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STUDY ON LUMPY SKIN DISEASE VACCINATION PRACTICE BASED ON FIELD SURVEY AND VACCINE EFFICACY BASED ON MOLECULAR CHARACTERIZATION OF IMMUNOGENIC GENES IN AND AROUND BISHOFTU TOWN, ETHIOPIA

MSc. Thesis

By

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Department of Microbiology, Immunology and Public Health

MSc Program in Veterinary Microbiology

June, 2023

Bishoftu, Ethiopia

STUDY ON LUMPY SKIN DISEASE VACCINATION PRACTICE BASED ON FIELD SURVEY AND VACCINE EFFICACY BASED ON MOLECULAR CHARACTERIZATION OF IMMUNOGENIC GENES IN AND AROUND BISHOFTU TOWN, 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 Science in Veterinary Microbiology

By

Tamirat Haile

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June, 2023

Bishoftu, Ethiopia

Study on Lumpy Skin Disease Vaccination Practice Based on Field Survey and Vaccine Efficacy Based on Molecular Characterization of Immunogenic Genes in and Around Bishoftu Town, Ethiopia

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STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

a.a.	Amino acid
AAU	Addis Ababa University
CSA	Central Statistical Authority
CaPV	Caprine Pox Virus
DNA	Deoxy Ribonucleic Acid
EEV	Extracellular Enveloped Virus
EPA	Environmental Protection Authority
EPO	Eosinophil peroxidase
GDP	Gross Domestic Product
GTPV	Goatpox Virus
IMV	Intracellular Matured Virus
KS	Kenyan Strain
LSD	Lumpy Skin Disease
LSDV	Lumpy Skin Disease Virus
NVI	National Veterinary Institute
OIE	Organization Interationale des Epizootics
ORF	Open Reading Frame
RNA	Ribo Neuclic Acid
SPPV	Sheeppox Virus
VACV	Vaccinia Virus
WFP	World Food Program

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ABSTRACT

Lumpy skin disease virus is a member of the *Capripoxvirus* genus of the *Poxviridae* family, which affects cattle and causes significant economic losses. It is controlled by vaccination with capripox live attenuated vaccines. The aim of this research was studying lumpy skin disease Vaccination practice based on field survey and vaccine efficacy based on molecular characterization of immunogenic genes in and around Bishoftu Town, Ethiopia. Questionnaires were prepared for 101 cattle owners and animal health professionals incorporating questions with a major emphasis on LSD vaccine and vaccination practices. From transport related factors, inappropriate vaccine handling and lack of transport equipment's and from storage related factors electric outage and refrigerator problems were taken as important factors which have negative impact on effective vaccination. Nodular skin lesions that have been collected in 2022 from the same study area by the same project were used to isolate the virus using lamb kidney cells at virology laboratory of Animal Health Institute (AHI) of Ethiopia. Polymerase chain reaction was performed targeting the immunogenic genes; LSDV 117(A27L), LSDV122 (A22R), LSD 060 (L1R) and LSD 141 (B5R). Additionally, both the field isolates and currently on use LSDV vaccine strains (KS-180) from National vaccine institute (NVI) were sequenced and analyzed. The amplicons of 7 isolates and 1 vaccine strain (KS-180) immunogenic genes (*LSDV*117, LSD122, LSDV060) were used for sequencing. The sequence from Neethling vaccine strain NI-2490 and NVI/CaPV vaccine strain (KS-180 have an overall similar sequence for LSD117 (A27L) but the sequences from the field sample (wild strains) have two nucleotides substituted at 325nt and 360nt positions 'A' by 'G' and 'G' by 'A', respectively. But a wide range of mutation was found on LSDV112 (EEV glycoprotein coding) gene sequence analysis. This amino acid change may have a mutative advantage for the wild strain over vaccine strains while it interacts with the immune system. Reference capripox viruses were obtained from GenBank to create the phylogenetic tree. It is recommended that genome sequence information of LSD virus circulating in a specified area (i.e. Bishoftu) should be considered in order to enhance the efficacy of the vaccine used prevent and control LSD disease in study area. Improving the transport, storage and handling of a vaccine is important for the success of disease prevention as well.

Key words: *Genome ; Sequence analysis ; phylogenetic analysis; lumpy skin disease; Capripoxvirus; Vaccine efficacy ;LSDV ; KS-180*

1. INTRODUCTION

Ethiopia has the largest livestock population in Africa, with 65 million cattle, 40 million sheep, 51 million goats, 8 million camels and 49 million chickens in 2020 (Central Statistics Agency, CSA, 2020a). Livestock is a major source of animal protein, power for crop cultivation, means of transportation, export commodities, manure for farmland and household energy, security in times of crop failure, and means of wealth accumulation. The sector contributed up to 40% of agricultural Gross Domestic Product (GDP), nearly 20% of total GDP, and 20% of national foreign exchange earnings in 2017 (World Bank, 2017).The Ethiopian livestock population is almost entirely composed of indigenous animals. Recent estimates showed that 97.8%, 1.9%, and 0.3% of cattle are indigenous, hybrid, and exotic breeds, respectively. (CSA, 2020a). Cattle breeds found in Ethiopia include the Boran, the Menz, and the Horro. These breeds are all well-adapted to the local environment, with their hardiness making them important in agricultural production.

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 Capri poxvirus 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 genus Capri poxvirus which comprises sheep pox, goat pox and LSD virus encodes putative 156 proteins in their genome, among which some of them are reported as potential immunogenic candidate genes (Kushwaha *et al*., 2019). Capri poxvirus has two virion forms, intracellular mature virions (IMV) and extracellular enveloped virions (EEV). The most abundant particle is the IMV, which accumulates in infected cells and is released as cells die (**Moss,** 2006). Thus, evaluating of the virion structures is required to develop knowledge regarding the targets of protective antibodies. It could be a significant achievement if the immuno dominant CaPV genes (IMV/EEV**)** identified and understood very well.

The most popular Vaccinia virus immunogenic genes majorly studied were IMV proteins (A27L, L1R, D8L, H3L and A17L), for EEV proteins (B5R, A33R, A34R, A36R and A56R) (Ramirez *et al.,* 2002; Kushwaha *et al*., 2019). Meanwhile, different study reports testified that at least six of the above mentioned genes products (proteins) (H3L, A27L, B5R, D8L, L1R) resulted protective neutralizing antibodies and (A33R) a protective but non-neutralizing antibody response in mice when used as vaccine (Rodriguez et al., 1987; Hsiao et al., 1999). The vaccinia virus Orthologs present in CaPVs, like the sequence analysis on CaPV L1R genes shown that it shares more than 99% and >96% identity both at nucleotide and amino acid levels with vaccine based on analysis done on some Indian isolates (Karki et al., 2018) which testifies a strong conserved region among the three CaPVs. Thus, its immunogenicity is expected the same too. The second IMV immunogenic protein is P32 protein which is homolog of VACV H3L. In the CaPVs (ORF074 coding) had shown VACV H3L homolog and reported in all the members namely SPPV, GTPV and LSDV as a major antigenic determinant (Gnanavel *et al*.2028). The other IMV protein is A27L protein identified by its (ORF 117) and Sequence analysis revealed a significant percentage of identity among GTPV, SPPV and LSD at both nucleotide and amino acid levels (Dashprakash *et al.* 2015). For extracellular enveloped virions (EEV) glycosylated protein B5R found to be a good candidate. Based on the study done for Vaccinia virus it was found more conserved among pox virus. Immunogenicity of GTPV homolog, B5R has been evaluated in combination with other protective immunogens and the result was promising (Zheng et al., 2009). In general, since the combination those immunogenic polyvalent formulation (A27L,L1R, B5R, H3L) from LSDV (IMV &EEV) and A4L core protein structurally share approximate similarity with the whole LSDV is most protective, polyvalent formulations with various combinations of five LSDV antigens can confer complete protection.

In Ethiopia lumpy skin 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 agro ecological 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). These are 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).

Although previous studies have explored the presence of LSDV in Ethiopia (MoA, 2022), there are also data on the molecular characterization of circulating LSD viruses which majorly depend on RPO30 subunit and GPCR genes for genotyping purpose. These data were essential for understanding the molecular epidemiology and vaccine design for disease control. More recently, there are some studies questioning the field efficacy of the Kenyan sheep and goat pox vaccine strain (KS-180) (Ayelet *et al*, 2013). Therefore, this study aimed at investigating this vaccine strains field efficacy through field survey and molecularly characterization of immunogenic genes recovered from Lumpy skin disease virus isolates and comparing with the vaccine strain currently on use in Ethiopia.

1.2.Objectives of the study

1.2.1. General Objectives

General objectives this study were "Study on Lumpy Skin Disease Vaccination Practice and Vaccine Efficacy Based on Molecular Characterization of Immunogenic Genes in and around Bishoftu town, Ethiopia "

1.2.2. Specific Objectives

Accordingly, Specific objectives of the study were;

- To investigate LSD Vaccination practice Based on Field Survey
- To isolate and identify LSD virus
- Molecular characterization of LSDV immunogens to evaluate vaccine strain deviation from filed strains

2. LITERATURE REVIEW

2.1. History and economic significance of LSD

Lumpy skin disease (LSD) is caused by lumpy skin disease virus (LSDV), a virus from the family Poxviridae, genus Capripoxvirus. Sheeppox virus and goatpox virus are the two other virus species in this genus.(woah,2022) 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**

According to Maclachlan and James (2017) the family Poxviridae is subdivided into two subfamilies: Chordopoxvirinae (poxviruses of vertebrates) and Entomopoxvirinae (poxviruses of insects). The subfamily Chordopoxvirinae is further subdivided into ten genera that include viruses that cause disease in domestic or laboratory animals (Table 1). A considerable and growing number of poxviruses await precise taxonomic assignment and are currently "unclassified."

Because of the large size and distinctive structure of poxvirus virions, negative-stain electron microscopic examination of lesion material is still used in veterinary and zoonotic virology laboratories for diagnosis—this method allows rapid visualization of poxviruses in various specimens, but it does not allow specific verification of virus species or variants. Hence, diagnostic specimens are frequently left with a diagnosis of "poxvirus," "orthopoxvirus," or "parapoxvirus," with further identification only pertaining to the species of origin. The application of molecular methods such as PCR, and especially real time PCR assays will help to rapidly confirm known poxviruses in diseased animals or to identify them in novel hosts. In addition, the use of next generation sequencing technologies will help to characterize additional pathogenic poxvirus species that have not been isolated and propagated in vitro, and to discover others that are new to science. (Maclachlan and James, 2017)

Based on phylogenetic reconstructions, a new virus order, Megavirales, has been recently proposed that would include the Poxviridae, the Asfarviridae, and other families of large DNA viruses, including giant viruses that infect protozoa. Most of these viruses typically replicate in so-called "virus factories" in the cytoplasm of infected cells (Maclachlan and James, 2017).

Table1: Eight genera of chordopoxvirinea

Genus	Virus members
Avipoxvirus	*Canarypox virus*
	Fowlpox
	Juncopox virus
	Mynahpox virus
	Pigeon pox virus
	Psittacinepox virus
	Quailpox virus
	Sparrowpox virus
	Starlingpox virus
	Turkeypox virus
Capripoxvirus	Goatpox virus
	Lumpy skin disease virus
	Sheeppox virus
Cervidpoxvirus	Mule deerpox virus
Crocodylidpoxvirus	Nile crocodilepox virus
Leporipoxvirus	Hare fibroma virus
	Myxoma virus
	Rabbit fibroma virus
	Squirrel fibroma virus
Molluscipoxvirus	Molluscum contagiosum virus[a]
Orthopoxvirus	Camelpox virus
	Cowpox virus
	Ectromelia virus
	Monkeypox virus
	Raccoonpox virus
	Skunkpox virus
	Taterapox virus
	Vaccinia virus[a]
	Variola virus
	Volepox virus
Parapoxvirus	Bovine papular stomatitis virus
	Orf virus
	Parapoxvirus of red deer in New Zealand
	Pseudocowpox virus
Suipoxvirus	Swinepox virus
Unassigned	*Squirrelpox virus*
Yatapoxvirus	Tanapox virus
	Yaba monkey tumour virus

Source; *(Tuppurainen et al., 2018 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.

