ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

LUMPY SKIN DISEASE (LSD): OUTBREAK INVESTIGATION, ISOLATION AND MOLECULAR DETECTION OF LSDV IN SELECTED AREAS OF EASTERN SHEWA, ETHIOPIA

BY

RGBE HAFTU

JUNE, 2012

DEBRE ZEIT, ETHIOPIA

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A thesis Submitted to the School of Graduate study of Addis Ababa University in partial Fulfillment of the requirement for the Degree of Masters Science in Veterinary Microbiology

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ACKNOWLEDGEMENTS

I would like to extend my best regards to my advisors Dr. Tesfaye Sisay and Dr. Gelagay Ayelet for their constructive advice, overall intellectual guidance, and provision of literature materials especially to Dr. Tesfaye Sisay for devotion of his precious time to correct the manuscripts.

Appreciations are due to the National Veterinary Institute that allowed me to use the laboratory facilities. I am well pleased to gratify Dr. Eseyas Gelaye and Ato Alebachew Belay for their Cooperativeness and unreserved technical support in all my laboratory works.

My sincere thanks go to Eastern Shewa Agricultural Zone Office and in particular to Dr.Ahmed and Ato Tesfaye. I am very much grateful to Dr. Kimya working at Adama federal quarantine office. Lots of thanks to Adama and Wenji fattening farm owners and animal attendants they allowed me to take samples from diseased animals.

Last but not least, I would like to thank my family for all of their support, particularly my brother Solomon, for his full financial support and unreserved love. I am exceptionally grateful to my dear Dr. Kinenet Atnafu for his unending support during period of my study.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
µl	Micro liter
CaPV	Capripoxviruses
ChPV	Chordopoxvirus
CPE	Cytopathic Effect
CSA	Central Statistical Agency
DNA	Deoxyribonucleic cid
dNTP	deoxy Nucleotide Tri Phosphate
DsDNA	Double stranded Deoxyribonucleic Acid
GDP	Gross Domestic Product
GMEM	Glasgow Minimum Essential Medium
IL	Inter leukin
Kbp	Kilo base pair
LSD	Lumpy Skin Disease
Min	Minute
NVI	National Veterinary Institute
ORF	Open Reading Frame
PBS	Phosphate Buffer Saline
PCR	Phosphate Buffer Saline
RNA	Ribonucleic Acid
Rpm	Revolution per minute
SGPV	Sheep and Got Pox Virus
SNNP	South Nations and Nationalities People
TAE	Tris-Acetate-EDTA
Taq	Thermus aquaticus
UN	United States
UV	Ultra Violate

ABSTRACT

The study was undertaken to investigate outbreaks of lumpy skin disease (LSD), isolate and identify LSDV at molecular level, to asses the economic significance of lumpy skin disease in the study area, and to asses the protection of the vaccine for lumpy skin disease given at field condition. The study was conducted from September 2011-April 2012 in selected areas of eastern Shewa. LSD Outbreaks were reported from 4 different areas of Eastern Shewa; Adama (5 intensive fattening farms), Wenji (4 intensive fattening farms), Mojo (small holders) and Welenchit (small holders). In total 2174 local Zebu breed cattle, the majority composed of males (98.8%), were visited and clinically diagnosed. From the total of visited cattle 296 (13.78%), 108 (5.03%) and 1 (3.84%) morbidity, mortality and abortion rates were recorded respectively. Analysis of the outbreak statistics revealed a relatively consistent morbidity rate with highest value in Adama (15.67%) followed by Wenji (10.26%), Wolenchit (8.8%), and Mojo (7.01%) was recorded respectivly. Highest mortality rate was observed in Adama (5.99%) followed by Wenji (3.42%), Welenchit (2.4%) and Mojo (0%) respectively. Isolation and identification of the agent was conducted based on cell culture and PCR results. LSDV genome was extracted and identified in all 22 skin nodules by PCR. Furthermore, LSD causes a great economic loss in the study area. Questionnaire survey based assessment on protection of the vaccine showed that the current vaccine (KSGPV) used in the area was not protective and high percentage of morbidity (15.1%) and mortality (5.37%) rates were reported among vaccinated animals but efficacy test of the vaccine under laboratory protocol in six seronegative calves showed that the vaccine was immunologically protective.

Key words; *Cattle, Outbreak, LSD, LSDV, Eastern Shewa, vaccine efficacy*

1. INTRODUCTION

Ethiopia has an estimated 53.4 million (55.2% are female and 44.8% are males) cattle distributed within the different agro-ecological zones (CSA, 2011); about 99% of cattle populations are of local Zebu breed. Genetically and geographically the main breed classifications in Ethiopia are Arsi, Fogera, Horo, Borana, Nuwer, Sheko and Afar breeds. The remaining 1% of exotic breeds is kept mainly for dairy production in and around urban areas (Gari *et al.*, 2010). The arid, semi-arid and sub-humid zones are homes for 14% of the cattle population each while 6% and 52% of the cattle population inhabit the humid zones and the highlands of the country, respectively. The majority of the cattle population is found in the highlands of Ethiopia where 43.6% of the human agricultural population is residing which indicates that cattle have a very important role in the Ethiopian economy (Jahnke, 1982). A total of 800,000 tones of milk and 220,000 tones of meat are produced by cattle annually which account for 30% the agricultural products that contribute 46% of the Gross Domestic Product (GDP). Livestock also has a significant share from the export earnings (15%), In addition, 14 million tones of manure are used annually primarily for heating and six million oxen provide the draught power required for the cultivation of grain crops (Azage and Alemu, 1997).

Livestock diseases are the major cause of economic losses to the peasant farmer and pastoralists in Ethiopia amounting to hundreds of millions of birr annually. Because livestock are the chief sources of cash income to small holders, up to 88% in the highland livestock-cropping system, diseases are an important cause of reduced productivity of meat and milk as well as draft, hides and dung fuel (Rashid and Shank, 1994). Domestic animals are often afflicted with various skin problems, some easy to cure others more complicated, and some even highly contagious to the human handlers (Alaa *et al.,* 2008).

Lumpy skin disease (LSD) is one of economically important viral disease of cattle and can produce a chronic debility in infected cattle. Severe and permanent damage to hides results from the skin lesions. Lesions in the mouth, pharynx and respiratory tract commonly occur, resulting in a rapid deterioration in condition and sometimes severe emaciation, which can persist for months.

The most effective method of transmission is mechanically through biting flies. The incidence of LSD occurrence is high during wet seasons when biting-fly populations are abundant and it decreases or ceases during the dry season (Gari *et al.*, 2010).

Lumpy skin disease is a pox disease of cattle characterized by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, oedema of the skin, and sometimes death (Alaa *et al.,* 2008; Vorster and Mapham, 2008). LSD is caused by strains of capripoxvirus that are antigenically indistinguishable from strains causing sheep pox and goat pox. However, LSD has a different geographical distribution to sheep and goat pox, suggesting that cattle strains of capripoxvirus do not infect and transmit between sheep and goats (Ahmed and Kawther, 2008).

The World Organization for Animal Health (OIE) categorizes LSD as a notifiable list A disease because of the substantial economic impact of an outbreak. 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. Temporary or permanent infertility may occur in cows and bulls. Emaciation of infected animals and a convalescence period lasting for several months may cause decreased growth rate in beef cattle (Weiss, 1968; Coetzer, 2004). Morbidity and mortality of the disease vary considerably depending on the breed of cattle, the immunological status of the population and insect vectors involved in the transmission. Morbidity rates generally varying between 1% and 20%. In a few outbreaks it was reported to be more than 50% although the mortality rates are usually less than 10%. Cows in 1% to 7% of cases may abort (Radostits *et al*., 2006; Vorster and Mapham, 2008).

In Ethiopia lumpy skine disease was first observed in the northwestern part of the country (southwest of Lake Tana) in 1983 (Mebratu *et al.*, 1984). It has now spread to almost all the regions and agroecological zones. Vaccination is classically used to control outbreaks whenever they occur. Because of the wide distribution of the disease and the size and structure of the cattle population in Ethiopia it is likely that LSD is one of the most economically important livestock diseases in the country (Gari *et al.*, 2010). Vaccination is the only effective method to control the disease in endemic countries like Ethiopia. Four live attenuated strains of capripoxvirus have

been used as vaccines specifically for the control of LSD (Carn and Kitching, 1995; Brenner *et al*., 2006; Kitching, 2003). 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 (OIE, 2010).

The control of LSD can be achieved by restriction of animal movement and stamping out of infected and exposed animals. This requires adequate financial, infrastructural, and human resources and adequate information system. However, under the current Ethiopian situation, these control strategies could not be implemented to control and eradicate the disease. Therefore, control measures through vaccination and restriction animal movement remain the most practical option in the country.

Therefore, to carry out an effective control of LSD through strategic vaccination the prerequisites are a thorough understanding of the epidemiology of the disease in the country. Hence, from the outset the epidemiological assessment of LSD should be implemented in order to envisage a rational plan for the control and eventual eradication of LSD from Ethiopia.

Accordingly, the objectives of this study were;

- To investigate outbreaks of lumpy skin disease
- To isolate and identify LSDV at molecular level,
- To asses the economic significance of lumpy skin disease in the study area
- To assess the protection of the vaccine for lumpy skin disease given at field condition.

2. LITERATURE REVIEW

2.1. History and economic significance of LSD

Lumpy skin disease also called Pseudo-urticaria, Neethling virus disease, exanthema nodularis bovis, and knopvelsiekt. LSD is an acute to chronic viral disease of cattle characterized by skin nodules that may have inverted conical necrosis (sitfast) with lymphadenitis accompanied by a persistent fever (Davies, 1991; Grooms, 2005; James, 2004; Alaa *et al*., 2008; Vorster and Mapham, 2008).

