	**16.33**
S17	03/Kallu	19.49
S18	03/Kallu	16.60
S19	07/Kallu	16.35
S20	07/Kallu	**Undet.**
S21	07/Kallu	**Undet.**
S22	07/Kallu	21.42
S23 LSD Positive control		**22.15**

Note: Undet. indicates undetermined Ct values

5. DISCUSSION

LSD is economically very important disease due to its large scale financial impact by downgrading skins, decreasing milk production, adding treatment costs, reduction in traction powers of oxen and death of the animals. It is listed as notifiable disease by OIE and is endemic in many African and Middle East countries including Ethiopia. In the present study three lumpy skin disease outbreaks from four administrative PAs of South Wollo zone were investigated and as of the objective, following the clinical examination of animals, isolation and characterization of the virus from outbreaks were done by cell culture and real time PCR, respectively.

Fever, skin nodules, enlarged lymph nodes, lameness, depression, lacrimation and salivation were the major and characteristic clinical features of LSD found during these outbreaks. Many other authors have also recorded the same symptoms in natural and experimental infections (Body *et al*., 2011; Agag *et al*., 1992; Jalali *et al*., 2017). Susceptibility of host animals due to factors like age, immunological status, and dose and route of virus inoculation also affected disease severity (Ayelet *et al*., 2014). In which animals with severe cases or died of LSD observed in these outbreaks had a poor body conditions which might affect the immunity to the disease.

The morbidity, mortality and case fatality rates of LSD were 15.71%, 2.86% and 18.18% respectively, indicating an important impact posed by the disease in the area. This may be due to the farming and management system practiced in the area which favors the vector transmission and poor nourishment of diseased animals which will die due to secondary disease. The morbidity rate was closer to reported from the study in central Ethiopia with 13.61% (Ayelet *et al*., 2014). Higher morbidity (21.2%) and mortality rates (4.5%) than the current study were previously reported by Wassie *et al* (2017) from Ethiopia. In addition, higher morbidity rate (26%), but lower mortality rates (1.9%) were also recorded in Jordan (Abutarbush *et al*., 2013). On the other hand, morbidity rate of the disease in the current study was higher than the reported results in north-eastern Ethiopia by Hailu *et al* (2014) who reported a morbidity rate of 7.4%, and others as 0.65% in Turkey (Ince *et al*., 2016), 9.11% in Iraq (Al-Salihi and Hassan, 2015) as well as 8.7% in

Greece (Tasioudi *et al*., 2016). These variations could be from the differences in geographic location and climate; the management conditions, breed, immune status and condition of the animals, virulence of the virus, and the number and types of putative insect vectors (Tuppurainen and Oura, 2011).

Different morbidity, mortality and case fatality rates were recorded in different PAs with the highest morbidity in Kalu/07 (21.42%) and lowest in Tehuledere/023 (11.76%). This might be due to the proximity of Kalu area to rivers which might be suitable for the replication of arthropod vectors.

In the present study, the morbidity rate of LSD indicated that calves were more susceptible to infection than adult cattle. This might have occurred due to the natural susceptibility of young animals which may also usually show more severe clinical disease (Coezer and Tuppurainen, 2004). This finding is in line with the reports of Ahmed and Zaher (2008) and Ayelet *et al* (2014) but it disagrees with the study done by Kasem *et al* (2018).

The mortality and case fatality rates were higher in male animals (3.29 and 28.57%) than in females (2.38 and 11.76%), while the morbidity was higher among female animals (20.23%) than male animals (11.53%). Even if the physiological conditions of female animals (like pregnancy and lactation) might affect their susceptibilities, evidenced by increase in morbidity rates, they are usually kept inside houses. The farmers in the area use oxen for ploughing their lands and stress and fatigue might have increased the mortality rates.

The study also shows that rates of morbidity and mortality were higher in cross breeds than local cattle with statistically significant differences. The genetic differences between breeds may have influenced susceptibility to the disease (Abera *et al*., 2015;Davies, 1991). In contrast, high case fatality rates were recorded in local cattle. This might be due to lack of proper nursing and treatment of diseased local animals because of their lower market values.

In the area, even if more number of cattle were vaccinated, the disease has been manifested by high morbidity and mortality rates, regardless of vaccination status. The observed vaccine failure may be due to lack of cross-protection of the vaccine strains against circulating virulent field strains in the area (Kasem *et al*., 2018). In addition, problems in vaccine management, including transport and storage may have influenced vaccine efficacy. Introduction of animals already incubating the virus or newly vaccinated animals becoming infected before they develop protective immunity due to untimely vaccine campaigns (vaccination after the out breaks have already occurred in the area) might have also a great contribution for vaccine failure (Ayelet *et al*., 2014).

LSDV can be propagated in a variety of primary cells or cell lines of bovine, ovine or caprine origin. The virus has been adapted to grow on the chorioallantoic membrane of embryonated chicken eggs and African green monkey kidney (Vero) cells (OIE, 2017). It grows slowly on cell cultures, and CPE can usually be detected four to six days after inoculation(Tuppurainen, 2017). A characteristic CPE consisting of retraction of the cell membrane from surrounding cells, and rounding of individual cells and margination of the nuclear chromatin can be seen (OIE, 2017). In this outbreak investigation the virus was isolated by growing on 31^{st} passage Vero cells and CPE were characterized by aggregated cells, destruction of cell monolayers and rounding of cells. Similar CPE characteristics were recorded by other authors (Ayelet *et al*., 2014; Lamya *et al*., 2017).