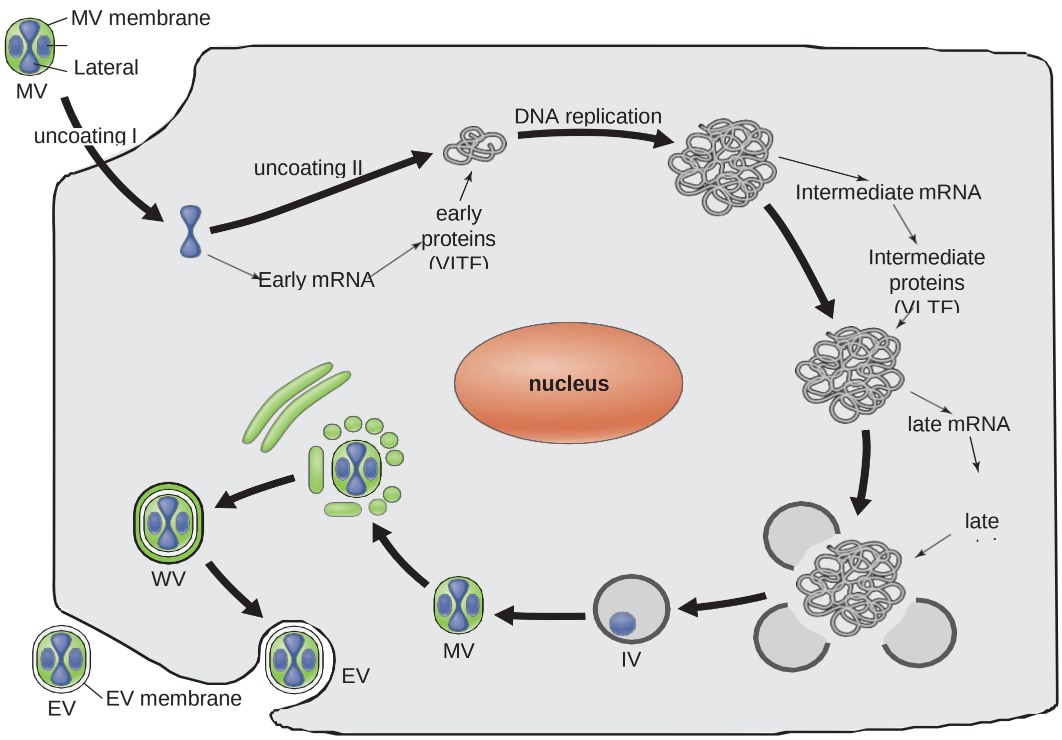


Figure .1. The vaccinia virus life cycle**.** See text for description. IV: immature virus; MV: mature virus; WV: wrapped virus; EV: enveloped virus; VETF: viral early transcription factors; VITF: viral intermediate transcription factors; VLTF: viral late transcription factors.

Source: (N.Acheson *et al*.,, 2011.)

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 Capripox virus

Capripox viruses are among the eight genera within the Chordopox virus (ChPV) subfamily of the Poxviridae. The capripox virus genus is currently composed 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 trans boundary 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 capripox virus 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 capripox viruses 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).

2.6. 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).

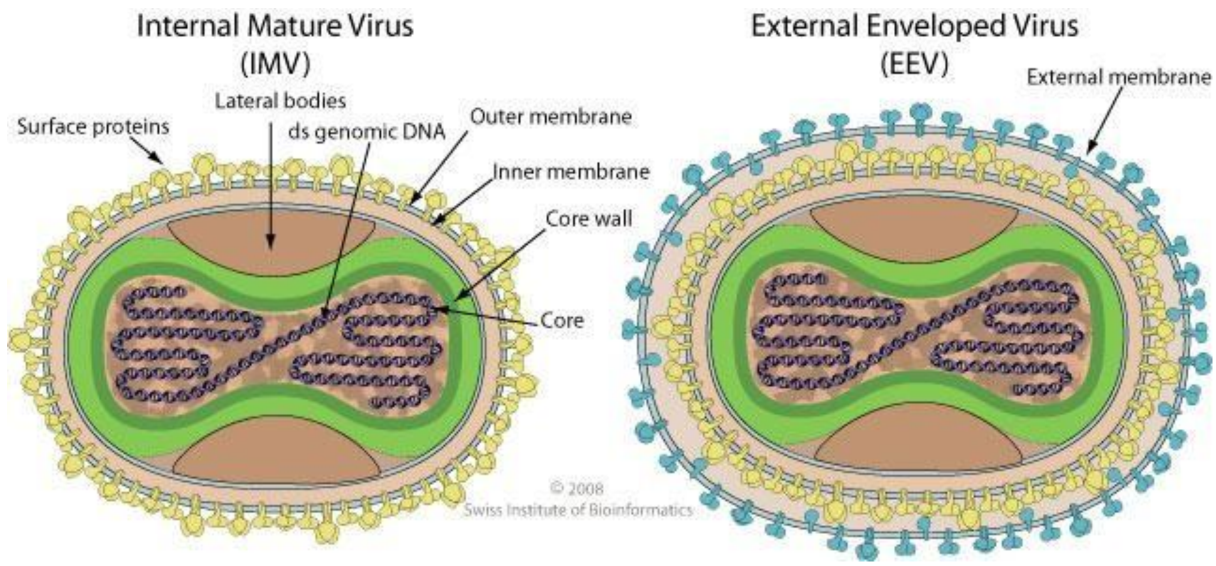


Figure .2: Morphological structure LSDV

2.7. 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 1000C 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).

2.8.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).

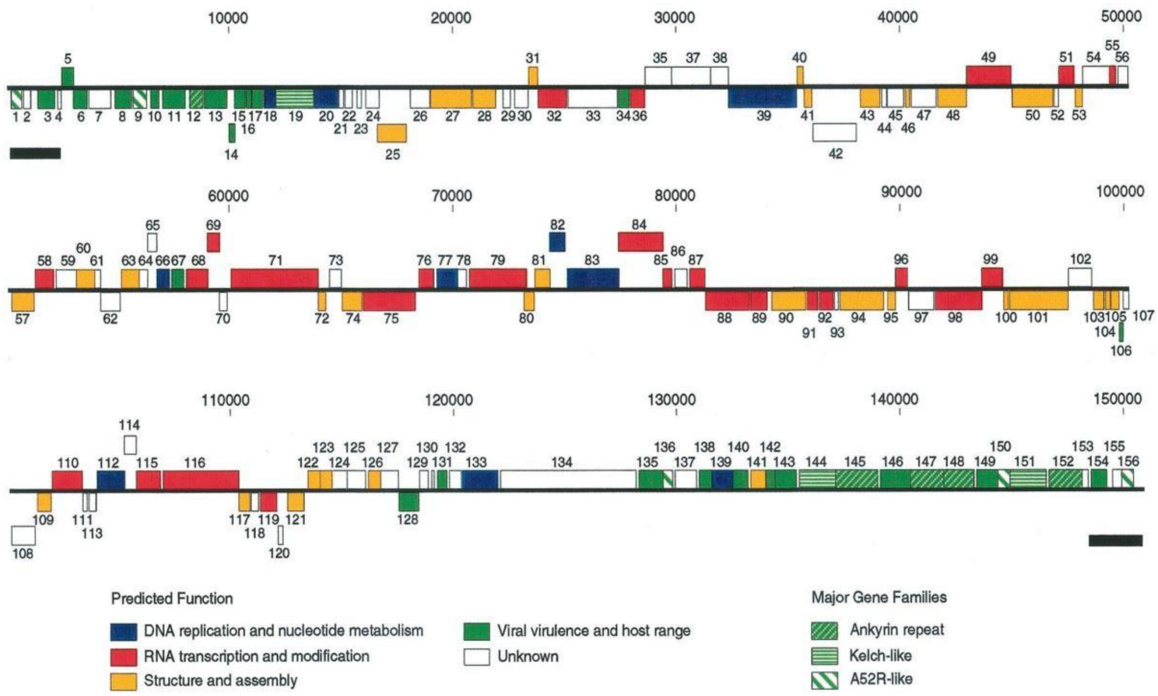


Figure 3: linear map of the LSDV genome. ORFs are numbered from left to right based on the position of the methionine initiation codon. ORFs transcribed to the right are located above the horizontal line; ORFs transcribed to the left are below. Genes with similar functions and members of gene families 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 leporipox viruses and, like the leporipox viruses, 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 immunomodulatory. 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 pox viral 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).

2.9.Epidemiology of LSD

2.9.1. Geographic distribution

Lumpy skin disease was known to circulate in the Central Africa for years (Thomas and Mare 1945) before it was reported in Zambia in 1929 (Thomas 1945; MacOwan 1959). After sweeping throughout and causing substantial economic losses for the cattle farming in the Southern African region in the 1940s, the disease continued to spread towards the north, and currently it is present practically everywhere in Africa, including Madagascar. Only Libya, Algeria, Morocco and Tunisia are still officially free of lumpy skin disease (LSD).(Tuppurainen *et al.*, 2018)

Many Middle Eastern countries import live cattle from the African Horn region (Shimshony and Economides 2006) where LSD is endemic. The first incursion of the disease in Egypt was reported in May 1988 followed by outbreaks in Israel in August 1989 (Yeruham et al. 1995). In 2006 LSD reoccurred in Egypt after being introduced by imported cattle from the Horn of Africa (El-Kholy et al. 2008). Outbreaks in the southern part of Israel swiftly followed (Brenner et al. 2009). In Oman LSD outbreaks occurred to such an extent in 2009 that it is currently considered endemic (Somasundaram 2011; Tageldin et al. 2014).

LSD is currently endemic in most of Africa, parts of the Middle East and Turkey. Since 2015, the disease has spread to most of the Balkan countries, the Caucasus and the Russian Federation, where the disease continues to spread, making the risk of an imminent incursion into other unaffected countries very high. Since 2019, several outbreaks of LSD have been reported by Members in Asia (Bangladesh, India, China, Chinese Taipei, Vietnam, Bhutan, Hong Kong (SAR-RPC), Nepal, Sri Lanka, Myanmar, Thailand- as of 02/6/2021).(WOAH,2022)

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.

2.9.2. Epidemiological risk factors

2.9.2.1. 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).

2.9.2.2. 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).

2.9.2.3. 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).

2.9.3. Origin of infection and transmission

Infected cattle 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 non-enzootic 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) .

2.10. 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 lymphatics 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 lifelong 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).

2.11. Diagnosis

2.11.1. 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).

2.11.2. 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 turbinates, trachea, lung and lymph nodes. specimens should arriving to laboratory within 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).

2.11.2.1.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).

2.11.2.2.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 pileo form carbon 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).

2.11.2.3.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).

2.11.2.4.Serological testes

All the viruses in the Capripox virus genus share a common major antigen for neutralising antibodies and it is thus not possible to distinguish strains of capripox virus 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).

2.12. 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).

The available vaccine for Lumpy skin Disease virus in Ethiopia is product of National Veterinary Institute at Debre Zeit. Vaccine is live freeze dried *Capri poxvirus* KSGP-0180 strain cultured on VERO-cells freeze dried (lyophilized) with a minimum titer of 3 $TCID_{50}$ per field dose. The vaccine should be stored at a temperature of -20ºc reconstitution and dilution of vaccine is in 100 ml of cool and sterile saline water. 5ml or 20ml vial of 100 doses 1 ml of diluted vaccine injected subcutaneously on back of neck for cattle and the inner face of the thigh both for sheep and goat. Immunity is develops eight days after vaccination and may last for two years (NVI, 2016).

2.13. Experimental Evaluation of the Efficacy of a Vaccine and Vaccination Effectiveness Studies

A challenge model for the evaluation of the efficacy of a vaccine against LSD has been described previously (Haegeman *et al.,* 2021 & Wolff *et al.,* 2020). A minimum of six to eight healthy animals should be included in a challenge trial and an additional five to six animals should serve as unvaccinated control. Most often, calves at the age of six to twelve months are used as experimental animals for practical reasons. In that age group, both male and female calves can be included. Prior to the onset of the experiment, cattle need to be tested to be sero-negative and free of ongoing LSD infection. Acclimatization to the controlled environment of one or two weeks is required to avoid the effect of stress-related factors. Vaccination should be undertaken strictly according to manufacturers' recommendations. Three to four weeks post-vaccination, vaccinated and control groups are infected with a highly virulent LSDV field strain (wild-type). Experiences obtained from previous animal experiments indicate that in order to produce visible clinical disease in half of the susceptible experimental cattle, the titer of the challenge virus needs to be between $10^{4.0}$ to $10^{6.5}$ $TCID_{50}$. Several different cells can be used to grow the virulent field strain. The application of the challenge virus via the intravenous route is important and can be combined with a local inoculation via the intradermal or subcutaneous route. After the challenge, the animals should be closely monitored and the findings recorded for up to four weeks for the appearance of clinical signs, such as fever, nasal and eye discharge, excessive salivation, enlarged lymph nodes, local or generalized skin nodules, and lesions in the muzzle and/or mucous membranes of the mouth and nasal cavities. Every second day, samples should be collected for PCR and virus isolation. EDTA blood, as well as saliva and nasal swabs are the recommended sample material to analyze viremia as well as virus excretion. In addition, biopsies from the typical skin nodules can be tested with polymerase chain reaction (PCR) and used for the virus isolation purpose. Molecular tools have been described to differentiate a vaccine from a field virus (Boumart *et al.,* 2016; Menasherow *et al.,* 2016; Sprygin *et al.,* 2018 and Vidanovic *et al.*, 2016)

A retrospective surveillance of vaccine effectiveness not only measures the efficacy of the vaccine, but also the implementation of the vaccination campaign. Active clinical surveillance is a good tool when combined with serological surveillance. The reliability of the clinical surveillance and sero-surveillance heavily builds on the ability to know which animals are vaccinated and which ones are not. If no animal health record databases/systems are available, a permanent way to mark the vaccinated animals should be developed for the purpose. During outbreaks with a virulent virus circulating in the region, it should be considered that it takes two to three weeks until the vaccine provides full protection, before that animals may still become infected and exhibit clinical signs typical for LSD.