Lumpy skin disease was first described in Northern Rhodesia (Zambia) in 1929 and spread in epizootics south then north and west. Initially, it was considered to be the result either of poisoning or a hypersensitivity to insect bites. Between 1943 and 1945, cases occurred in Botswana (Bechuanaland), Zimbabwe (Southern Rhodesia) and the Republic of South Africa. The infectious nature of the disease was recognized at this time (Davies, 1991; OIE 2008). Until 1988 LSD was confined to sub-Saharan Africa, but then spread into Egypt. There have been only two laboratory-confirmed outbreaks of LSD outside Africa: in Israel in 1989, which was eliminated by slaughter of all infected and in-contact cattle, and vaccination and in Bahrain in 1993. There was an outbreak in 2000 in cattle imported into Mauritius; the diagnosis was confirmed by electron microscopy (Murphy *et al*., 1999; OIE 2008). And more recent outbreaks of LSD outside Africa have been reported in Israel (2006 and 2007), Palestine (2007 and 2008) and Bahrain (2006-2009) (OIE, 2010; Anonymous, 2011; Body *et al*., 2012).

The office international des epizootics consider LSD as list A'' disease that has the potential for rapid spread with ability to cause serious economic loss (Castro and Heuschele, 1992). Morbidity and mortality of the disease vary considerably depending on the breed of cattle, the immunological status of the population and insect vectors involved in the transmission. Morbidity rates generally varying between 1% and 20%. In a few outbreaks it was reported to be more than 50% although the mortality rates are usually less than 10%. Cows in 1% to 7% of cases may abort. LSD causes severe economic losses due to permanent damage to hides, a

prolonged debilitating clinical course, reduced weight gain, temporary or permanent loss of milk production, temporary or permanent infertility or even sterility in bulls, and abortion of pregnant cows (Radostits *et al*., 2006; Vorster and Mapham, 2008).

2.2. Classification

LSDV is grouped under the family of *poxviridae.* The family *Poxviridae* is subdivided into two subfamilies: *Chordopoxvirinae* (poxviruses of vertebrates) and *Entomopoxvirinae* (poxviruses of insects). The subfamily *Chordopoxvirinae* is subdivided into eight genera i.e. *orthopoxvirus, parapoxvirus, capripoxvirus, suipoxvirus, leporipoxvirus, yatapoxvirus, avipoxvirus and molluscipoxvirus* and the genus *capripoxvirus* contains three important viruses; i.e. lumpy skin disease virus, Sheeppox virus and Goatpox virus (Murphy *et al*., 1999; Van Regenmortel *et al*., 2000; Lefkowitz *et al*., 2006; Howley *et al*., 2007). The eight genera of *chordopoxvirinea* are summarized in table 1.

Table1: Eight genera of *chordopoxvirinea*

Genus	species	Strain	Abbreviation
Avipoxviru	Canarypox virus	ATCCVR-111	CNPV
	Fowlpox virus		FPV
Capripoxvirus	*Sheeppox virus*	TU-V02127	SPPV
	Goatpox virus	Pellor	GTPV
	LSD virus	Neethling 2490	LSDV
Leporipoxvirus	*Myxoma virus*	Lausanne	MYXV
	Rabbit fibroma virus	Kaza	RFV
	Molluscum contagiosum virus	Subtype 1	MOCV
Molluscipoxvirus			
	Variola (smallpox)	India-1967	VARV
	Vaccinia virus	Copenhagen	VACV
Orthopoxvis	Cowpox virus	Brighton Red	CPXV
	Camelpoxvirus	M-96	CMLV
	Ectromelia virus	Moscow	ECTV
	Monkeypox virus	Zaire-96-I-16	MPXV
Parapoxvirus	Bov.Papular stomatitis virus	BV-AR02	BPSV
	Orf virus	OV-SA00	ORFV
Suipoxvirus	*Swinepox virus*	17077-99	SWPV
Yatapoxvirus	Yaba-like disease virus		YLDV

Source**;** (Caroline *et al*., 2004; Ke X. *et al*., 2006); complete genomic sequences of poxvirus.

2.3. Viral replication LSDV

As LSDV a poxvirus; all viruses grouped under family of *poxviridae* have the same way of replication (Howley *et al*., 2007). Replication of the poxvirus involves several stages. The first thing the virus does is to bind to a receptor on the host cell surface; the receptors for the poxvirus are thought to be Glycosaminoglycans (GAGs). After binding to the receptor, the virus enters the cell where it uncoats. Uncoating of the virus is a two step process. Firstly the outer membrane is removed as the particle enters the cell; secondly the virus particle (without the outer membrane) is uncoated further to release the core into the cytoplasm. The pox viral genes are expressed in two phases. The early genes are expressed first. These genes encode the non-structural protein, including proteins necessary for replication of the viral genome, and are expressed before the genome is replicated. The late genes are expressed after the genome has been replicated and encode the structural proteins to make the virus particle. The assembly of the virus particle occurs in the cytoskeleton of the cell and is a complex process that is poorly understood but is currently being researched. Considering the fact that this virus is large and complex, replication is relatively quick taking approximately 12 hours until the host cell dies by the release of viruses, About 10,000 viral particles are produced by infected cell and are released upon lysis of cell (Kara *et al*., 2003; Howley *et al*., 2007).

The replication of poxvirus is unusual for a virus with double-stranded genomic DNA because it occurs in the cytoplasm. Poxvirus encodes its own machinery for genome transcription, a DNA dependent RNA polymerase, which makes replication in the cytoplasm possible. Most dsDNA viruses require the host cell's proteins to perform transcription. These host proteins are found in the nucleus, and therefore most dsDNA viruses carry out a part of their infection cycle within the host cell's nucleus (Kara *et al*., 2003; Howley *et al*., 2007).

2.4. Diseases caused by genus *Capripoxvirus*

Capripoxviruses represent one of eight genera within the *Chordopoxvirus* (ChPV) subfamily of the *Poxviridae*. The *capripoxvirus* genus is currently comprised of lumpy skin disease virus, Sheeppox virus, and Goatpox virus. These viruses are responsible for some of the most economically significant diseases of domestic ruminants in Africa and Asia. CaPV infections are generally host specific and they have specific geographic distributions. CaPVs are, however, serologically indistinguishable from each other, able to induce heterologous cross-protection, and able in some instances to experimentally cross-infect (Davies, 1991; Tulman *et al.,* 2001; Alaa *et al*., 2008).

CaPVs induce highly economic important diseases of sheep, goat and cattle causing significant production losses in endemic countries. Sheep pox and goatpox cause reduced milk production, decreased weight gain, abortion, damage to wool and skin, increased susceptibility to pneumonia and fly strike and mortality (Bhanuprakash *et al*., 2006; Alaa *et al*., 2008). A production loss by LSD is also similar in cattle causing skin damage with occasional fatality. CaPVs diseases can be introduced into the countries where the diseases are exotic, the economic costs because of trade restrictions and the need of disease eradication would be substantial and comparable to a Foot and Mouth disease outbreak (Babiuk *et al*., 2008). *Capripox* diseases are considered as transboundary diseases which have significant impendent on livestock market and animal products. In addition *Capripoxviruses* are listed by the OIE and US Department of Agriculture as Select Agents Legislation on the National Select Agent Registry List A and are considered as potential economic bioterrorism agents (Castro and Heuschele, 1992; Babiuk *et al*., 2008).

2.5. Etiology of lumpy skin disease

Lumpy skin disease virus (LSDV) is the causative agent Lumpy skin disease, belonging to the family of *poxviridae*, It belongs to the genus *capripoxvirus* that includes sheep pox virus and goat pox virus. There is only one serotype of LSDV Neethling strain (James, 2004; Vorster and Mapham, 2008). Restriction endonuclease studies of *capripoxviruses* indicate that LSDV strains

are essentially identical with each other and with a Kenyan strain (O 240/KSGP) of sheep and goat pox virus (SGPV). Other strains of SGPV from Kenya were different from the O 240/KSGP strain but similar to each other and resembled strains of SGPV from the Arabian Peninsula. The Kenyan group of SGPV strains showed differences when compared with ones from India, Iraq, and Nigeria (Kitching *et al*., 1998; James, 2004).

2.5.1. Morphological structure LSDV

LSDV has a Linear, dsDNA genome of about 151kb. The genome is flanked by inverted terminal repeat (ITR) sequences which are covalently-closed at their extremities. And the virion is enveloped, brick-shaped, 300×270x200nm. The surface membrane displays surface tubules or surface filaments. Two distinct infectious virus particles exist, the intracellular mature virus (IMV) and the extracellular enveloped virus (GPBR, 2008; Yehuda *et al.*, 2011).