Real time PCR technique was used for identification of the virus responsible for the outbreaks and out of 20 samples 17 (85% of the samples) were positive. The Ct values taken from the positive samples indicate lower numbers lying around the Ct values of positive controls. No or higher values were indicated as negatives in which lower or no loads of the virus are present. This is in agreement with the other finding which reported Ct values less than 37 as positives (Salnicove *et al*., 2018).

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6. CONCLUSION AND RECOMMENDATIONS

In the present study three LSD outbreaks in South Wollo zone were investigated. The disease affected all age groups of cattle regardless of the differences in breed, sex and vaccination status and already caused great economic loss due to high mortality, morbidity and case fatality rates. Cell culture and real time PCR conducted confirmed that the outbreaks were due to LSDV. Vaccine failure which could be due to problems in vaccine storage and efficacy of produced vaccines against the current field strain in the area needs concern. Therefore the following recommendations were forwarded from the above conclusions.

- Broader and detailed investigation including sequence characterisation and determination of their evolutionary relationship of the viruses as compared to the vaccine strain in use
- Regular annual vaccinations by effective and well managed vaccines,
- Prevention of movement of the diseased animals to new areas and vector control.
- Annual surveillances on the status of the disease.
- Awareness creation for cattle owners to vaccinate healthy animals for control and nourish the diseased ones to prevent death and disease transmission.

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8. ANNEXES

Annex 1: Cell culture procedures

1. Thaw the biopsy samples at room temperature and wash three times in sterile phosphate-buffered saline (PBS, pH 7.2).
2. Take approximately 1 g washed tissue sample mix with 9 ml sterile PBS containing antibiotic (0.1% gentamicin, Sigma-Aldrich, Germany) and ground using a sterile mortar and pestle.
3. Centrifuge the tissue suspension at 600 x *g* for 15 min and filter the supernatant through a membrane of pore size 0.45 µm (Millipore, United States of America [USA]).
4. Inoculate approximately 1 ml filtered supernatant onto a monolayer of Vero cells in 25 cm2 tissue culture flasks.
5. Incubate at 37°C for an hour for adsorption, and then add9 ml Glasgow modified minimum essential medium (GMEM, Sigma-Aldrich), containing 0.1% gentamicin and 2% fetal calf serum (Sigma- Aldrich).
6. Incubate the inoculated flasks at 37°C in a humidified incubator with 5% CO2.
7. Monitor the cells daily for 14 days for evidence of CPE.

Annex 2: DNA extraction (Quagen)

1. Cut the tissue sample in to pieces and grind it with sand by adding PBS buffer.
2. After centrifugation at 2000 rpm for 2 minutes, collect the supernatant in to new micro centrifuge tubes after that
3. Take 200µl of it put in to new micro centrifuge tube and add20 µl of proteinase K and mix by vortex. To ensure efficiency of lysis add 200 µl of AL buffer (lysis buffer) and mix it with pulse vortexing for 15 sec.
4. Incubate it at 56°C for 10 minutes then briefly centrifuge
5. Add 200µl ethanol (96-100%) and mix thoroughly for 5 sec by vortex mixer and briefly centrifuge it.
6. Apply this mixture to the QIAamp mini spin column and centrifuge at 6000 x g (8000rpm) for 1 minute.
7. Transfer the spin column in to 2 ml collection tube and add 500µl buffer AW1and centrifuge it at 8000rpm for 1 min.
8. Discard the collection tube, transfer the spin column in to new 2 ml collection tube and add 500µlbuffer AW2 and centrifuge at full speed 14000 rpm for 3 min.
9. Carefully transfer the spin column in to a new 2 ml collection tube and discard it.
10. Centrifuge the old collection tube with the filtrate for 1 min and add200 µl of buffer AE and incubate at room temperature for 1 minute and continue with centrifugation at 8000rpm for 1 min and this step was repeated to get the finale extract.
11. Collect the extracted DNA and store at -20°C until use.

Annex 3: Data collection sheet

Affected cattle

Animal id					
Date of collection					
Area					
Age					
Sex					
Breed					
Vaccinated/not					

Susceptible cattle

Id					
Area					
Age					
Sex					
Breed					
Vaccinated/not					
No of animals in the house hold					

Animals died

Area		
Age		
Sex		
Breed		
Vaccinated/not		

Annex 4: photos of sick animals and sample collection



Annex 5: Ethical clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/18/05/10/2018

Name of Applicant: Betelihem Tegegne (DVM, MVSc fellow)

Address: College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: Outbreak investigation of Lumpy skin disease: Isolation and molecular characterization of the virus in south Wollo zone, North Ethiopia

Date of application: **15/10/2017**
Nature of the project: **mildly-invasive**
Target animal species: **cattle**
Number of animals involved: **depends on case availability**
Study area: **South Wollo, North Ethiopia**

Minutes No. and date of review: **VM/ERC/05/10/018, 03/01/2018**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is allowed to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee whenever this is deemed necessary

Dr Getachew Terefe
Chairman

Signature

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