Serological surveys after vaccination campaigns are complicated by the fact that some vaccinated animals, and those individuals showing a mild disease, may develop only low antibody levels although they are fully protected (Weiss, 1968 and Kitiching *et al.,* 1992). The unclear meaning of sero-negative animals decreases the value of sero-surveillance as a sole method. Sero-negative animals in a vaccinated herd may or may not be protected by the vaccine or they may have been missed during the vaccination campaign. The most reliable results are obtained when the serum samples are collected not earlier than one month and not later than five months after vaccination (Milovanovic *et al.,* 2019). The sample size calculations need to take into consideration the percentage of animals that are expected not to develop any detectable antibody levels after vaccination. Passive surveillance provides an additional tool if the awareness levels regarding LSD are high amongst cattle farming stakeholders and if farmers are willing to report. Commercially available pancapripoxvirus ELISA is available for large-scale testing, allowing easier monitoring of the duration of humoral responses in vaccinated herds than using a serum neutralization method.

Currently, there are no DIVA (differentiating infected from vaccinated animals) vaccines with associated marker tests commercially available for LSDV. Therefore, it is not possible to differentiate between vaccinated and naturally infected animals based on serology. Even if a DIVA strategy would be available, it is questionable whether it would be affordable or feasible for low-resource countries, most likely due to the lack of animal identification/vaccination/recording systems and the extra costs and resources required by the surveillance programs. Measuring the cytokine (e.g., IFN-γ) response, such as in blood samples collected from vaccinated animals, could potentially provide an additional diagnostic tool in the future. Currently, standard operating procedures and commercially available tests validated for this approach are still missing.

2.14. LSD Vaccine types and vaccination in Ethiopia

According to Boumart *et al*., (2016) vaccines produced against LSD have different types including 'live' attenuated and inactivated vaccines. Comparatively, the inactivated one are considered to be less effective. However, 'live' attenuated vaccines are relatively effective, cheap and available with recorded herd immunity (over 80%) through annual vaccination (Tuppurainen *et al*., 2017a). There are four CaPVs strains of 'live' vaccines namely Yugoslavian RM 65 sheep pox strain, Kenyan sheep and goat pox (KSGP) virus, Romanian sheep pox strain and LSDV strain from South Africa, used to control the spread of LSD (OIE, 2017). Regardless of its availability and suggested efficacy, vaccine failure is reported in different areas that pose its effect on the effective control of LSD (Gari *et al*., 2015). Even in effective vaccination outcome, the existence of large local reaction at injection site was reported as a side effect (Davies, 1991). The skin reaction sometimes can affect the cattle owners' attitude and discourage the use of vaccine though the loss to the production and productivity from the disease outbreak is enormous (OIE, 2017).

In general control and prevention methods include vaccination, animal control movement and slaughter of affected and in contact animals. However, some of these methods are not affordable practices especially in developing countries. Vaccination is considered to be a realistic approach to control the disease in endemic regions and considered cost-effective compared to the abovementioned one (Tuppurainen *et al*., 2012). LSD as endemic disease to Ethiopia is approached with annual vaccination scheme and following an outbreak (Molla *et al*., 2017). Kenyan sheep and goat pox (KSGP) virus is a vaccine produced by Ethiopian National Veterinary Institute (NVI) and available when needed with affordable price (NVI, 2023). However, there have been repeated complaints towards vaccine effectiveness due to the occurrence of outbreak in vaccinated cattle population (Ayelet *et al*., 2013; Molla *et al*., 2017)

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in purposely selected dairy cattle herders in Bishoftu town and surrounding rural areas of Ada'a district, of East Shewa zone. The selection considers presence of previous outbreak of LSD in the sites aiming to collect some retrospective information and accessibility to reach both sites for sampling. LSD outbreak was reported in the farms in different times. The samples used for LSD isolates was collected 2022 from the same study area from the same project and stored at NAHDIC.

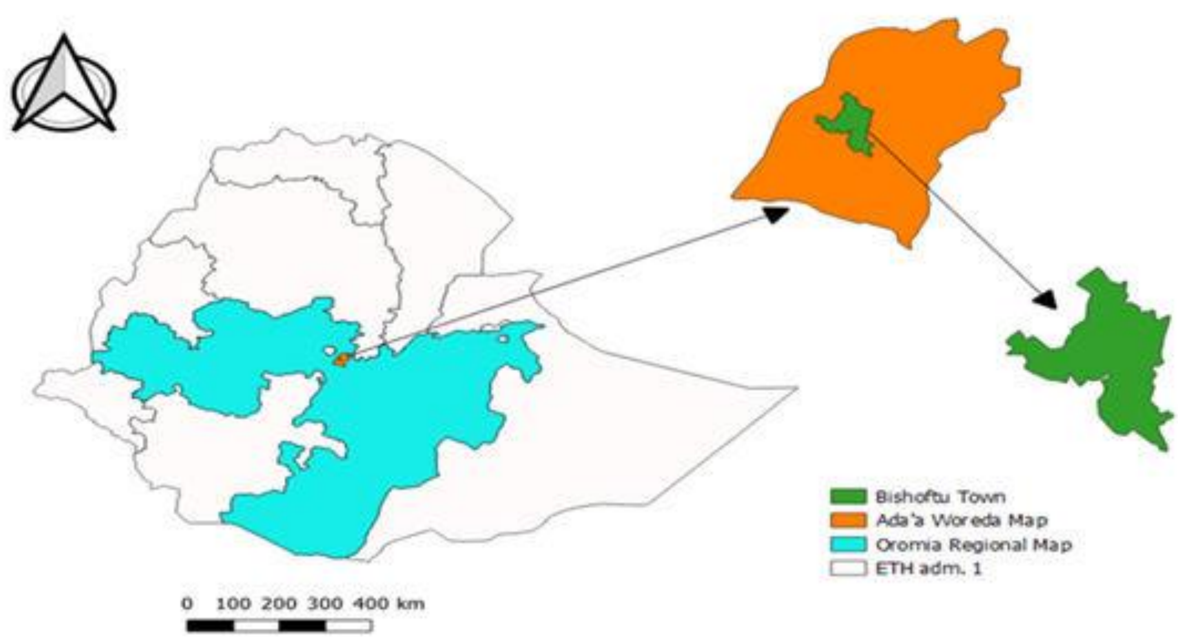


Figure: 4. Map of Study Area

The town is located in the East Shewa Zone of the Oromia National Regional State, and has an elevation of 1,999 meters, latitude of $8^0$45'0 N, and longitude of $38^0$58' E. It is the capital town of Ada'a District, the largest District in East Shewa Zone. One of the district in this zone is Ada'a, central Ethiopia that has a bimodal rainfall season: the long rainfall season from late June to late September and the short season from February to April, with a mean annual rainfall range of 450 mm to 1,000 mm and temperature range of 17°C to 30°C (Eastern Shewa Agricultural Bureau report, 2014). The town is surrounded by Dire, Dhankaka, and Godino rural kebeles of Ada'a District in the north, the east and the south, respectively, and Dukam Town in the west. The total cattle population in Ada'a district was 172, 356 (Ada'a district animal health office, 2023).

3.1.Study population

This study respondents were cattle owners who had brought cattle having of clinical sign of LSD to the wereda veterinary clinic and animal health professionals in and around Bishoftu and Ada'a district. Cattle production system in the study area is both extensive and intensive production system. The intensive production system is market oriented dairy farms and fattening. The rest is majorly small holders farms. The breed types are including local (indigenous), exotic and crossbred cattle.

3.2.Study design and sample size

From October 2022 to April 2023 a cross-sectional study design was carried out. The study was done purposively based on 2021-2022 LSD outbreak reports the study areas. This study designed based on both retrospectively (2022) and prospectively with employed questionnaire survey used to generate information related to LSD outbreak occurrence, status of vaccination, challenges on vaccination delivery ,and disease associated losses. Additionally, Tissue samples collected from the same area by 2022 processed for molecular characterization.

To get information on LSD vaccination practice, 101 cattle owners and Clinical professionals were subjected to respond to the questionnaire prepared.

3.3.Sample collection

The tissue and whole blood sample collection was performed during the weeks of reported outbreaks. And about 2-5 grams of nodular skin lesions were collected by using a sterile scalpel blade and an incision was made to reach the epidermis and the dermis of the skin. The skin biopsy was placed in the Virus transport media (VTM) which contains 10% antibiotics.

whole blood samples were collected for the serum with a plain tube (Tuppurainen *et al*, 2017; OIE, 2017). The tissue samples with VTM in a falcon tube and the blood sample were transported within an ice box to PROCFARM in the laboratory (AHI) and stored at -20^{0}C until used for the next laboratory processes.

Questionnaires were prepared for cattle owners and animal health professionals incorporating questions with a major emphasis on LSD vaccine and vaccination practices which include issues on the management, storage, transportation, administration of the vaccine and related questions. Additionally, background data were also collected in each locality clinics and farms where the outbreak occurred, including the date of data collection, age, sex, and previous history of vaccination using these questionnaires. Information were obtained from face to face interview with farm owners and animal health professionals.

3.4.Laboratory Diagnosis

3.4.1. Virus isolation

The nodular tissue samples were thawed at room temperature and washed three times in sterile phosphate-buffered saline (PBS, pH 7.2). With 9 ml sterile PBS containing antibiotic (0.1% gentamicin, Sigma-Aldrich, Germany) and about 1 g of washed tissue sample was mixed and ground using a sterile mortar and pestle. Following this tissue suspension was centrifuged at 3000rpm for 15 min and the supernatant was filtered through a membrane of pore size 0.45 µm (Millipore, United States of America). Approximately 100 µL of filtered supernatant was inoculated onto a monolayer of (Lamb kidney cells). Primary lamb kidney cells were established and propagated in a Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Germany) supplemented with antibiotics and 10% fetal bovine serum. And then the cells were aliquoted into different flasks and used when required by adding trypsin and incubating for ten minutes. When detachment takes much time the cells were then manually washed from the flask using the pipette. Then the cells were transferred to the 24 micro well plates. The cells were then reconstituted with the growth maintenance medium (DMEM) supplemented with 1% fetal bovine serum and cultured incubated at 37°C in a humidified incubator with 5% CO2. The cells were then checked for the 80% confluence. After checking the confluence of the cell, DMEM was added to the cells by removing the first media then, virus suspension was added to the cells. The whole process of the cell culture was performed under the biosafety cabinet level 2.

Virus suspension was inoculated on the cell after removing the media from the cell and washing the cell using 100µl phosphate buffers. The inoculation was done on a 24-well plate and incubated for 1 hour at 37°C for the adsorption time**.** Maintenance media was added and the culture was followed for up to 14 days for the presence of CPE. The absence of CPE shows presence of protective antibody in the test sera. The negative samples were then made to continue for the consecutive 2^{nd} to 3rd blind passages until the CPE was observed. This was done by freeze-thawing so that virus-cell particles are released from the culture cell. Then the collected suspension was vortexed and transferred to a new fresh cell, and monitored daily using an inverted microscope for the evidence of virus-induced CPEs.

3.4.2. DNA extraction

DNA of LSDV from tissue samples was extracted by QIAamp DNA Mini Extraction Kit (Qiagen, Germany) for blood and tissue based on the protocol set for DNA extraction in its manual for user. The skin nodular samples were processed by grinding using a sterile pestle and mortem and by adding sterile coarse sand. Following grinding the suspensions were centrifuged at 2000 rpm for 2 minutes and the 200µl of supernatant was transferred into a sterile microcentrifuge tube and 20 µl proteinase k was added to digest the cell. Afterwards, 200 µl of AL (lysis buffer) was added, mixed by vortex and incubated at 56°C for ten minutes in a water bath. Then 200µl of 95% ethanol was added to it and mixed thoroughly, this was to ensure the binding process. The mixture was transferred to the mini spin column in a 2ml collection tube and centrifuged at 6000 xg (8000 rpm) for 1 minute. The first wash was done using washing buffer AW1 with an amount of 200 µl on a mini-spin column in a new collection tube. That was then centrifuged at 8000 rpm for 1 minute. The next washing was done using AW2 (washing buffer 2) with 200 µl and centrifuged at 14000 rpm for 3 minutes. Finally, the collection tube was discarded, and the min-spin column was carefully transferred into a new 1.5ml microcentrifuge tube with 200 µl elution buffers and incubated for 1 minute at room temperature before centrifuging at 14000rpm for 3 minutes until all the liquid has completely passed through. The eluted DNA was labelled and stored in a -20C freezer until downstream activities start.

3.4.3. Polymerase chain reaction (PCR)

A conventional polymerase chain reaction was performed to detect four LSDV immunogens (LSD117, LSD122, LSD141 and LSDV060); using the specific primers listed under table 2. The primers (forward and reverse) designed based on the lumpy skin disease NI-2490, complete genome (Acc no=NC_003027) (Tulman *et al*, 2001), retrieved from NCBI data bank. The primers were designed by using snapGene viewer software to generate the targeted genes. All PCRs were conducted in a reaction volume of 25 µL containing; 0.5µL of F primer, 0.5µL of REV primer, 0.5µL of dNTPs, 2.5µL of 10x PCR Buffer (Qiagen), 0.125 µL Taq polymerase (Qiagen), 17.875µL Milli-Q water and 3 µL template DNA. The PCR run set was with the first denaturation at 95°C for 2 minutes, followed by 40 cycles of elongation at 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. Aliquots of PCR products were checked using electrophoresis on a 1% agarose used Midori Green Advance (NIPPON Genetics EUROPE, inc.) in gel staining for 40 minutes at 100v.