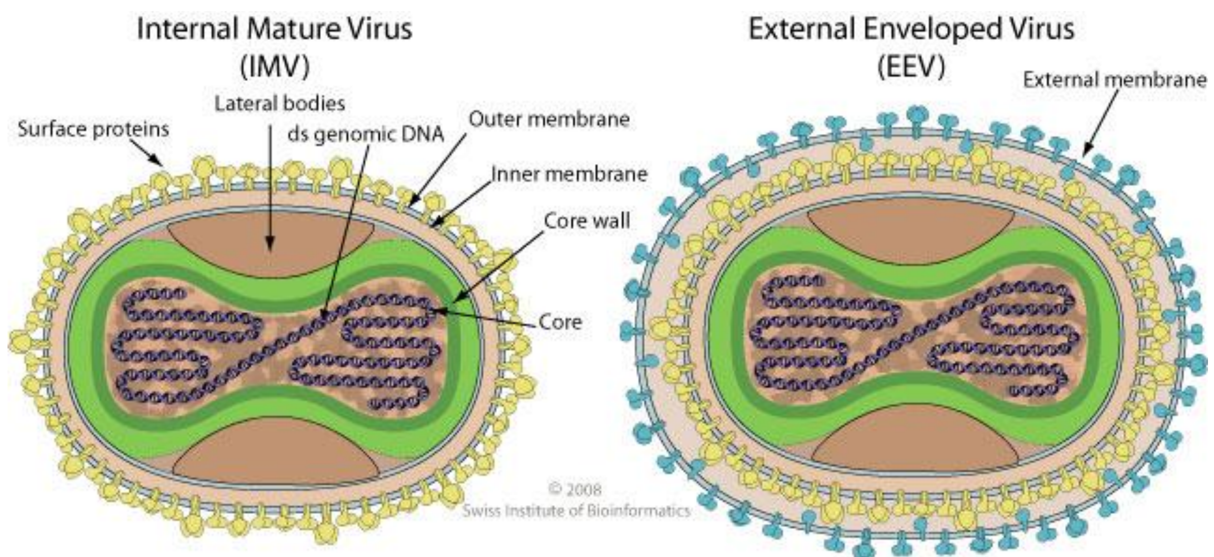

Figure 6: Morphological structure LSDV

2.5.2. Physico-chemical property of LSDV

LSDV is very resistant to physical and chemical agents. The virus persists in necrotic skin for at least 33 days and remains viable in lesions in air-dried hides for at least 18 days at ambient temperature. The virus is remarkably stable and it can survive in skin nodules kept at –80 °C for ten years and from infected tissue culture fluid stored at 4 °C for six months (Vorster and Mapham, 2008). Purified virus resists 100^0C dry heat for 5-10 min, but is destroyed by moist heat (60C) within 10 min**.** LSDV is susceptible to sun light and detergents containing lipid (Babiuk *et al.*, 2008; Lefevre and Gourreau, 2010).

2.5.3. Genome organization and protein processing

The genome of LSDV is 151-kbp; this genome consists of a central coding region bounded by identical 2.4 kbp-inverted terminal repeats and contains 156 putative genes. Comparison of LSDV with *chordopoxviruses* of other genera reveals 146 conserved genes which encode proteins involved in transcription and mRNA biogenesis, nucleotide metabolism, DNA replication, protein processing, virion structure and assembly, and viral virulence and host range (Tulman *et al.,* 2001).

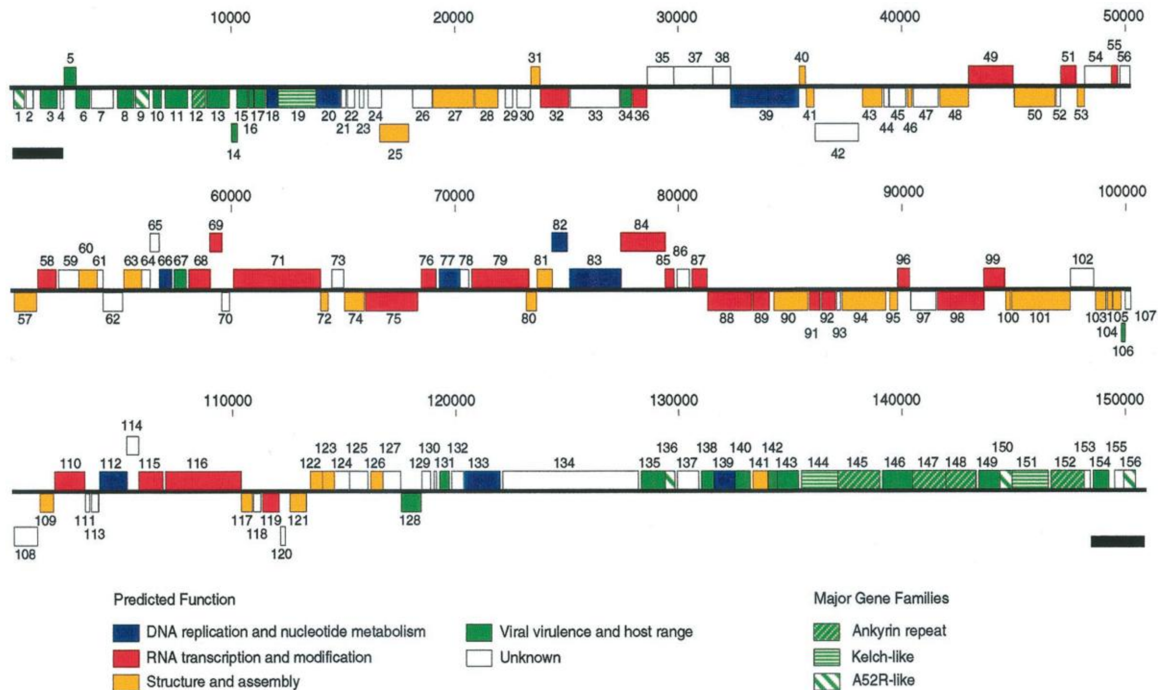

Figure 7: linear map of the LSDV genome. ORFs are numbered from left to right based on the position of the methionine initiation codon. ORFs transcribed to the right are located above the horizontal line; ORFs transcribed to the left are below. Genes with similar functions and members of gene families are colored according to the figure key. ITRs are represented as black bars below the ORF map (Tulman *et al.,* 2001).

LSDV contains a number of potential host range genes with likely functions in modulation or evasion of host immune responses, in modulation or inhibition of host cell apoptosis, and in aspects of cell and/or tissue tropism. Many potential LSDV host range genes are similar in sequence and in terminal genomic location to genes present in other poxviruses. However, LSDV encodes unique complement genes which dictate its specific host range properties (Tulman *et al.,* 2001). LSDV also contains the same complement of nucleotide metabolism genes found in the leporipoxviruses and, like the leporipoxviruses, it lacks a large subunit of ribonucleotide reductase, this shared complement likely reflects phylogenetic relatedness but may also be significant in cell and/or tissue tropism (Willer *et al*., 1999; Tulman *et al.,* 2001; Kara *et al*., 2003; Le Goff *et al*., 2009).

LSDV encodes at least 30 homologues of poxviral protein known to be structural or involved in virion morphogenesis and assembly. These include proteins present in the virion core; proteins present in the intracellular mature virus (IMV) and associated membranes; potential enzymes involved in protein modification, DNA packaging, and redox activity; and at least four Vaccinal virus proteins found in or associated with the release of extracellular enveloped virions (Tulman *et al.,* 2001).

LSDV secretes six proteins potentially and likely involved in the disruption or modulation of host immune responses, as indicated by the presence of potential signal peptide sequences and/or similarity to other secreted immunomodulators. These include homologues of cellular and viral interleukin-10 (IL-10), gamma interferon (IFN-g) receptor (R), IL-1R, IFN- α / β binding protein, and IL-18 binding protein. LSDV is the first poxvirus known to encode two proteins, in addition to poxvirus IFN- α / β binding proteins, with similarity to IL-1 R (Tulman *et al.,* 2001). LSDV also contains four potentially membrane localized, immunomodulatory proteins. Homologues of a G protein-coupled β-Chemokine receptor (GPCR), CD47, and poxvirus OX-2- like proteins potentially bind extracellular factors and/or influence intracellular signal transduction mechanisms to affect immune mechanisms or host range (Lalani *et al*., 1999; Tulman *et al.,* 2001; Kara *et al*., 2003). Several LSDV proteins are likely to have intracellular roles in immune modulation or immune evasion. These include homologues of vaccinia virus PKR inhibitors which confer resistance to the antiviral effects of IFN. LSDV encodes six homologues of other poxviral proteins known to affect virus virulence, virus growth in specific cell types, and/or cellular apoptotic responses. These include homologues of epidermal growth factor (EGF), VV C7L host range, N1L virulence, and A14.5L virulence proteins, MYX M004 and M011L anti-apoptosis proteins, and the rabbit fibroma virus (RFV) N1R/ectromelia virus p28 host range factor (Tulman *et al.,* 2001).

2.6. Epidemiology of LSD

2.6.1. Geographic distribution

Lumpy skin disease is generally confined to Africa. Until the 1980s, this disease was only found south of the Sahara desert and in Madagascar, but in 1988, it spread into Egypt. It also occurs in other Middle Eastern countries. In 1989, an outbreak in Israel was eradicated by slaughter and vaccination (OIE, 2008) and more recent outbreaks of LSD outside Africa have been reported in Israel (2006 and 2007), Palestine (2007 and 2008) and Bahrain (2006-2009). Some field outbreaks are associated with severe and generalized infections and a high mortality, while with others there are few obviously affected animals and no deaths but in general outbreaks are more severe with the initial introduction of the infection to a region and then abate, probably associated with the development of widespread immunity (OIE, 2010; Anonymous, 2011; Body *et al*., 2012).

In Ethiopia LSD was first observed in the northwestern part of the country (southwest of Lake Tana) in 1983 (Mebratu *et al.*, 1984). It has now spread to almost all the regions and agroecological zones. Because of the wide distribution of the disease and the size and structure of the cattle population in Ethiopia it is likely that LSD is one of the most economically important livestock diseases in the country (Gari *et al.*, 2010). Data investigations from the national disease outbreak report database during the period 2000-2009 showed that major epidemic outbreaks of LSD occurred in 2000/2001 in the northern parts of the country in Amhara and West Oromia regions. Then it extended to the central and the southern parts of the country in 2003 and 2004 covering large parts of Oromia and Southern Nation, Nationalities and Peoples (SNNP) regions. In 2006 and 2007 another extensive outbreak reappeared in Tigray, Amhara and Benishangul regions in the northern and north-western parts of the country. From 2007 up to 2009 the outbreak number progressively increased in Oromia Region situated in the central part of the country while it seemed to be gradually decreasing in the northern part of the country including Tigray, Amhara and Benishangul regions. This showed that an epidemic reoccurs after an interval of 5-6 years cycle in unvaccinated cattle population (Gari, 2011).

According to 2010 annual report of Ministry of Agriculture, animal and plant health regulatory directorate in the department of epidemiology prevalence of the disease in different regional state of the country shows us; 1.63%, 0.49%, 5.2%, 2.69%, 0.37%, 0.7%, and 3.8% in Addis Abeba, Amhara, Gambela, Oromia, SNNP, Somali and Tigray regions respectively. The 2011 annual report shows prevalence of; 0.36%, 1.13%, 0.22%, 0.65%, 0.24% and 0.30% in Amhara, Gambela, Oromia, SNNP, Somali and Tigray regions respectively. This is an indicative that how much the disease distributes throughout the country.

2.6.2. Epidemiological risk factors

Host risk factor

All ages and types of cattle are susceptible to the causative virus, except animals recently recovered from an attack, in which case there is a solid immunity. In outbreaks, very young calves, lactating and malnourished cattle develop more severe clinical disease. British breeds, particularly Channel Island breeds, are much more susceptible than zebu types, both in numbers affected and the severity of the disease because of their thin skin. Wildlife species are not affected in natural outbreaks, although there is concern that they might be reservoir hosts. Serological evidence of naturally acquired infection has been observed only in African buffalo (Syncerus caffer). There is only one report of the natural occurrence of LSD in a species other than cattle, in water buffalo (Bubalis), but no further such cases are recorded (Radostits *et al*., 2006; Vorster and Mapham, 2008).

Environmental risk factor

Outbreaks tend to follow waterways and extensive epizootics are associated with high rainfall and concomitant high levels of insect activity with a peak of disease in the late summer and early autumn (Radostits *et al*., 2006). Other environmental risk factors associated with spread of LSD were found to be worm humid agro-climate, communal grazing/watering and introduction of new animals in a herd. The incidence of LSD occurrence is high during wet seasons when biting-fly populations are abundant and it decreases or ceases during the dry season (Gari *et al.,* 2010).

Pathogen risk factor

LSDV is generally resistant to drying, survive freezing and thawing. Resistance to heat is variable but most are inactivated at temperatures above 60°C (Radostits *et al*., 2006). LSDV is very resistant to physical and chemical agents. The virus persists in necrotic skin for at least 33 days and remains viable in lesions in air-dried hides for at least 18 days at ambient temperature (Vorster and Mapham, 2008).

2.6.3. Origin of infection and transmission

Infected cattle's are the main source of LSDV infection. Transmission of LSD among cattle is inefficient, and arthropod-vectored transmission may be significant in epizootic outbreaks and in the spread of LSD into nonenzootic regions and direct contact could be a minor source of infection (Alaa *et al.,* 2008). LSDV can be found in cutaneous lesions, saliva, respiratory secretions, milk and semen. Shedding in semen may be prolonged; viral DNA has been found in the semen of some bulls for at least 5 months after infection (Carn and Kitching, 1995).

The most likely way for LSD to enter a new area is by introduction of infected animals. Extensive livestock production system allows maximum chance for different herd mixing during utilization of communal grazing lands and watering points. Under this prevailing system it is likely to speculate that the introduction and spread of LSD infection could have favorable environment. Uncontrolled cattle movements due to trade, pastoralism, vector insects population and dynamic, wet climate which favors insect multiplications and other reasons of cattle movement from place to place could render potential risk factors for the transmission of the disease from herd to herd and from place to place (Toma *et al*., 1999). Animals can be infected experimentally by inoculation with material from cutaneous nodules or blood, or by ingestion of feed and water contaminated with saliva. LSDV has been proven to be transmissible to calves through infected milk (Vorster and Mapham, 2008).

Biting insects including flies (*Stomoxys* and *Tabunus*) and mosquito (*Culex* and *aedes*), are mechanical vectors and the major means of LSD virus transmission (Castro and Heuschele, 1992). And also LSD virus has been isolated from *Stomoxys calcitrans* and *Musca confiscata* and

transmitted experimentally using *Stomoxys calcitrans* but other vectors are also suspect including *Biomyia, Culicoides, Glossina* and *Musca* species. However, in a recent study, despite the detection of virus in mosquitoes (Anopheles stephensi, Culex quinquefascuatus) the stable fly and a biting midge (*Culicoides nebeculosis*) after they had fed on cattle with lumpy skin disease, the infection did not transmit to susceptible cattle when these arthropods were allowed to re-feed on them (Radostits *et al*., 2006). In a recent study researchers found molecular evidence suggesting that LSD can be transmitted through hard (Ixodid) ticks (*Rhipicephalus decoloratus, Rhipicephalus appendiculatus* and *Amblyomma hebraeum*) (Tuppurainen *et al.,* 2011) .

2.7. Pathogenesis and clinical sign of the disease

The basic pathogenic mechanism by which the virus seems to cause lesions is viral replication in cells such as the pericytes and endothelial cells in lymphatic's and blood vessels walls; giving rise to vasculitis and lymphangitis. In some more severe cases thrombosis and infarction may be the end result. Other cells such as macrophages, fibroblasts and keratinocytes may also be infected. Most animals that recover from clinical disease seem to develop a life long immunity. Immunity to LSD seems mostly cell- mediated but maternal antibodies acquired by calves may protect them from clinical diseases for approximately six months (Vorster and Mapham, 2008).

An incubation period of 2-4 weeks is common in field outbreaks and 7-14 days following experimental challenges. The clinical signs range from in apparent to severe. Host susceptibility, dose and route of virus inoculation affect the severity of disease (Knopvelsiekte, 2008). In severe cases there is an initial rise of temperature, which lasts for over a week, sometimes accompanied by lacrimation, nasal discharge, salivation, and lameness. Multiple nodules appear suddenly about a week later, the first ones usually appearing in the perineum. They are round and firm, varying from 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, in most cases, are confined to the skin area. Other manifestations that may be observed in severe cases include lesions in the nostrils and on the turbinates, causing mucopurulent nasal discharge, respiratory obstruction and snoring; plaques, later ulcers, in the mouth causing salivation; nodules on the conjunctiva,

causing severe lacrimation, and on the prepuce or vulva, and spreading to nearby mucosal surfaces. The limbs may become grossly distended with edema fluid (Radostits *et al.*, 2006; Salib and Osman, 2011).

Feed intake decreases in affected cattle, milk yield can drop markedly, and animals may become emaciated. Rhinitis, conjunctivitis and keratitis can also be seen; ocular and nasal discharges are initially serous but become mucopurulent (Knopvelsiekte, 2008). Secondary bacterial infections can cause permanent damage to the tendons, joints, teats and mammary gland. Abortions and temporary or permanent sterility may occur in both bulls and cows. A few animals die, but the majority slowly recovers. Recovery can take several months, and some skin lesions may take a year or two to resolve. Deep holes or scars are often left in the skin (Grooms, 2005).

The post mortem lesions can be extensive. Characteristic grayish-pink deep nodules with necrotic centers are found in the skin (Grooms, 2005). Similar lesions on the skin are present in the mouth, pharynx, trachea, skeletal muscle, bronchi and stomachs, and there may be accompanying pneumonia. The superficial lymph nodes are usually enlarged. Respiratory distress and death are often the result of respiratory obstruction by the necrotic ulcers and surrounding inflammation in the upper respiratory tract and/or concurrent aspiration pneumonia (Radostits *et al.*, 2006).

2.8. Diagnosis

2.8.1. Field diagnosis

Field diagnosis of LSD is often based on characteristic clinical signs of the disease. However, mild and subclinical forms require rapid and reliable laboratory testing to confirm diagnosis (Alaa *et al.,* 2008; Knopvelsiekte, 2008).

2.8.2. Laboratory diagnosis

Samples submitted for laboratory diagnosis of LSD includes; take biopsy specimen at least two early lesions (for viral isolation), clipped and cleansed with a none-disinfectant soap; if a punch biopsy is used, specimens must be collected at the lesions edge. An enlarged LN can be aspirated aseptically with a syringe and 16- gage needle or a biopsy can be taken. Organ samples should be sealed in screw-caped vials and taped shut. Tissue specimens should include all organs with emphasis on those showing lesions i.e., skin turbinate's, trachea, lung and lymph nodes. specimens should arriving to laboratory with in 24 hours ship with wet ice; if more than one day shipment is required dry ice should be used (Castro and Heuschele, 1992).

Tests for the specific diagnosis of a viral infection are of two types: (1) those that demonstrate the presence of infectious virus, viral antigen, or viral nucleic acid and (2) those that demonstrate the presence of viral antibody (Murphy *et al*., 1999). Generally LSDV diagnostic tests can be grouped into 3 categories (1) direct detection, (2) indirect examination (virus isolation), and (3) serology. In direct examination, the clinical specimen is examined directly for the presence of virus particles, virus antigen or viral nucleic acids. In indirect examination, the specimen into cell culture, eggs or animals in an attempt to grow the virus: this is called virus isolation. Serology actually constitute far the bulk of the work of any virology laboratory to demonstrate the presence antibody against the virus infection (Vorster and Mapham, 2008; OIE, 2010; Tuppurainen *et al*., 2011).

Virus isolation

LSD virus can grow in tissue culture of bovine, ovine or caprine origin, although primary or secondary culture of bovine dermis cells or lamb testis (LT) cells are considered to be the most susceptible, particularly those derived from a breed of wool sheep. Infected cells develop a characteristic CPE consisting of retraction of the cell membrane from surrounding cells, and eventually rounding of cells and margination of the nuclear chromatin. At first only small areas of CPE can be seen, sometimes as soon as 2 days after infection; over the following 4–6 days these expand to involve the whole cell sheet. If no CPE is apparent by day 14, the culture should

be freeze–thawed three times, and clarified supernatant inoculated on to fresh LT culture (OIE, 2010).