3.4.4. Sequencing and Phylogenetic analysis

Following the amplification of the LSDV immunogens (LSD117, LSD122, LSD141 and LSDV060) with observation of a clear band through the gel were selected and sent for sequencing. Then, the remaining PCR product was cleaned by using the Wizard™ SV Gel and PCR Clean-Up kit (Promega, Germany). Each purified product's concentration which was above 20 ng/µl was prepared for sequencing. The purified PCR products were mixed with the amplification/sequencing primers and submitted for sequencing to Eurofins Scientific SE (Luxembourg, EURO).

Table 2. Genes selection and primer design for PCR amplification and Sanger sequencing

No	**LSDV-ORF**	**Pair of primers**	**Annealing Temperature**	**Gene fragment length (bp)**
1	LSD117 (A27L)	F: 5'-TCATAGTGTTGTACTTCGGCT-3'	49 °C	447
		Rev: 5'- AATGGACAGAGCTTTATCAATC-3'		
2	LSD122 (A33R)	F: 5'-ATAGTTGATATTCAAAAG-3'	50 °C	594
		RE: 5'-ATTTATAGTAATATAATAAT-3'		

3	LSD141 (B5R)	F: 5'-AAAAATATACATATGTTATTG-3'	48 ^{o}C	678
		Rev: 5'- ATTATTTATAATATTTTATACTATC-3')		
4	LSDV060 (L1R)	F: 5'-AGAGCAGCCGCAAGTATAC-3'	57 ^{o}C	738
		Rev. 5'-ATTATTTTTCTATGATTGTTGGTGT-3'		

3.5.Data management and analysis

The data collected by a Questionnaire data were entered in MS- excel, edited to make it fit for analysis and transferred to R software package with version (R.0.s) for descriptive analysis. Pearson X^2 test was applied to test the relatedness of the variables.

Molecular sequence data received from Eurofins Scientific SE were edited and cleaned to get the consensus sequence, and then all the sequence data was analysed using a bioinformatics tool. The comparative sequence analysis of the RPO30 gene subunit of, Ethiopian Lumpy skin disease virus isolates was carried out along with those of other Capri poxviruses available in the NCBI database (https://www.ncbi.nlm.nih.gov/). The nucleotide sequences of the RPO30 gene subunit generated by the Sanger sequence and other publicly available sequences retrieved from NCBI data bank were transported to BioEdit (version 7.2) software package (https://bioedit.software.informer.com/7.2/), and were edited and joined together for each of the isolates. Finally, multiple sequence alignments were performed using the ClustalW algorithm implemented in **BioEdi**t software package to compare the RPO30 genes sequences of the outbreak isolates and the reference strain retrieved from the gene NCBI database, and to that of the vaccine strain. Moreover, the sequences obtained from the RPO30 gene amplicons were translated into corresponding amino acid sequences and were checked if any of the unique signatures associated with LSDV happened.

For phylogenetic analysis, the Maximum Likelihood method in MEGA 11 was used to construct the phylogenetic tree. Bootstrap analysis with 1000 replications was used to estimate the confidence of the branching patterns of the trees.

4. RESULTS

4.1. Questionnaire Survey

A total of 101 households in K'oftu , Dire , Bekejo , Golo dheertu , Akako, Hidi , Denkaka of Ada'a district(i.e. Surrounding of Bishoftu town) and Kebele 02, Kebele 08, Kebele 03, Kebele 15 , Kebele 05 and Kebele 01 of Bishoftu town. The higher number of Livestock was recorded in respondents of Denkaka Kebele of Ada'a district. During the study period outbreak of LSD was not reported. But there were report of outbreak in the times preceded the study period (September to November 2022).

4.1.1. General cattle population at risk owned by respondents years LSD outbreak seen in the last 8-10 years

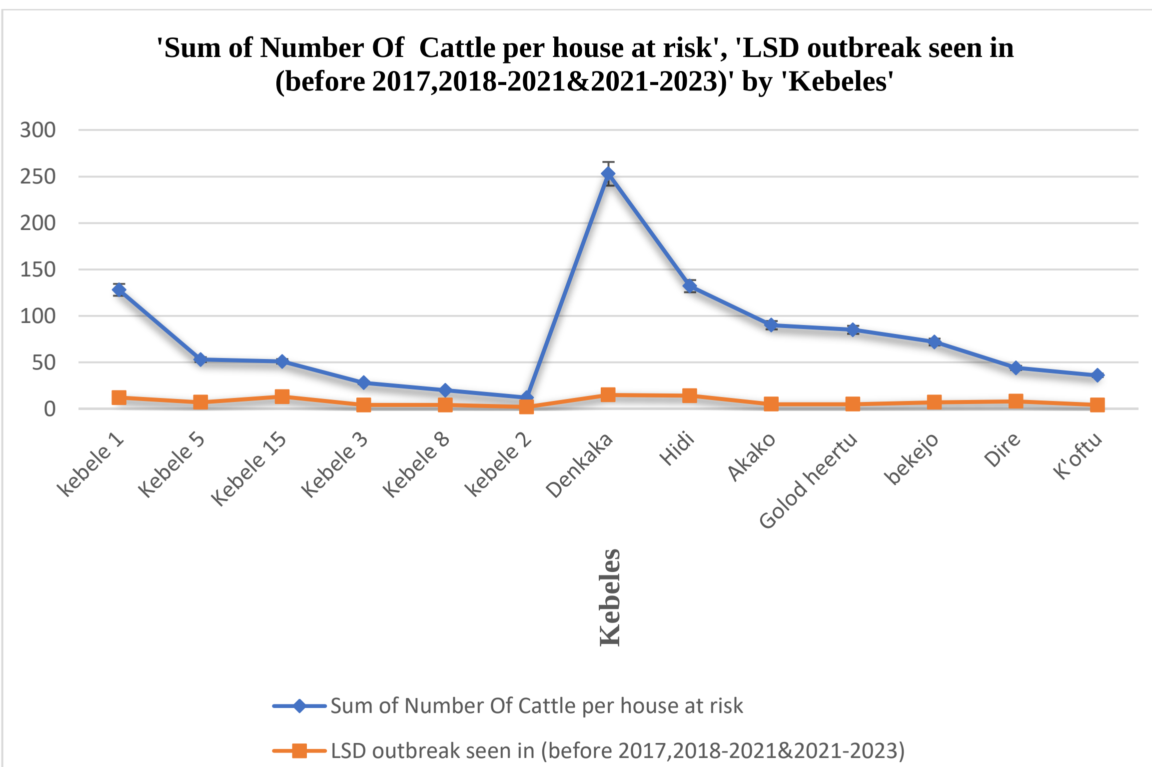


Figure 5: *General cattle population at risk owned by respondents and years LSD outbreak seen in the last 8-10 years ; bars with blue shading cattle population at risk and bars with pink shade number of outbreaks*

As depicted in the figure (Figure 5) above the outbreak of LSD in the past 10 years are increasing time to time.

4.1.2. Variables affecting vaccination and LSD outbreak

Table 3: Variables affecting vaccination and LSD outbreak

Variables	vaccination	LSD outbreak occurrence observed by the respondents		Pearson's- X^2 Value	P-value
Vaccination Status of		2021-2023	2017-2021		
LSD	unvaccinated	13	18	0.3	0.587
	vaccinated	25	44		
Vaccination period	After outbreak	17	40	6.3	0.043
	At time of an outbreak	8	4		
	No vaccination given	13	16		
Vaccine delivered by	Public Vet.clinics	15	27	1.25	0.534
	private Vet.practitioners	8	17		
	None	15	18		
Vaccination status vrs recurrence of LSD outbreak					
Vaccination Status of LSD		repeated occurrence	First Time occurrence		
	unvaccinated	49	20	3.54	0.06
	vaccinated	16	15		

As depicted in Table 3 the factors responsible for previous out breaks were found to be Vaccination period at time occurrence of an outbreak or After Occurrence of an out Break P value = 0.043.

4.1.3. General LSD vaccine and vaccination related challenges affecting the its efficacy and effectiveness in the study areas based on respondents(users)

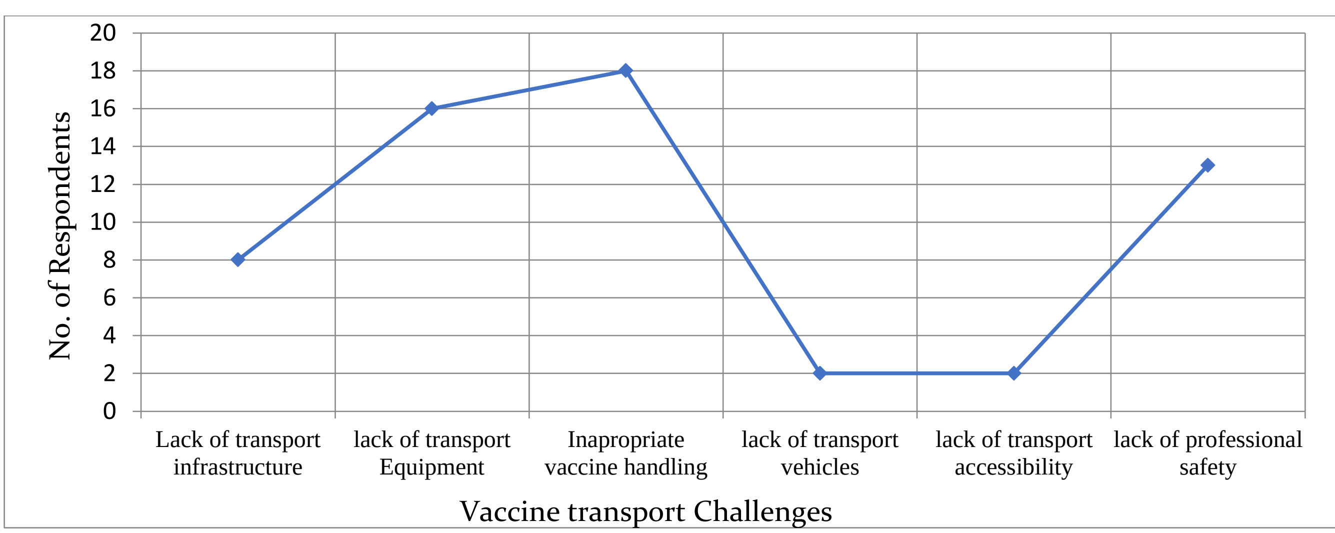


Figure 6: LSD vaccine transport Challenges

As we can see on the table Figure above (Figure 6) , the vet clinic professionals responds about the general LSD vaccine and vaccination related challenges and effectiveness of the vaccine from inappropriate vaccine handling were taken as a main(first) factor , lack of transport equipment(ice box) taken as a second factor and lack of professional safety hold third factor . Additionally, from LSD vaccine storage challenges in local Veterinary Clinics electricity challenges (e.g. Power cuts) was mentioned as the main constraint and lack of properly working refrigerators and unfit vaccine storage rooms were assumed to be important factors for the respondents. Furthermore, the regarding general challenge related with LSD vaccine delivery was claimed to be problem during vaccine delivery and old equipment like syringes.

4.1.4. LSD Vaccine storage challenges in local Veterinary clinics

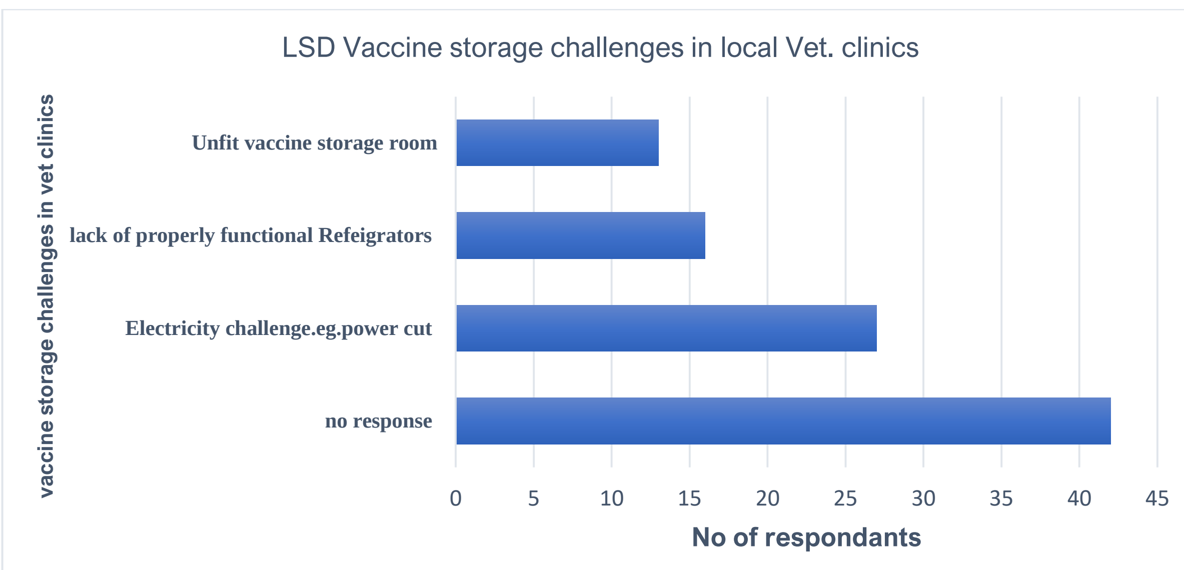


Figure 7: LSD vaccine storage challenges

As illustrated in the figure 7 Electricity challenges such as electric power cut seen as the major challenge for LSD vaccine storage followed by lack of properly functional Refrigerator.

4.1.5. General challenges during LSD vaccine delivery at field

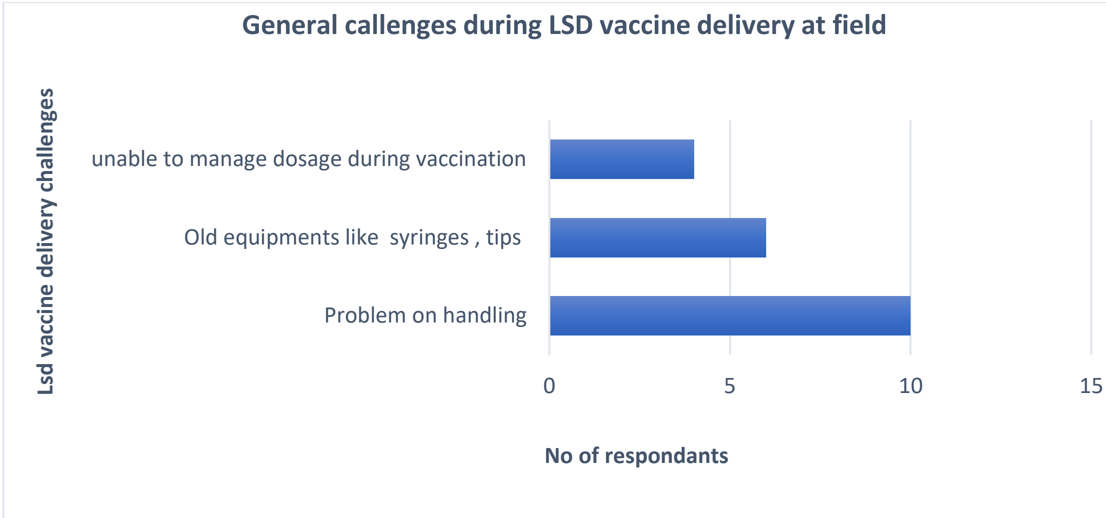


Figure 8: General challenges during LSD vaccine delivery at field

As shown in figure 8 among the general challenges during LSD vaccine delivery at field problem in handling of the vaccine is seen as the biggest challenge and followed by old equipment like vaccination syringe and tips.

4.2. Polymerase Chain Reaction

PCR was run for four samples four each of the targeted genes (4*4=8) and the expected amplicons sizes (figure 1) were detected here on all the samples. The strong running bands produced by Ethidium Bromide-stained Agarose Gel were detected. And the bands after cleaned from mix prepared for downstream analysis (sequencing).

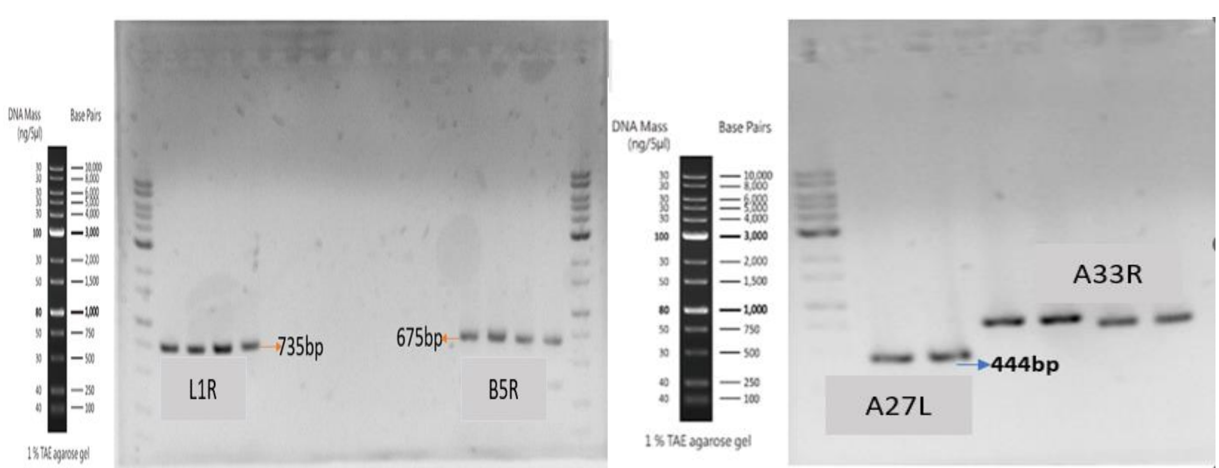
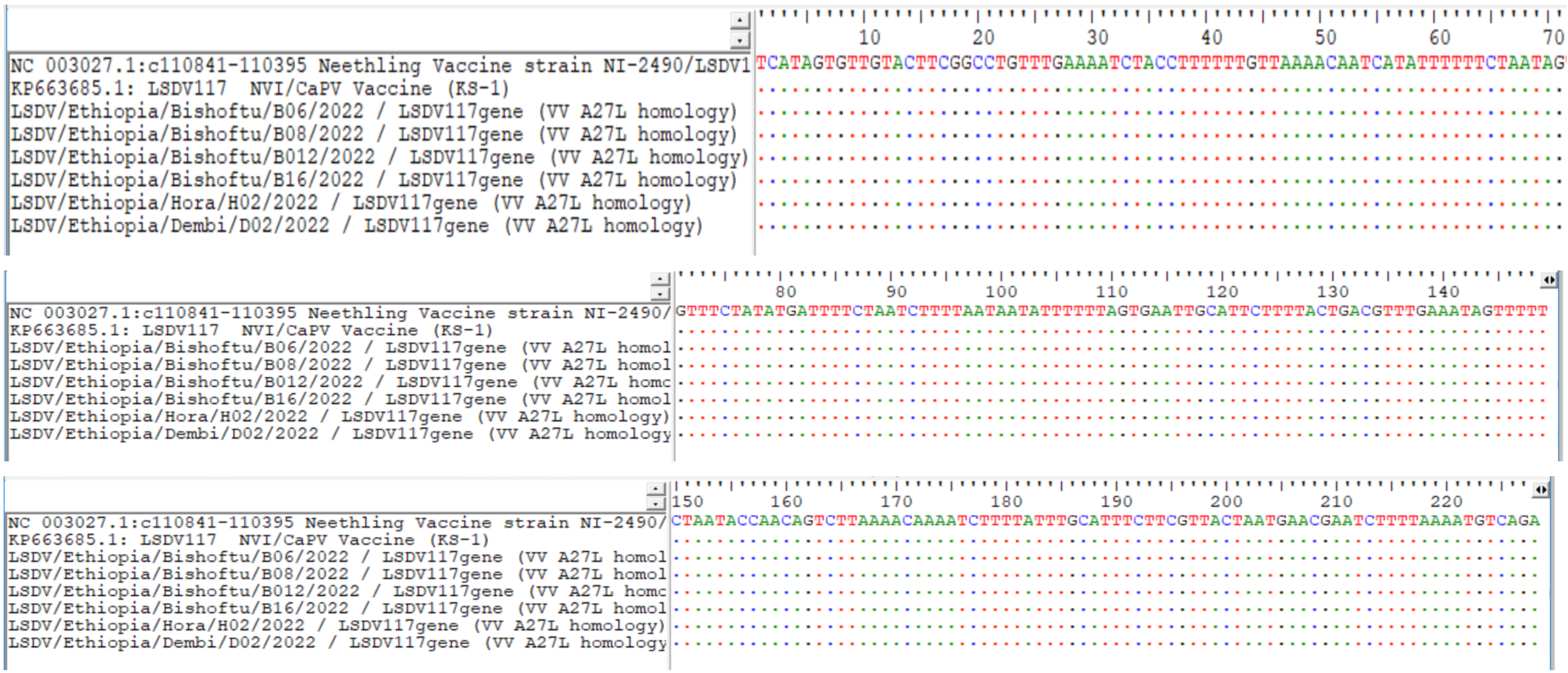


Fig.9. conventional PCR gel products for the four LSDV immunogens (LSD117, LSD122, LSD141 and LSDV060); The first Lane on the left and right side denotes DNA ladder of a 1kp (1000bp)

4.3.Sequences analysis Results

1. LSDV117(VVA27L homology)



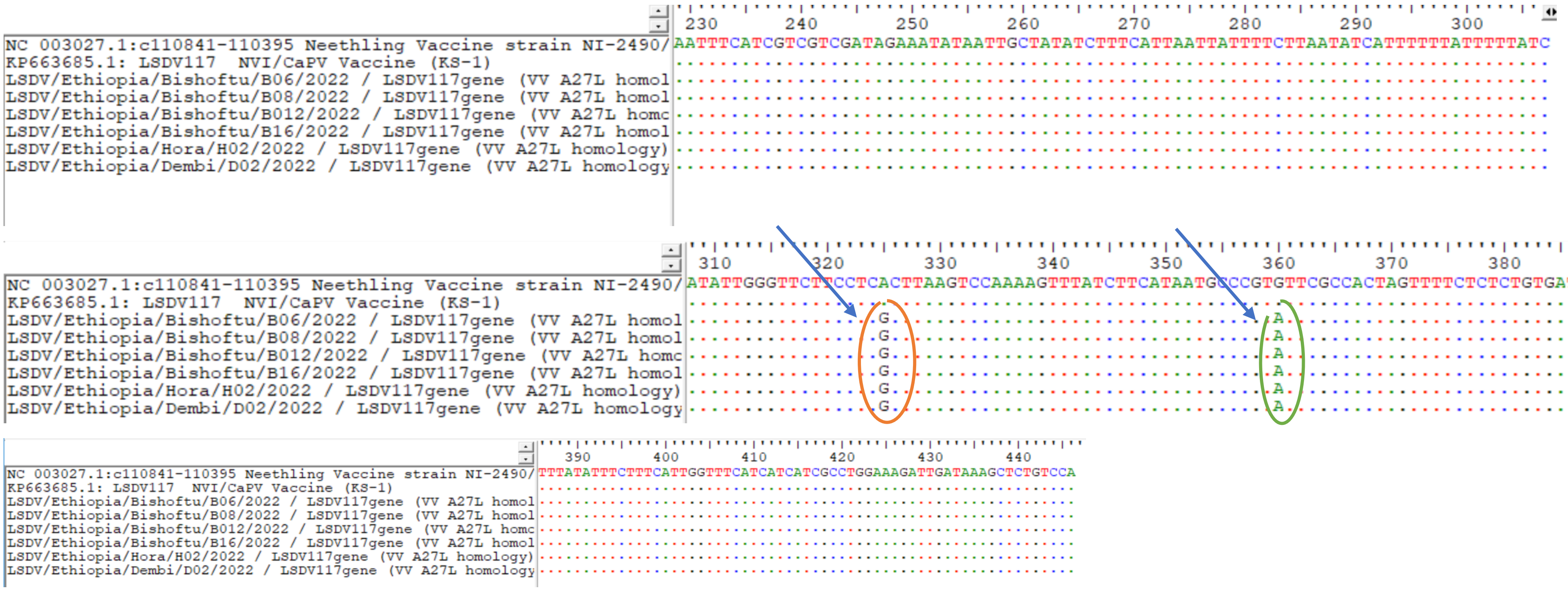


Fig 10. LSDV117 (VVA27L homology)

The sequence from the vaccines (Neethling vaccine strain NI-2490 and NVI/CaPV vaccine strain (KS-180) have an overall similar sequence for ORF117 (A27L) but the sequences from the field sample (wild strains) have two nucleotides substituted at 325nt and 360nt positions ‘A’ by ‘G’ and ‘G’ by ‘A’, respectively. Since all nucleotide changes may not have any effect on the codons (amino acids).