Virus Identification

LSDV can be identified using transmission electron microscopy. Material from the original biopsy suspension is prepared for examination under the transmission electron microscope by floating a 400-mesh hexagon electron microscope grid, with pileoformcarbon substrate activated by glow discharge in pentylamine vapour, on to a drop of the suspension placed on parafilm or a wax plate. After 1 minute, the grid is transferred to a drop of Tris/EDTA buffer, pH 7.8, for 20 seconds and then to a drop of 1% phosphotungstic acid, pH 7.2, for 10 seconds. The grid is drained using filter paper, air-dried and placed in the electron microscope. The capripox virion is brick shaped, covered in short tubular elements and measures approximately 290 × 270 nm. A host-cell-derived membrane may surround some of the virions, and as many as possible should be examined to confirm their appearance (Kitching and Smale, 1986).

Viral Nucleic acid identification

The polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The conventional gel-based PCR method is a simple, fast and sensitive method for the detection of capripoxvirus genome. In EDTA blood, biopsy, semen or tissue culture samples. However, it does not allow differentiation between LSD and sheep and goat pox viruses. Primers for the viral attachment protein gene and the viral fusion protein gene (Ireland and Binepal, 1998) are specific for all the strains within the genus *Capripoxvirus*. By the use of sequence and phylogenetic analysis; strains of virus can be identified (Le Goff *et al*., 2009).

Serological testes

All the viruses in the *Capripoxvirus* genus share a common major antigen for neutralising antibodies and it is thus not possible to distinguish strains of capripoxvirus from cattle, sheep or goats using serological techniques. Virus neutralization, Agar gel immunodiffusion, Indirect fluorescent antibody test are sensitive serological diagnostic system for the detection of antibody to *Capripoxvirus* structural proteins (OIE, 2010).

2.9. Prevention and control

The most likely way for LSD to enter a new area is by introduction of infected animals. Biting insects that have fed on infected cattle may travel and be blown for substantial distances. The movement of contaminated hides represents another potential means for this resistant virus to move (Davies, 1991). Vaccination is the only effective method to control the disease in endemic countries like Ethiopia. The experience in the major parts of the country showed that the vaccination approach is commonly chosen and is often that of ring vaccination around a local foci outbreak when it occurs. Animals that recover from virulent LSD infection generate lifelong immunity consisting both of a humoral and cell mediated protective immunity (Kitching *et al*., 1987). Maternal immunity provides protection from LSD in calves at least for 6 months (Davies 1991). In previously LSD-free countries, in the event of an outbreak, the rapid confirmation of a clinical diagnosis is essential so that eradication measures, such as quarantine, slaughter-out of affected and in-contact animals, proper disposal of carcasses, cleaning and disinfection of the premises and insect control and ring vaccinations can be implemented as soon as possible (Tuppurainen *et al*., 2005; Radostits *et al.*, 2006).

Live attenuated vaccines of different capripoxvirus strain origins are available to protect cattle, sheep and goats. Four live attenuated strains of capripoxvirus have been used as vaccines specifically for the control of LSD (Carn and Kitching, 1995; Brenner *et al*., 2006; Kitching, 2003). A strain of Kenyan sheep and goat pox virus passaged 18 times in LT or fetal calf muscle cells, Yugoslavian RM 65 sheep pox strain, Romanian sheep pox strain and lumpy skin disease virus strain from South Africa, passaged 60 times in lamb kidney cells and 20 times on the chorioallantoic membrane of embryonated chicken eggs (OIE, 2010). It is likely that many of these vaccine strains available in different parts of the world would be suitable for the prophylaxis of LSD. These live attenuated vaccines are mainly stimulating the cell mediated immune response (Davies, 1991; Kitching, 2003).

In Ethiopia Kenyan SGPV strain, Romanian sheep pox strain and South African Neethling vaccinal strains were used for production of LSD vaccine at the National Veterinary Institute (NVI). But in the current time only the vaccine that produced from Kenyan SGPV strain is widely used for vaccination of all cattle, sheep and goats populations in the country.

3. MATERIALS AND METHODS

3.1. Study area

This study was conduct from October 2011 to April 2012 in selected areas of Eastern Shewa (in and around Adama, Mojo, Welenchit and Wenji). East Shewa is one of the 12 Zones of the Ethiopian Region of Oromia. This zone is located in the middle of Oromia, connecting the western regions to the eastern ones. And it is bordered on the south and southwest by the Southern Nations, Nationalities and Peoples Region, on the west by Southwest Shewa, on the northwest by North Shewa, on the north by the Amhara Region, on the northeast by the Afar Region, and on the southwest by Arsi (CSA, 2007).

According to planning office of eastern shewa zone of agriculture, eastern shewa zone has three agro-climatic zones; "Weyna Dega" (midland), "Kola" (lowland) and "Dega" (highland) zones representing 70%, 25% and 5% of the total area respectively (Eastern Shewa Agricultural Zone, 2011).

Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this Zone has a total population of 1,356,342, of whom 696,350 are men and 659,992 women. The dominant livestock population in the area is cattle (1,130,386) followed by sheep (521,654) and goat (522,058) populations with an area of 8,370.90 square kilometers (CSA, 2007).

Eastern Shewa zone state farm metrological station recorded two rainy seasons in the area; the long rainy season that extends from late June to late November and the short rainy season extends from February to April with mean annual rainfall of 450-1000mm and mean temperature of 17-30°c (Eastern Shewa Agricultural Zone, 2011).

Administrative Zones and regional states of Ethiopia

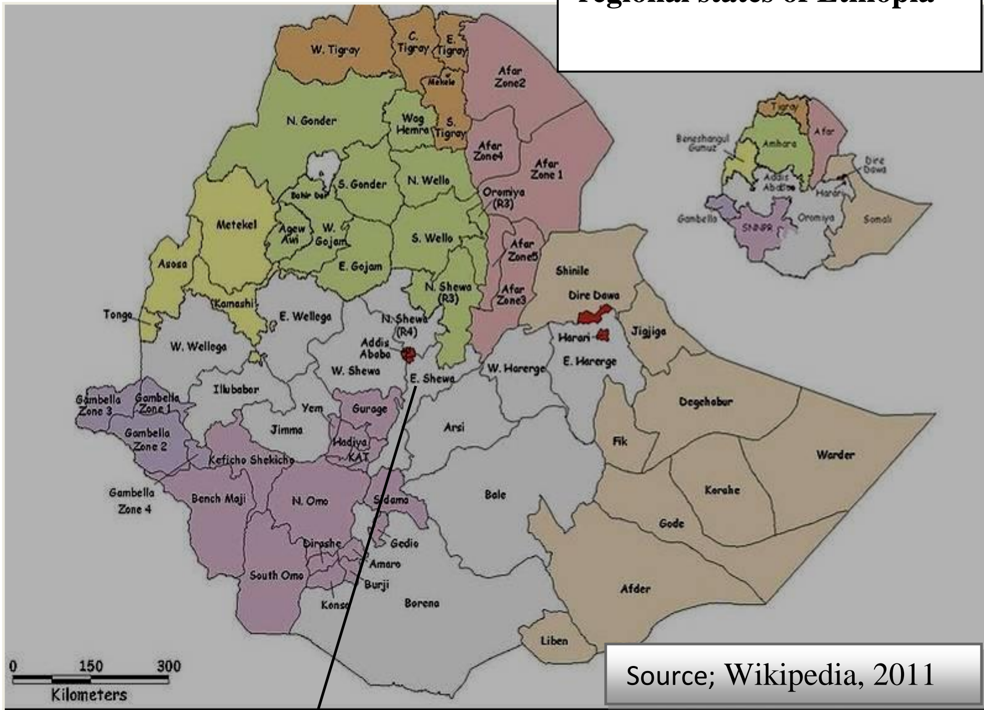

Figure 8: Map of the study area

3.2. Study design

An outbreak investigation was conducted starting from September 2011 to April 2012 to estimate the outbreaks of the disease, cases, and deaths and to sketch the seasons for the occurrence of LSD in the area. Active outbreaks were assessed together with veterinary professionals who are working in the zonal veterinary laboratories and district veterinary clinics. When an active outbreak of LSD was encountered or reported, field investigations were conducted and information was gathered by interviewing cattle owners and district animal health workers. In searching outbreak, even there was at least one case of LSD in an area, it was considered as an outbreak. Clinical and epidemiological data were recorded and samples were collected for virus isolation, identification.

3.3. Questionnaire survey and Epidemiological data collection

A structured questionnaire format was prepared to interviewed individual owners of cattle. The investigation process involves seasonality and occurrence of out break, milk reduction, abortion in pregnant cows, and presence of death due to the disease. The investigation also assessed the difference between vaccinated and non vaccinated animals that became affected by the disease. Relevant data was gathered by observing clinically sick animals and interviewing cattle owners and animal health workers working at the field. Information was carefully recorded on a designed format (Annex 1).

3.4. Sample collection

According to the procedures of OIE (2010), samples for virus isolation and antigen detection were collected by biopsy from skin nodules. Skin nodules from 22 representative cattle which developed severe clinical sign of the disease were taken aseptically by washing and cleaning the area and removing the hairs with the help of sterile scalpel blade. Tissue samples were placed in

the sterilized universal bottle and transported to National Veterinary Institute virology laboratory with in 4 to 8 hrs of collection with in icebox and kept at -20 ^{0}C until processed.

3.5. Virus isolation

Vero cell was used for LSD virus isolation. The biopsy samples were thawed at room temperature and washed three times using sterile PBS at a PH of 7.2 under laminar air flow hood class II. About 1 gm of each samples were grounded using sterile mortar and pestle by adding 9 ml of sterile PBS containing antibiotic (0.1% Gentamicine). The tissue suspension was centrifuged three times at 1500 rpm for 15 min. The supernatant was collected and filtered by Millipore filter of 0.45µm pore size. About 1ml of filtered tissue suspension was inoculated on Vero monolayer cells grown on 25cm^2 tissue culture flasks and then flashed with 9 ml of suitable medium of GMEM, containing antibiotic and 2 % fetal calf serums and incubated at $37^{o}{}_{C}$ and 5% CO_2 in a humidified incubator for 48hrs. Cells were monitored daily for 14 days for evidence of Cytopathic effect (CPE) under 10x power of inverted microscope, and frozen when CPE was exhibited.