2. Proteins sequence analysis for LSDV117(VVA27L homology)

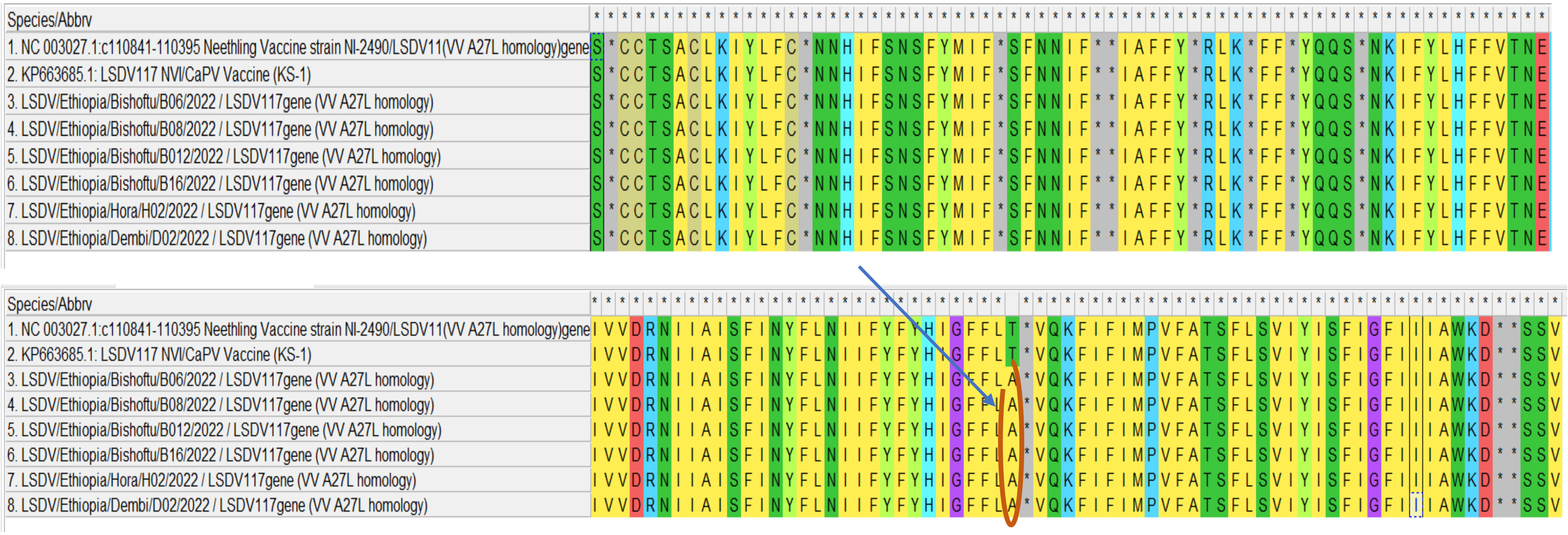
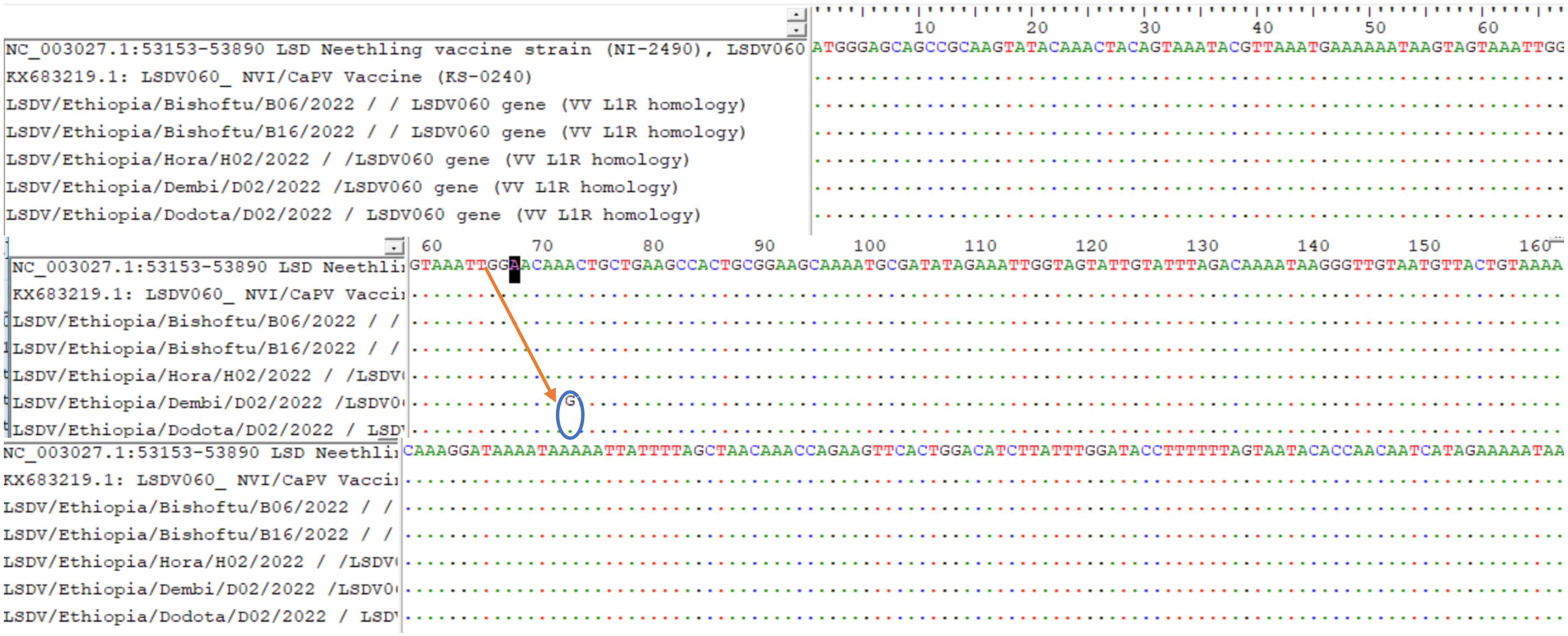


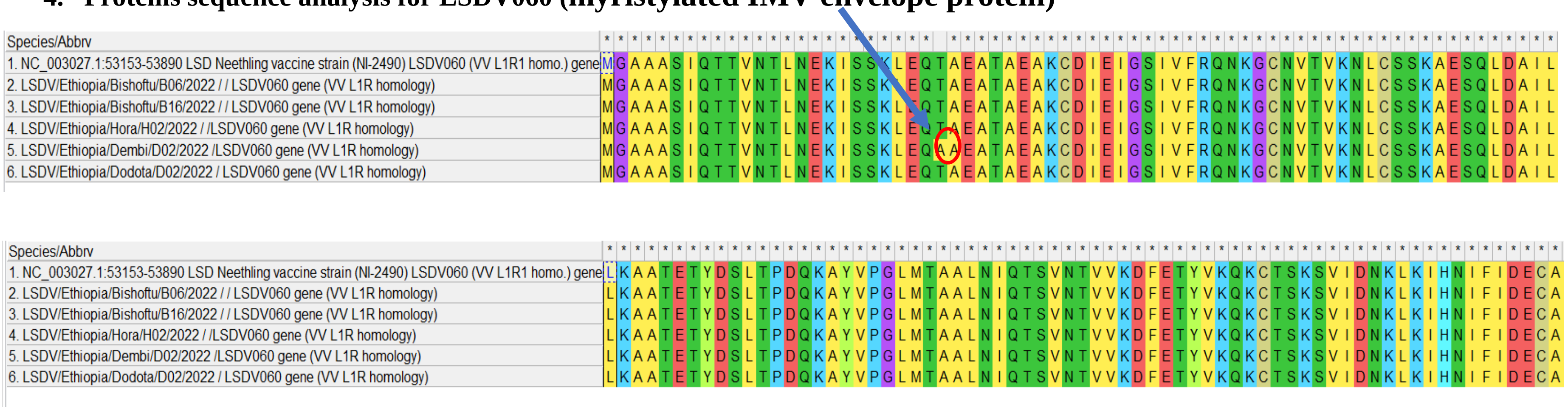
Figure 11. Proteins sequence analysis for LSDV117

There complete similarity between the two vaccine strains but the vaccine strains amino acid at number 108 a.a sequence which is threonine (T) is replaced by Alanine in the sample (wild strain) of lumpy skin disease virus. This amino acid change may have a mutative advantage for the wild strain over vaccine strains while it interacts with immune system. But further detail study needed on this protein at the structure level.

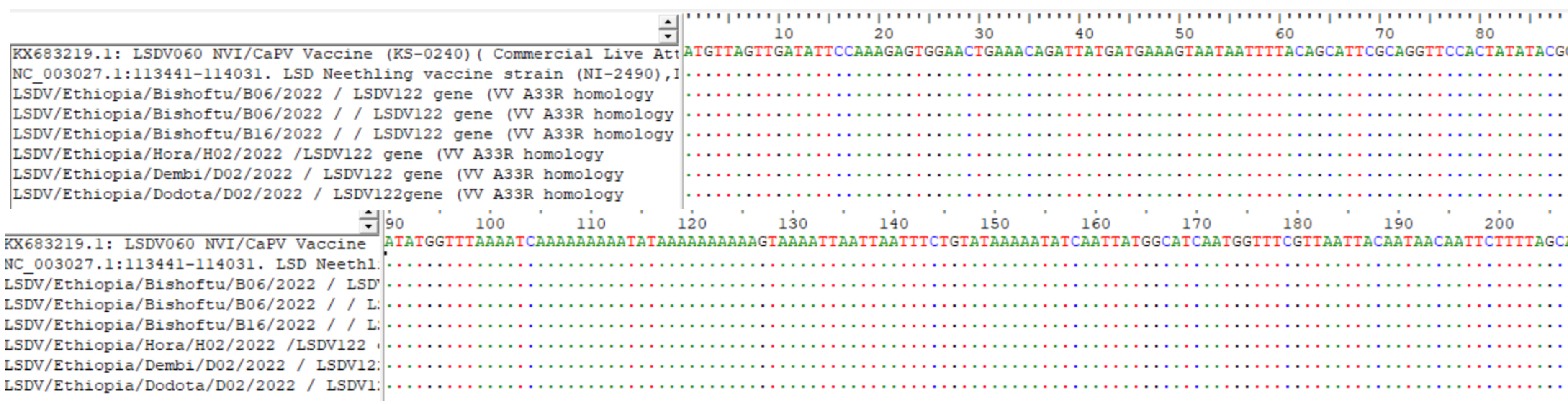
3. LSDV060 (VV L1R homologous)



4. **Proteins sequence analysis for LSDV060 (myristylated IMV envelope protein)**



5. LSDV112 (EEV glycoprotein coding)gene sequence analysis



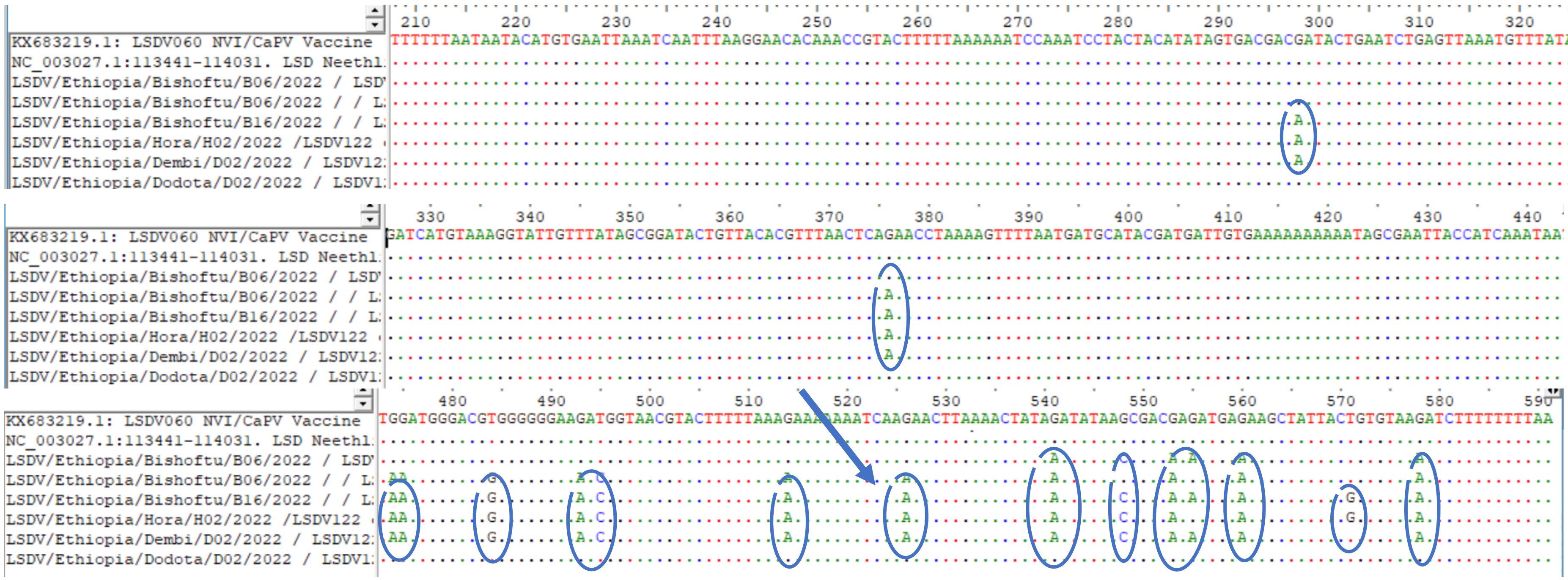


Fig. 12. Sequence alignment of LSDV112 (EEV glycoprotein coding)gene sequence

6. Proteins sequence analysis for LSDV112 (EEV glycoprotein coding)

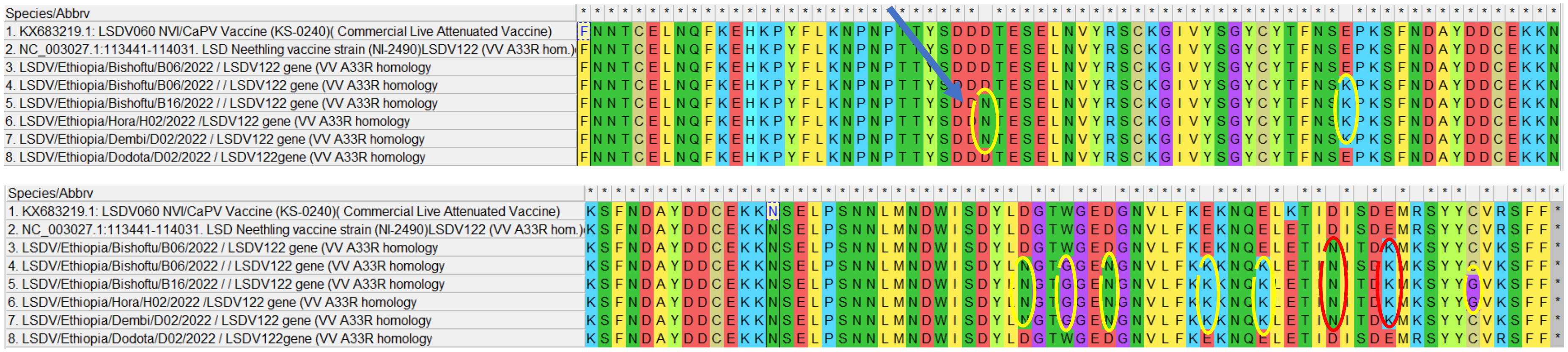


Fig 13. *Protein translate sequence alignment of LSDV112 (EEV glycoprotein coding) gene*

There is a complete similarity between the two vaccine strains. But almost 10 amino acid substitutions on sample isolates compared to vaccine strains. But the isolate from DoDota/2022 (Aris, Oromia region) is very similar to the two vaccine strains. The major a.as substitutions were Aspartic acid (D) to Asparagine (N), Glutamic acid (E) to Lysine (K), Tryptophan (W) to Glycine (G) and Cysteine (C) to Glycine (G). This amino acid change may have a mutative advantage for the wild strain over vaccine strains while it interacts with the immune system. But further detailed study is needed on this protein at the structure level.