3.6. Extraction of viral DNA

DNA extraction was performed using DNeasy Blood and tissue kit (Qiagen, Rocm and Haas Company, USA) according to the procedures of the manufacturer. DNA was extracted from 22 specimens; 180 µl crude virus in culture supernatant from the LSDV infected cells were pipated in to 2 ml of micro centrifuge tube clarified by centrifugation at 8000 rpm for 5 min. 200 µl of AL buffer was added in to the centrifuged virus suspension and mixed by vortexing and incubated at 56 ^{0}c for 10 min. 200 µl of ethanol (100%) was added and mixed thoroughly with the help of vortex mixer. the mixture was transferred in to DNeasy min spin column in 2ml collection tube and centrifuged at 8000 rpm for 5 min. the spin column was transferred in to a new 2 ml collection tube and 500 µl of AW_1 centrifuged for 1 min at 8000 rpm. after this the collection tube was discarded and the min spin column placed in a new 2 ml collection tube and 500 µl of AW2 was added and centrifuged for 3 min at 14,000 rpm, min spin column transferred

carefully in to a new 2 ml of micro centrifuge tube and 200 µl of elution buffer (AE buffer) added and incubated for 1 min at room temperature centrifuged for 1 min at 8000 rpm. The spin column was discarded and the DNA containing micro centrifuge tube was placed at -21°C until amplification process started.

3.7. Polymerase chain reaction and primer used

A polymerase chain reaction (PCR) was carried out for the confirmation of the disease by using commercial *capripoxviru* PCR kit' (Vienna, Australia) with the sequence of forward primer (SpGpRNAPol F) 5'-TCTATGTCTTGATATGTGGTGGTAG-3' and reverse primer (SpGpRNAPol R) 5'-AGTGATTAGGTGGTGTATTATTTTCC-3', amplifies CaPV homologues of the vaccinia virus E4L gen which encodes the 30 KDa DNA-dependent RNA polymerase subunit (Charles *et al*., 2011). DNA amplification was carried out in a final volume of 50 µl containing the following: 5 µl of (10Mm) PCR buffer, 1.5 µl of $MgCl_2$ (25Mm), 1 µl of dNTP mixture (10 Mm), 1 µl of (50 Mm) forward primer, 1 µl of (50 Mm) reverse primer, 5 µl of DNA template, 0.5 µl of Taq DNA polymerase and 35 µl of RNAas free water. All PCR experiments performed in a PTC-100 thermal cycler (MJResearch, Inc., Waltham, Massachusetts, USA) using the following amplification program: initial denaturation at 95°C for 1 min; 40 cycles of denaturation at 95°C for 30 s, annealingat 55°C for 30 s and elongation at 72°C for 1 min. An additional elongation step was performed at 72°C for 5 min and the PCR products were stored at 4°C until analysis.

3.8. Agaros gel electrophoresis

Amplified products were analyzed using a gen ruler^{TM} 100bp plus DNA ladder (Fermentes, Germeny) as a molecular marker on 2% agarose gels prepared in Tris/Acetate/EDTA (TAE) buffer. Twenty µl of PCR product was mixed with 5 µl loading buffer and loaded to wells in previously prepared gel and run at 100 volt for about 60 minutes in parallel with DNA molecular weight marker in electropherosis apparatus until the DNA samples have migrated a sufficient

distance through the gel. DNA bands were visualized using an UV transilluminator at a wavelength of 590 nm, and positive results were confirmed according to the size of the bands formed on agarose gel. The PCR results were considered positive for LSD virus DNA when a 172bp product was observed.

3.9. Vaccine Efficacy test

Only live attenuated vaccines against LSD are currently commercially available. Because of antigenic homology and cross-protection between sheep pox, goat pox and LSD viruses, any of these viruses can be used as a vaccine strain to protect cattle against LSD (Kitching, 1983). KSGPV vaccinal strain of capripox is used for vaccine production of lumpy skin disease in Ethiopia.

Vaccine efficacy test of the current vaccine used in the country was performed on six selected seronegative young Holstein-Friesian calves with an age of six to seven months. Four calves were inoculated subcutaneously with the recommended field dose of lumpy skin disease virus vaccine which is produced at NVI. Two calves were remained in contact unvaccinated as a control. On day 22 all of the calves challenged intradermally with virus isolates from the field. Daily follow up was performed for 14 days and results were well recorded on designed format. And the following formula was used to evaluate the efficacy of the vaccine

$$VE = \frac{(ARU - ARV)}{ARU} \times 100$$

Where

VE = vaccine efficacy
ARU = attack rate in the unvaccinated population and
ARV = attack rate in the vaccinated population.

3.10. Data Management and Analysis

The collected data was entered and stored into Microsoft Excel spread sheet 2007. The data were thoroughly screened and properly coded before subjecting to statistical analysis. The data were imported from the Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) software version 19.0. Descriptive statistics was also used to quantify the results on number of outbreaks, cases and deaths during the outbreaks. In all the analyses, confidence level was at 95% and $p \leq 0.05$ was set for significance

4. RESULTS

4.1. Out break investigations and Clinical signs observed

During the study period, a total of two outbreaks were investigated in the study period of time. One of the outbreaks was occurred on September 2011 in Adama and Wenji and the other out break were occurred between December and January 2011 in Mojo and Welenchit areas. In total 2174 local Zebu breed cattle the majority composed males (98.8%) were investigated. According to the information obtained from cattle owners, veterinarians and animal health assistants who are working in the outbreak areas, a total of 296 cattle were affected by LSD, from which 108 cattle were died, with 13.78% and 5.03% morbidity and mortality rates respectively. The most commonly observed signs of LSD on the clinically sick cattle were fever, nodules on the skin with different size (Figure 4), enlarged peripheral lymph nodes (Figure 5), necrotic nodules and deep scab formation (Figure 6), dullness, lameness, and lacrimation.

Analysis of the outbreak statistics based on each study area (Table 2) revealed a relatively consistent morbidity rate with highest value in Adama (15.67%) followed by Wenji (10.26%), Wolenchiti (8.8%), and Mojo (7.01%). Highest mortality rate was observed in Adama (5.99%) followed by Wenji (3.42%), Welenchiti (2.4%) and Mojo (0%). Case fatality rate also higher in Adama (38.2%) and low in Mojo (0%).

Based on the descriptive statistics result age, sex and vaccination status were found to be significantly ($P<0.05$) associated with the occurrence of lumpy skin disease (Table 3).

The sex specific morbidity rate was higher in female (23.07%) than male (13.5%) which is statistically significant, but sex specific mortality was high in males (4.99%) than females (3.8) and also the case fatality rate was higher for males with 36.9% and which is also statistically significant (Table 4).

The age was categorized into three exclusive age groups as calve (< 2 years), young (2 - 4 years) and adult (> 4 years). Morbidity rate of cattle in different age groups were higher in calves (18.03%) and lower in adults (10.8%) and also the age specific mortality and case fatality rate were higher in calves with 9.84% and 54.62% respectively (Table 4).

The vaccine specific morbidity (15.1%) and mortality (5.37%) rates were found to be higher in vaccinated cattle than unvaccinated cattle in the study area (Table 4).

Table 2: Morbidity and Mortality rates and Case fatality of lumpy skin disease outbreaks in each study area.

Area	Susceptible cattle	Affected cattle	Death	Morbidity rate (%)	Mortality rate (%)	Case fatality rate%
Adama	1495	230	88	15.38	5.89	38.2
Wenji	497	51	17	10.26	3.42	33.3
Mojo	57	4	0	7.01	0	00
Welenchiti	125	11	3	8.8	2.40	27.2
Total	2174	296	108	13.78	5.03	24.67

Table 3: Occurrence of lumpy skin disease with different risk factors; sex, age and vaccination status of the cattle.

variable	Susceptible cattle	Affected cattle	prevalence	X^2	p-value
Sex					
Male	2148	290	13.5		
Female	26	6	23.07	1.98	0.02
Age(years)					
<2	599	108	18.03		
2-4	1283	156	12.15		
>4	292	32	10.8	0.71	0.01
Vaccination status					
Vaccinated	1601	242	15.1		
unvaccinated	573	54	9.4	11.62	0.001

Significant (P<0.05)

Table 4: Morbidity, Mortality and Case fatality of LSD based on the sex, age and vaccination status of the cattle

Variable	Susceptible cattle	Affected cattle	Morbidity rate%	Death	Mortality rate%	Case fatality rate%
Sex						
female	26	6	23.07	1	3.8	3.84
male	2148	290	13.5	107	4.98	36.9
Age(years)						
<2	599	108	18.03	59	9.84	54.62
2-4	1283	156	12.15	47	3.6	30.12
>4	292	32	10.8	2	0.68	6.25
Vaccination status						
Vaccinated	1601	242	15.1	86	5.37	35.5
Unvaccinated	573	54	9.4	22	3.8	40.7

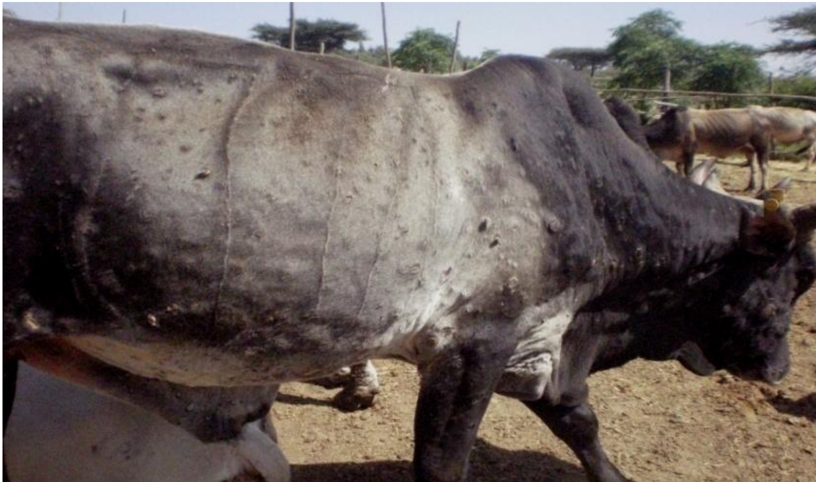

Figure 4: Large circumscribed skin nodules in LSD affected cattle

Figure 5: Enlargement of peripheral lymph nodes

Figure 6: Progression of lumpy skin disease; necrotic nodules and formation of deep scab

Figure 7**:** Large circumscribed skin nodules in LSD affected cattle and deep eroded lesion after removal of the skin nodule

4.2. Virus Isolation

Both non infected (negative control) and infected Vero cells with 1ml filtered LSDV suspected tissue suspensions were thoroughly investigated in parallel under 10x magnification of inverted microscope for the development of CPE. CPE (Figure 9) was observed in all 22 Vero cells infected with suspected sample starting from the 5th day up to 11th days post incubation at $37^{O}{}_{C}$ and 5% CO_2 in a humidified incubator. The CPE was characterized by destruction of monolayer, infected cells were rounded formed singly and aggregation of died cells was investigated. None of the negative controls produced any CPE (Figure 8).