4.4.Phylogenetic analysis for LSDV117 (VVA27L homology) sequence

Phylogenetic analysis

Phylogenic analysis was done to evaluate the evolutionary relationship between the sample DNA sequences with the two vaccine strains (Neethling vaccine strain NI-2490 and CaPV Vaccine (KS-1). Thus, to construct the phylogenic tree, first multiple sequence alignments were performed to align the sequence result using the Muscle algorithm in MEGA11 (https://www.megasoftware.net/). The Neighbor-Joining statistical method was used, along with the maximum composite likelihood nucleotide substitution model and the pair-wise deletion option was used to remove all ambiguous positions for each sequence pair (Tamura *et al.*, 2011).

Figure.14: Phylogenetic analysis of LSD 117

5. DISCUSSION

Vaccination is the sole control strategy for so long time in Ethiopia. The vaccine type used as vaccine control against LSD has been live attenuated KS1 O-180 vaccine. However, some studies (Ayelet *et al*.2013 , Garia *et al*., 2015 and Molla *et al*., 2017), reported that KS1 O-180 based vaccine strain protection against natural LSD infections and severity of the disease are failed. Therefore, this study aimed at investigating LSD Vaccine Efficacy based on Field vaccination practices and drawbacks in specific area in and around Bishoftu town and Adaá districts which purposefully selected based on previous repeated outbreaks and vaccination delivery. The other objective which, as far as our knowledge is concerned, was not done before for LSDV was characterization of the vaccinia virus immunogens homologies and analysis of the translated proteins for real mutation.

5.1.Questionnaire survey

The questionnaire survey shows that in almost all respondents (small holder farmer as well as large scale dairy farms), LSD outbreak occurred in the last 10 years in Bishoftu town and the surrounding areas (Adaá district). In general the survey covered 13 kebeles from the urban, semi-urban and rural areas in the study area by involving 101 participants. From the respondents two rural kebeles' (Denkaka and Hidi) and one Bishoftu town Kebele (kebele 01) have high cattle population per household at LSD risk.

In the current study, according to the respondents in the survey, the general LSD vaccine and vaccination related challenges affecting its efficacy and effectiveness were dominantly related to vaccine transport challenges, vaccine storage challenges in vet clinics and related to vaccine delivery system to the animals. From the first factor (vaccine transport challenges) inappropriate handling of vaccine, the animals may not get a vaccine with proper quality due the veterinarian administer to the cattle. On the second part related to vaccination delivery challenges, most respondents had no confidence on appropriate vaccine delivery either from competency of vaccinator or vaccine delivery equipment like malfunctioning of vaccination syringes. This is in agreement with the report of Molla *et al*. (2017) which suspected one cause of vaccine failure in his study area (in the central and north-western parts of the country) might be related to the administration of the vaccine by incompetent practitioners. In general, other major challenges were also lack of transport equipment (ice box), lack of electricity or Power cuts in Vet, clinics and lack of properly working refrigerators. In this study vaccination status was also compared with other factors which might have contribution for vaccine protection failure in the study area. One of these factors was vaccination period in relation to LSD outbreak occurrence i.e LSD vaccination is usually given after an observation of at least the first case of the disease. Therefore, the vaccine delivery after or before LSD outbreak occurrence has a statistically significant ($P<0.05$) effect on the status of the LSD expansion which might affect the vaccination efficiency (Table 3). This also in line with the previous study by Molla *et al*. (2017) "LSD vaccination is commonly carried out at the face of the outbreak to control the disease occurrence". Meanwhile, vaccinating animals during an LSD outbreak may worsen the transmission the disease and may cause genetic recombination (Hunter and Wallace, 2001).

5.2.Molecular characterization LSD immunogenic genes

In these study four LSD immunogenic genes namely LSD117, LSD122, LSD141 and LSDV060 were detected by polymerase chain reaction. The genome length of each of these four genes were confirmed based on sequence annotated for Lumpy skin disease virus NI-2490, vaccine strain complete genome accessed from Gene bank data base with accession number (NCBI Reference Sequence: NC_003027.1) (https://www.ncbi.nlm.nih.gov/nuccore/NC_003027.1).

After agarose gel band purification, three of the immunogenic genes (LSD117, LSD122, and LSDV060) were sequenced. And from the sequence analysis and alignment of LSD117 gene, the sequence from the vaccines (Neethling vaccine strain NI-2490 and NVI/CaPV vaccine strain (KS-180) have an overall similar sequence for LSD117 (A27L) but the sequences from the field sample (wild strains) have two nucleotides substituted at 325nt and 360nt positions 'A' by 'G' and 'G' by 'A', respectively. Since all nucleotide changes may not have any effect on the codons (amino acids), nucleotide translation was done for the alignment. And even though there was a similarity between the two vaccine strains but the vaccine strains amino acid at number 108 a.a sequence which is threonine (T) is replaced by Alanine in the sample (wild strain) of lumpy skin disease virus. This amino acid change may have a mutative advantage for the wild strain over vaccine strains while it interacts with immune system. But further detail study needed on this protein at the structure level. Meanwhile , there were no significant changes on nucleotide sequence as well as its translated amino acids analysis for LSDV060 (myristylated IMV envelope protein) except a single nucleotide and amino acid substitution ('A' by 'G') and ('threonine' by 'Alanine') ,respectively on a single isolate (Dembi/02). But a wide range of mutation was found on LSDV112 (EEV glycoprotein coding) gene sequence analysis. There is a complete similarity between the two vaccine strains. But almost 10 amino acid substitutions on sample isolates compared to vaccine strains. But the isolate from DoDota/2022 (Aris, Oromia region) is very similar to the two vaccine strains. The major a.as substitutions were Aspartic acid (D) to Asparagine (N), Glutamic acid (E) to Lysine (K), Tryptophan (W) to Glycine (G) and Cysteine (C) to Glycine (G). This amino acid change may have a mutative advantage for the wild strain over vaccine strains while it interacts with the immune system. But further detailed study is needed on this protein at the structure level.

The phylogenetic tree was built for analysis of the local isolates, vaccine strains and other *Capripoxvirus* strains to compare relatedness only for LSD117 gene only. That was because of the absence of sequences for other Capripoxvirus *virus* strains (*Sheep pox virus and Goat pox virus*) from the database. The phylogenetic relationship (Fig13) and Sequence distance of the LSD117 genes showed a high identity overlap of the field isolated LSD viruses and with the tested vaccine strains: 100% among the isolates, 83% with KS-180, and Neethling vaccine strain, NI-2490.

6. CONCLUSIONS AND RECOMMENDATIONS

Vaccination is the sole control strategy for so long time in Ethiopia. The vaccine type used as vaccine control against LSD has been live attenuated KS1 O-180 vaccine. The questionnaire survey shows that in almost all respondents (small holder farmer as well as large scale dairy farms), LSD outbreak occurred in the last 10 years in Bishoftu town and the surrounding areas (Adaá district).According to the respondents in the survey, the general LSD vaccine and vaccination related challenges affecting its efficacy and effectiveness were dominantly related to vaccine transport challenges, vaccine storage challenges in vet clinics and related to vaccine delivery system to the animals. In this study four LSD immunogenic genes namely LSD117, LSD122, LSD141 and LSDV060 were detected by polymerase chain reaction. From the sequence analysis and alignment of filed strains and LSDV060 (IMV) have slight (single mutation) difference with vaccine ones. Meanwhile the sequence from LSD122 of the vaccines (Neethling vaccine strain NI-2490 and NVI/CaPV vaccine strain (KS-180) vaccines have significant variation (multiple mutation) at nucleotide as well as a.as sequence lebvel. This wide range of mutation found on LSDV112 (EEV glycoprotein coding)gene sequence may have an implication for immune response. This amino acid change may have a mutative advantage for the wild strain over vaccine strains while it interacts with the immune system. But further detailed study is needed on this protein at the structure level needed. The phylogenetic relationship (Fig13) and Sequence distance of the LSD117 genes showed a high identity overlap of the field isolated LSD viruses and with the tested vaccine strains: 100% among the isolates, 83% with KS-180, and Neethling vaccine strain, NI-2490.

Recommendations

- Vaccination strategy, especially given after onset of outbreak, must not be given and awareness creation trainings should be provided for clinical professionals.
- Proper handling of a vaccine during transportation, injection to the cattle and disposition should be made in accordance vaccination regulations and safety rules.
- Further studies needed on immunogenic genes and on their translates at the proteins structural level and their immunogenic status at animal experimentation level
- Improving the transport, storage and handling of a vaccine is important for the success of disease prevention

7. REFERENCES

A. U., ... & Sadekuzzaman, M. (2021): Molecular characterization of lumpy skin disease virus (LSDV) emerged in Bangladesh reveals unique genetic features compared to contemporary field strains. BMC veterinary research, 17(1), 1-11.

Agianniotaki, E.I.; Chaintoutis, S.C.; Haegeman, A.; Tasioudi, K.E.; De Leeuw, I.; Katsoulos, P.D.; Sachpatzidis, A.; De Clercq, K.; Alexandropoulos, T.; Polizopoulou, Z.S.; et al. Development and validation of a TaqMan probe-based real-time PCR method for the differentiation of wild type lumpy skin disease virus from vaccine virus strains. J. Virol. Methods 2017, 249, 48–57.

Allam, A. M., Elbayoumy, M. K., Abdel-Rahman, E. H., Hegazi, A. G., & Farag, T. K. (2020): Molecular characterization of the 2018 outbreak of lumpy skin disease in cattle in Upper Egypt. Veterinary World, 13(7), 1262.

Amin, D. M., Shehab, G., Emran, R., Hassanien, R. T., Alagmy, G. N., Hagag, N. M., ... &

Ayelet, G., Abate, Y., Sisay, T., Nigussie, H., Gelaye, E., Jemberie, S., Asmare, K., 2013. 446 Lumpy skin disease: preliminary vaccine efficacy assessment and overview on outbreak impact in dairy cattle at Debre Zeit, central Ethiopia. Antivir. Res. 98, 261-265.

Ayelet, G., Abate, Y., Sisay, T., Nigussie, H., Gelaye, E., Jemberie, S., & Asmare, K. (2013): Lumpy skin disease: preliminary vaccine efficacy assessment and overview on outbreak impact in dairy cattle at Debre Zeit, central Ethiopia. Antiviral research, 98(2), 261-265. https://doi.org/10.1016/j.antiviral.2013.02.008

Ayelet, G., Haftu, R., Jemberie, S., Belay, A., Gelaye, E., Sibhat, B., ... & Asmare, K. (2014): Lumpy skin disease in cattle in central Ethiopia: outbreak investigation and isolation and molecular detection of the virus. Rev. Sci. Tech, 33(3), 877 87.https://doi.org/10.20506/rst.33.3

Babiuk, S. (2018): Treatment of lumpy skin disease. In Lumpy Skin Disease (pp. 81-81).

Badhy, S.C., Chowdhury, M.G.A., Settypalli, T.B.K., Cattoli, G., Lamien, C.E., Fakir, M.A.U., Akter, S., Osmani, M.G., Talukdar, F., Begum, N., Khan, I.A., Rashid, M.B., Sadekuzzaman, M., 2021. Molecular characterization of lumpy skin disease virus (LSDV) emerged in Bangladesh reveals unique genetic features compared to contemporary field strains. BMC Vet. Res. 17, 1–11. https://doi.org/10.1186/s12917-021-02751-x

Bamouh, Z., Hamdi, J., Fellahi, S., Khayi, S., Jazouli, M., Tadlaoui, K.O., Fihri, O.F., Tuppurainen, E., Elharrak, M., (2021): Investigation of post vaccination reactions of two live attenuated vaccines against lumpy skin disease of cattle. Vaccines 9, 1–15. https://doi.org/10.3390/vaccines9060621

Brenner, J., Bellaiche, M., Gross, E., Elad, D., Oved, Z., Haimovitz, M., Wasserman, A., Friedgut, O., Stram, Y., Bumbarov, V., Yadin, H., (2009):Appearance of skin lesions in cattle populations vaccinated against lumpy skin disease: Statutory challenge. Vaccine 27, 1500–1503. https://doi.org/10.1016/j.vaccine.2009.01.020

C. P., DeClercq, K., Gubbins, S., Klement, E., & Stegeman, A. (2019): Lumpy skin disease.

Calistri, P., DeClercq, K., Gubbins, S., Klement, E., Stegeman, A., Cortiñas Abrahantes, J., Antoniou, S.E., Broglia, A., Gogin, A., (2019): Lumpy skin disease: III. Data collection and analysis. EFSA J. 17. https://doi.org/10.2903/j.efsa.2019.5638

Chand, P., 1992. Molecular and immunological characterization of amajor envelope protein of capripoxvirus. Ph.D. Thesis. University of Surrey, Surrey, UK.