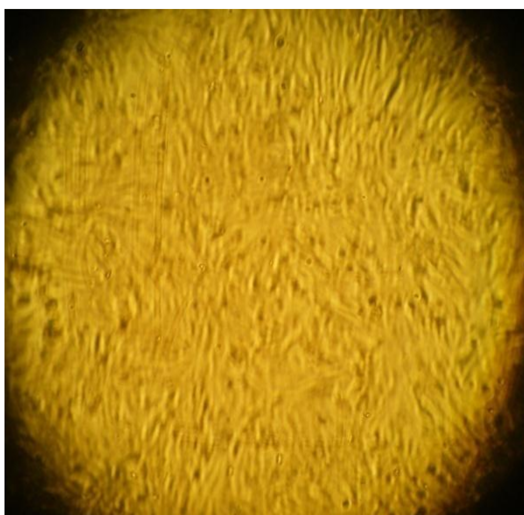

Figure 8: None infected Vero cell (Negative control)

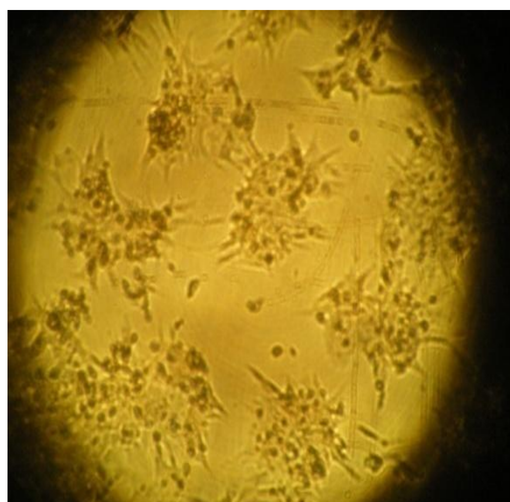

Figure 9: CPE formation on day nine.

4.3. PCR

DNA was extracted from twenty two samples from skin nodules, collected from 22 local Zebu cattle. Subsequently, the extracted DNA was amplified and visualized by convectional PCR assay to detect LSDV genomes. The specific primers set amplified a DNA fragment of 172 bp equivalent to the expected amplification product (amplicon) size from LSDV. All of the 22 skin biopsy samples (100 %) were positive for this PCR assay. None of the negative controls

produced any amplicon (Figure 10). Subsequently, it was certain that these specimens contained DNA of LSDV.

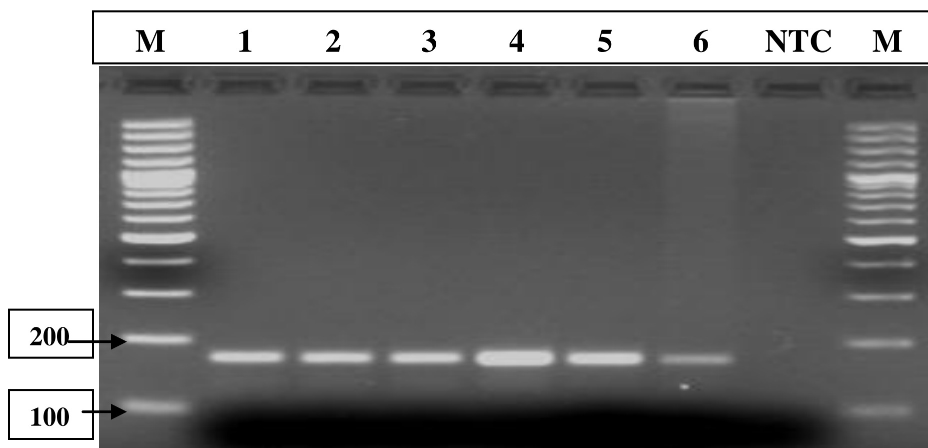

Figure 10**:** PCR based detection of LSDV in samples taken from skin nodules of infected animals. Lanes M: DNA ladder; lane NTC :Negative Template Control ; Lanes 1and 2 represent positive samples from Adama; Lanes 3 and 4 represent positive samples from Wengi; Lanes 5 represent positive sample from Mojo; Lane 6 is a positive sample from Welenchit.

4.4. Questionnaire survey

The result of questionnaire survey from interviews conducted with 49 animal owners and animal attendants in the study area indicates that the dominant live stock population in the study area is cattle followed by sheep and goat raised under traditional condition management system. Average number of cattle per population per house hold is four.

``Fentata`` is the local name of lumpy skin disease in those areas. Before 2009 the disease occurred rarely as sporadic form; but starting from June 2009 LSD occurred as endemic form in the area. The respondents also replied that the incidence of the disease increases during rainy season.

Concerning the possible source of infection, they responded as follows: due to contact with sick animals (19/49), introduction of sick animals to the herd (7/49) and the rest (23/49) responded that they did not know the source of infection

The effect on milk yield based on 4 LSD positive lactating cows before and after infection indicated that LSD caused reduction in milk yield of the cows by an average of 1.5 lit./cow/day, which is equivalent to 45 lit./cow/month. In terms of Ethiopian birr it causes loss of 360 birr/cow/month from the sell of milk (Since the current milk price is eight birr/ lit.).

Furthermore one of 5 LSD positive pregnant cows aborted. This means abortion rate of the disease in the study area was about 3.84% per total susceptible cows in the current study.

The survey also assessed economic loss due to death of animals from LSD. Production loss due to mortality was computed based on the weighted average price, determined for each breed, sex, and age group, of animals that had died of LSD. An average cost of a single ox died from this disease is 9,000 of Ethiopian birr (at fattening farms). This means the average economic loss from LSD in the study area in the present study is around 972,000 Ethiopian birr (since 108 cattle were dead in this study period).

The estimated total expense incurred for the treatment of LSD was also assessed. An average of 16.50 birr/animal was incurred for treatment of LSD with frequency of treatment of once per month. High economic loss due to additional feed cost for diseased animals until their recovery was also reported in intensive fattening farms.

A result from the survey also shows LSD causes in reduction of draught power of oxen in terms of in time of plough. The valuation of the draft power loss depended on the point in the crop season that an ox fell sick and on the corresponding demand for draft power during that specific season.

The importance of vaccination against LSD with KSGP vaccinal strain of capripox virus, which is currently used for the production of lumpy skin disease vaccine at National Veterinary Institute showed that from the total diseased and dead ones about 81.75% and 79.6% of cattle were vaccinated with this vaccine respectively.

4.5. Vaccine efficacy test

KSGPV vaccinal strain of *caprepoxvirus* is used for production LSD vaccine in Ethiopia but complaints have been reported from different corners of the country about the effectiveness of the vaccine. Even in the present study the result of the questionnaire survey on vaccine protection assessment showed us high percentage of morbidity and mortality rates were recorded among the vaccinated ones.

Vaccine efficacy test on six different seronegative young calves were performed based on the procedures on the OIE (2010) manual. The result showed that among those calves that were challenged with field strain of LSDV only the two controls developed clinical signs of the disease. Those signs were local swelling on the administration site, small circular skin nodules, decreasing body weight and lacrimation. In addition, one calf from the control group died on day ten after challenging. This means;

$$VE = \frac{(ARU - ARV)}{ARU} \times 100$$

$$= \frac{2-0}{2} \times 100$$

$$= 100\%$$

Therefore based on this experiment we can say that this vaccine is 100% protective against LSD.

5. DISCUSSION

Based on this study using clinical, cell culture, and PCR diagnosis and participatory epidemiological methods, LSD was found to be one of the major cattle health problems in the study area. The local name given to LSD by the farmers is ‘Fentata’. According to the questionnaire survey result, before 2009 LSD occurs rarely as sporadic forms but starting June 2009 LSD occurs as endemic form and seasonal occurrence of the disease is high at rainy season and decreases in the dry season. This is in agreement with reports other researchers who showed incidence of LSD is high during wet seasons when biting-fly populations are abundant and it decreases during the dry season, (Radostits *et al*., 2006; Gari *et al.,* 2010).

In total 2174 local Zebu breed cattle were visited and clinically diagnosed. Morbidity (13.78%) and mortality (5.073%) rates observed during this outbreak were in agreement with other authors who reported that LSD is a disease with high morbidity (1-50%) and low mortality (<10%) rates (Radostits *et al*., 2006; Alaa *et al*., 2008; Vorster and Mapham, 2008; 2008; Salib and Osman, 2011) and these values can fluctuate according to geography, climate, management conditions, immune status of animal, breed, strain of virus involved and insect vectors involved in the transmission (Vorster and Mapham, 2008; Tuppurainen *et al.,* 2011). Based on the questionnaire survey result the abortion rate (3.84%) per susceptible cows in the study area is in agreement with the authors who reported that lumpy skin disease can cause abortion rate of 1% to 7% (Vorster and Mapham, 2008). High morbidity and mortality rate of the disease was observed in intensive fattening farms this can be due to introduction of new animals from another place. Because most of the cattle in fattening farms in the present study were coming from the market. This is in similar with the documents that reported by (Davies, 1991) who reported that the most likely way for LSD to enter a new area is by introduction of infected animals.

Age specific morbidity, mortality and case fatality rates of cattle in different age groups were higher in calves and lower in adults in the present study. The result indicated that calves were more susceptible for LSD infection than adult. The finding is in agreement with the report (Grooms, 2005; Radostits *et al*., 2006; Fayez and Ahmed, 2011).

Clinical picture of the disease led to the speculations about occurrence of LSD at affected areas. Outstanding features of the disease, i.e. fever, characteristic skin nodules, enlarged lymph nodes, lacrimation and salivation, were in agreement with those documented by (Coetzer, 2004; Grooms, 2005; Radostits *et al.,* 2006; Alaa 2008; Salib and Osman, 2011). Host susceptibility, age, immunological status of the animal, dose and route of virus inoculation affect the severity of disease (Knopvelsiekte, 2008).

Laboratory confirmation was made upon the results of virus isolation on cell culture and LSDV genome identification based on positive PCR reaction. On cell culture, CPE characterized by rounded infected cells that formed singly, aggregation of cells and destruction of monolayer was investigated. Positive PCR reaction is a quick, sensitive, reliable method as antigenic resemblance of LSD virus with sheep and goat poxvirus makes the diagnosis through routine serological tests impossible (Tuppurainen *et al.,* 2005; Anonymous, 2010). PCR is also preferred over other reliable methods of virus isolation and electron microscopy because these are not readily available and time consuming (Vorster and Mapham, 2008).

The office international des epizootics and US Department of Agriculture as Select Agents Legislation on the National Select Agent Registry consider LSD as list A and are considered as potential economic bioterrorism agents. LSD causes severe economic losses due to permanent damage to hides, a prolonged debilitating clinical course, reduced weight gain, temporary or permanent loss of milk production, temporary or permanent infertility or even sterility in bulls, and abortion of pregnant cows (Vorster and Mapham, 2008; Radostits *et al*., 2006), this was supported by the result of the present study on economic significance of the disease in infected areas. The result of survey assessment on economic loss of lumpy skin disease in the study area showed that there was high economic losses due to abortion of pregnant cows, milk reduction, cost of dead animals, cost of treatment, additional feed cost for diseased animals until their recovery and reduction of draught power of oxen in terms of time of plough since most of Ethiopian farmer uses oxen plough system.

Reports of vaccine breakdown and short duration of protection have emerged as a serious problem for efficient control of the lumpy skin disease (Kara *et al.*, 2003; Brenner *et al.*, 2009). In Ethiopia vaccination is given for control of LSD in sporadic and endemic areas with the vaccine prepared from Kenyan sheep and goat pox strain produced in National Veterinary Institute. But high percentage of morbidity and mortality rates in these study areas were reported among vaccinated animals. Similar complaints on the vaccine was reported by different authors, occurrence of LSD after the cattle were vaccinated with sheep pox vaccinal strain in Egypt by (Fayez and Ahmed, 2011) and in Israel by (Brenner *et al*., 2009). This could be the result of the above obstacle for efficient control of the disease that is reported by the researchers (Kara *et al.*, 2003; Brenner *et al.*, 2009).

Vaccine efficacy test of the vaccine that is currently used in the country for control of the disease was performed in 6 different seronegative calves. But the result of this test showed that there is no defect on immunological protection of the vaccine, all the calves except the controls developed immunological protection against the disease. From my point of view this needs further study in order to answer why the disease is occurred among vaccinated ones? It might be due to inappropriate storage of vaccine or since a failure in one or more steps of the cold chain may occur, or the vaccine may be inactivated because of exposure to direct sunlight or high environmental temperatures during the vaccination process, improper use of protective dose and poorly administration, vaccination of infected cattle (cattle which are at the incubation period of the disease that does not show clinical sign) and using of needles or diluents contaminated with virulent LSDV during the actual vaccination procedure may transmit the virus.

6. CONCLUSION AND RECOMMENDATIONS

LSD was found to be the major cattle health problem causes severe economic loss due to permanent damage to hides, a prolonged debilitating clinical course, reduced weight gain, temporary or permanent loss of milk production, temporary or permanent infertility or even sterility in bulls, and abortion of pregnant cows. In the present study LSD causes high morbidity (13.78%), mortality (5.073%) and abortion (3.84%) rate with a great economic loss followed by abortion of pregnant cows, milk yield reduction, cost of dead animals, cost of treatment and additional feed cost for diseased animals until their recovery. Vaccination is the only effective method to control the disease in endemic countries like Ethiopia. In Ethiopia vaccination is given for control of LSD in sporadic and endemic area with the vaccine that prepared from Kenyan sheep and goat pox strain but high morbidity and mortality rates reported among vaccinated ones. The efficacy test of the vaccine performed on six calves under laboratory protocol revealed that the vaccine is immunologically protective. Based on the above conclusion the following recommendations are forwarded.

- ✓ Lumpy skin disease is considered as transboundary and trade band disease which has significant impendent on livestock market and animal products. This disease not only economically affects individual cattle owners but also affects the foreign currency of the country from exporting of live animal and animal product. So the government should establish strategic policies for effective control and eradication of the disease, i.e. restriction of livestock movement, strategic vaccination program and depopulation of infected and in contact animals.

- ✓ Further isolation and molecular characterization of LSDV should be conducted so as to identify strain of the virus in the country in order to produce new vaccine.

- ✓ Further assessment of the vaccine should be performed during transportation, storage and period of protection and time of vaccination.

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8. ANNEXES

Annex 1: Questionnaire format

1. Background information:

    District_______________________________

    Kebele _____________

    Owner's name_______________________

2. Animal description

    Animal name or tag. No__________________

3. Number of cattle per house holds____________

4. Occurrence of LSD infection

    2.1. Occurrence

First time	yes ☐	No☐
Commonly occurred	yes ☐	No☐

    2.2. Season of occurrence

Dry season	yes ☐	No☐
Rainy season	yes ☐	No☐
Any season	yes ☐	No☐

    2.3. The last outbreak of LSD in the village occurred in

Before 2007	yes ☐	No☐
2007-2009	yes ☐	No☐
In 2008-2010	yes ☐	No☐
None	yes ☐	No☐

5. Animals at risk

Species	Breed	sex	Age group			Total
			<2 years	2-4 years	>4 years	
		M				
		F				

6. Animals affected

Species	Breed	sex	Age group			Total
			<2 years	2-4 years	>4 years	
		M				
		F				

7. Total number of animal dead

Species	Breed	sex	Age group			Total
			<2 years	2-4 years	>4 years	
		M				
		F				

8. Likely source of outbreaks

Introduction of infected animals yes ☐ No ☐

Contact at communal points yes ☐ No ☐

Movement of infected animals yes ☐ No ☐

9. Vaccination status

Have you vaccinated your animals against LSD? Yes ☐ No ☐

When the vaccination performed? _____________

Who gave the vaccine? ___________________

Total No of animals get sick (No sick/total No of cattle) ___________

No of animals get sick from vaccinated (No sick/total No vaccinated) _____

Opinion of the owner about the vaccine__________________________

10. Economic Impact of LSD

Do you milk LSD infected cows? Yes ☐ No ☐

Do LSD infected pregnant cows abort? Yes ☐ No ☐

11. Estimated Cost for treatment for individual diseased animals_________________________

12. No Dead (No died/total No of cattle) ___________

13. Estimated cost of dead animals_________________________

Annex 2: Cell culture procedures

1. Thawed the tissue sample at room temperature and wash three times using sterile washing buffer
2. Take enough tissue sample and grounded by sterile mortar and pestle by adding washing buffer containing antibiotic.
3. Put the grounded tissue suspension in to test tube and centrifuged three times at 1500 rpm for 15 min.
4. Collect and filter the supernatant in to sterile container
5. Inoculate filtered tissue suspension in to Vero monolayer cells and then flashed With suitable medium
6. Incubate it at $37^{o}{}_{C}$ and 5% CO_2 in a humidified incubator for 48hrs. Cells monitored daily for 14 days for evidence of CPE

Annex 3: Procedure of Agaros Gel Electrophoresis

1. Prepare 100 ml of 1.5% agarose in 1 x TBE buffer.
2. Either heat in microwave for 2 min on full power or place in a beaker of boiling water until melted.
3. Allow to cool to about 45 ^{0}C and add 10 µl of ethidium bromide (stock=mg/µl) per 10 ml, giving a final concentration of 0.5µg/ml. this can be increased to 15µg/ml if no ethidium bromide is added to the buffer
4. Pour the gel and insert well former (comb). Allow to set on a flat surface for about 15min.
5. Pour buffer 1 x TBE (containing 0.55µg/ml ethidium bromide, i.e. 1 µl of 5 mg/ml stock to every 10 ml of buffer) in to tank and remove comb from gel.
6. Prepare sample in tubes, a multi well plate or on parafilm.

    1 µl of loading buffer

    5 µl of PCR product
7. Prepare molecular weight marker.

    0.5 µl molecular weight marker

    1 µl loading buffer

    4.5 µl H_2O
8. Loading sample in to the wells formed in the gel. It is often useful to load the molecular weight marker in both the first and the last lanes.
9. Electrophorese at 120 volts for 60 min.
10. View and photograph the gel on an UV-transilluminator.