Characterisation and Vaccine Effectiveness Study of Lumpy Skin Disease Virus in Selected Diary Farms of Central Ethiopia. J. Biol. Agric. Healthc. 37, 764–775. https://doi.org/10.7176/jbah/9-11-01

Chibssa, T. R., Grabherr, R., Loitsch, A., Settypalli, T. B. K., Tuppurainen, E., Nwankpa, N.,... & Lamien, C. E. (2018): A gel-based PCR method to differentiate sheeppox virus field isolates from vaccine strains. Virology journal, 15(1), 1-7.https://doi.org/10.1186/s12985- 018-0969-8

Chibssa, T. R., Settypalli, T. B. K., Berguido, F. J., Grabherr, R., Loitsch, A., Tuppurainen, E.,... & Lamien, C. E. (2019): An HRM assay to differentiate sheeppox virus vaccine strains from sheeppox virus field isolates and other capripoxvirus species. Scientific reports, 9(1), 1-9.https://doi.org/10.1038/s41598-019-43158-x

Chibssa, T. R., Sombo, M., Lichoti, J. K., Adam, T. I. B., Liu, Y., Elraouf, Y. A., ... & Lamien, C. E. (2021): Molecular analysis of East African lumpy skin disease viruses reveals a mixed isolate with feature of both vaccine and field isolates. Microorganisms, 9 (6), 1142. https://doi.org/10.3390/microorganisms9061142

CSA, 2020. Agricultural sampling survey. Rep. Livest. Livest. Charact. II, 1–215.

Dao, T. D., Tran, L. H., Nguyen, H. D., Hoang, T. T., Nguyen, G. H., Tran, K. V. D., ... & Bui, V. N. (2022): Characterization of Lumpy skin disease virus isolated from a giraffe in Vietnam. Transboundary and Emerging Diseases.https://doi.org/10.1111/tbed.14583

Das, M., Chowdhury, M. S. R., Akter, S., Mondal, A. K., Uddin, M. J., Rahman, M. M., & Rahman, M. M. (2021): An updated review on lumpy skin disease: Perspective of southeast asian countries. J. Adv. Biotechnol. Exp. Ther, 4(3), 322-333. https://doi.org/10.5455/JABET.2021.D133

FAO, 2017. Lumpy Skin Disease Special Issue, Empres-Animal Health 360.

Ferris, R. D., & Plowright, W. (1958): Simplified methods for the production of monolayers of testis cells from domestic animals, and for the serial examination of monolayer cultures. Journal of Pathology and Bacteriology, 75(2),313-18. https://doi.org/10.1002/path.1700750210

Feyisa, A. F. (2018): A case report on clinical management of lumpy skin disease in bull. Journal of Veterinary Science & Technology, 9(3), 538. https://doi.org/10.4172/2157-7579.1000538

G.F. El-Bagoury, E.M. El-Nahas, A.S. El-Habbaa, and E.T.A.-N., (2009): Isolation and Molecular Characterization of Lumpy Skin Disease Virus from Kalubia Governorate. Benha Veterinary Medical Journal 20, 109–119.

Gamal Elden, I. M., Kamoura, N. A. E., & Ibrahim, M. A. (2021): Virological, Molecular And Immuno-Biochemical Studies Of Lumpy Skin Disease In Naturally Infected Cattle. Journal of Applied Veterinary Sciences, 6(1), 28-37.

Gari, G., Abie, G., Gizaw, D., Wubete, A., Kidane, M., Asgedom, H., Bayissa, B., Ayelet, G., Oura, C.A., Roger, F., Tuppurainen, E.S., 2015. Evaluation of the safety, immunogenicity and efficacy of three capripoxvirus vaccine strains against lumpy skin disease virus. Vaccine 33, 3256-3261.

Gari, G., Biteau-Coroller, F., LeGoff, C., Caufour, P., & Roger, F. (2008): Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method. Veterinary microbiology, 129(3-4), 269-280. https://doi.org/10.1016/j.vetmic.2007.12.005

Gari, G., Bonnet, P., Roger, F., & Waret-Szkuta, A. (2011): Epidemiological aspects and financial impact of lumpy skin disease inEthiopia. Preventive veterinary medicine, 102(4)

Gari, G., Waret-Szkuta, A., Grosbois, V., Jacquiet, P., & Roger, F. (2010): Risk factors associated with observed clinical lumpy skin disease in Ethiopia. Epidemiology & Infection, 138(11), 1657-1666. https://doi.org/10.1017/S0950268810000506

Gelaye, E., Belay, A., Ayelet, G., Jenberie, S., Yami, M., Loitsch, A., ... & Lamien, C. E. (2015): Capripox disease in Ethiopia: Genetic differences between field isolates and vaccine strain, and implications for vaccination failure. Antiviral Research, 119, 28-35.https://doi.org/10.1016/j.antiviral.2015.04.008

Gelaye, E., Belay, A., Ayelet, G., Jenberie, S., Yami, M., Loitsch, A., ... & Lamien, C. E. (2015): Capripox disease in Ethiopia: Genetic differences between field isolates and vaccine strain, and implications for vaccination failure. Antiviral Research, 119, 28 35.https://doi.org/10.1007/s11250-021-02593-7

Gelaye, E., Lamien, C.E., Silber, R., Tuppurainen, E.S.M., Grabherr, R., Diallo, A., (2013): Development of a Cost-Effective Method for Capripoxvirus Genotyping Using Snapback Primer and dsDNA Intercalating Dye. PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0075971

Gumbe, A. A. F. (2018): Review on lumpy skin disease and its economic impacts in Ethiopia. J. Dairy Vet. Anim. Res, 7(2), 39-46. https://doi.org/10.15406/jdvar.2018.07.00187

Haegeman, A., De Leeuw, I., Mostin, L., Van Campe, W., Aerts, L., Vastag, M., & De Clercq,

Haegeman, A.; De Leeuw, I.; Mostin, L.; Van Campe, W.; Aerts, L.; Venter, E.; Tuppurainen, E.; Saegerman, C.; De Clercq, K. Comparative Evaluation of Lumpy Skin Disease Virus-Based Live Attenuated Vaccines. Vaccines 2021, 9, 473.

Hedayati, Z., Varshovi, H. R., Mohammadi, A., & Tabatabaei, M. (2021): Molecular characterization of lumpy skin disease virus in Iran (2014–2018). Archives of Virology, 166(8), 2279-2283. https://doi.org/10.1007/s00705-021-05119-6

Hsiao, J.C., Chung, C.S. and Chang, W., 1999. Vaccinia virus envelope D8L protein binds to cell surface chondroitin sulfate and mediates the adsorption of intracellular mature virions to cells. J. Virol. 73(10): 8750-8761.

Hunter, P., Wallace, D., 2001. Lumpy skin disease in southern Africa: a review of the disease and aspects of control. J. South Afr. Vet. Ass. 72, 68–71.

Jiang, C., Tao, D., Geng, Y., Yang, H., Xu, B., Chen, Y., ... & Guo, A. (2022): Sensitive and Specific Detection of Lumpy Skin Disease Virus in Cattle by CRISPR-Cas12a Fluorescent Assay Coupled with Recombinase Polymerase Amplification. Genes, 13(5), 734. https://doi.org/10.3390/genes13050734

K. (2020): An Immunoperoxidase Monolayer Assay (IPMA) for the detection of lumpy skin disease antibodies. Journal of virological methods, 277, 113800. https://doi.org/10.1016/j.jviromet.2019.113800

Kara, P. D., Afonso, C. L., Wallace, D. B., Kutish, G. F., Abolnik, C., Lu, Z., ... & Rock, D. L. (2003): Comparative sequence analysis of the South African vaccine strain and two virulent field isolates of lumpy skin disease virus. Archives of virology, 148(7), 1335-1356. https://doi.org/10.1007/s00705-003-0102-0

Karki, M., A. Kumar, G. Venkatesan, S. Arya and A.B. Pandey. 2018. Genetic analysis of L1R myristoylated protein of Capripoxviruses reveals structural homogeneity among poxviruses. Infect. Genet. Evol. 58: 224-231. https://doi.org/10.1016/j.meegid.2018.01.001

Kasem, S., Saleh, M., Qasim, I., Hashim, O., Alkarar, A., Abu-Obeida, A., ... & Abdelatif, M.

(2018): Outbreak investigation and molecular diagnosis of Lumpy skin disease among livestock in Saudi Arabia 2016. Transboundary and emerging diseases, 65(2), e494-e500.https://doi.org/10.1111/tbed.12769

Kitching, R.P.; Hammond, J.M. Poxvirus, infection and immunity. In Encyclopaedia of Immunology; Roitt, I.M., Delves, P.J., Eds.; Academic Press: London, UK, 1992; pp. 1261–1264.

Kononova, S., Kononov, A., Shumilova, I., Byadovskaya, O., Nesterov, A., Prutnikov, P., ... & Sprygin, A. (2021): A lumpy skin disease virus which underwent a recombination event demonstrates more aggressive growth in primary cells and cattle than the classical field isolate. Transboundary and Emerging Diseases, 68(3), 1377-1383. https://doi.org/10.1111/tbed.13798

Krešić, N., Šimić, I., Bedeković, T., Acinger-Rogić, Ž., & Lojkić, I. (2020): Evaluation of serological tests for detection of antibodies against lumpy skin disease virus. Journal of Clinical Microbiology, 58(9), e00348-20.https://doi.org/10.1128/JCM.00348-20

Kumar, N., Chander, Y., Kumar, R., Khandelwal, N., Riyesh, T., Chaudhary, K., ... & Tripathi, B. N. (2021): Isolation and characterization of lumpy skin disease virus from cattle in India. PLoS One, 16(1), e0241022.https://doi.org/10.1371/journal.pone.0241022

Lamien, C. E., Le Goff, C., Silber, R., Wallace, D. B., Gulyaz, V., Tuppurainen, E., ... & Diallo, A. (2011): Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: Development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. Veterinary microbiology, 149(1-2), 30-39. https://doi.org/10.1016/j.vetmic.2010.09.038

Lamien, C. E., Lelenta, M., Goger, W., Silber, R., Tuppurainen, E., Matijevic, M., ... & Diallo, A. (2011): Real time PCR method for simultaneous detection, quantitation and differentiation of capripoxviruses. Journal of virological methods, 171(1), 134-140.https://doi.org/10.1016/j.jviromet.2010.10.014

Le, T., Sun, C., Chang, J., Zhang, G., & Yin, X. (2022): mRNA Vaccine Development for Emerging Animal and Zoonotic Diseases. Viruses, 14(2), 401.

Leliso, S. A., Bari, F. D., & Chibssa, T. R. (2021): Molecular Characterization of Lumpy Skin Disease Virus Isolates from Outbreak Cases in Cattle from Sawena District of Bale Zone, Oromia, Ethiopia. Veterinary MedicineInternational, 2021. https://doi.org/10.1155/2021/8862180

Limon, G., Gamawa, A.A., Ahmed, A. I., Lyons, N. A., & Beard, P.M. (2020): Epidemiological characteristics and economic impact of lumpy skin disease, sheeppox and goatpox among subsistence farmers in northeast Nigeria. Frontiers in veterinary science, 7, 8.https://doi.org/10.3389/fvets.2020.00008

MacDonald, R.A.S. (1931): Pseudo-urticaria of cattle.Annual Report for 1930. Department of Animal Health, Northern Rhodesia, 20–21.

Maclachlan, N.J., Dubovi, E.J., (2010): Fenner's Veterinary Virology: Fourth Edition, Fenner's Veterinary Virology: Fourth Edition. https://doi.org/10.1016/C2009-0-01816-9.

Mebratu, G.Y., Kassa, B., Fikre, Y., & Berhanu, B. (1984): Observation on the outbreak of lumpy skin disease in Ethiopia. Journal of Animal Husbandry and Veterinary Medicine in Tropical Countries , 37 (4), 395-399. https://doi.org/10.19182/remvt.8346

Menasherow, S.; Erster, O.; Rubinstein-Giuni, M.; Kovtunenko, A.; Eyngor, E.; Gelman, B.; Khinich, E.; Stram, Y. A high-resolution melting (HRM) assay for the differentiation between Israeli field and Neethling vaccine lumpy skin disease viruses. J. Virol. Methods 2016, 232, 12–15. [CrossRef]

Milovanovic, M.; Dietze, K.; Milicevic, V.; Radojicic, S.; Valcic, M.; Moritz, T.; Hoffmann, B. Humoral immune response to repeated lumpy skin disease virus vaccination and performance of serological tests. BMC Vet. Res. 2019, 15, 80.

Mitiku Tamire(2022): Current status of Lumpy skin disease and its Economic impacts in Ethiopia. Journal of vaccine research 1:103

Molini, U., Boshoff, E., Niel, A. P., Phillips, J., Khaiseb, S., Settypalli, T. B., ... & Lamien, C. E. (2021): Detection of Lumpy Skin Disease Virus in an Asymptomatic Eland (Taurotragus oryx) in Namibia. Journal of Wildlife Diseases, 57(3),708-711. https://doi.org/10.7589/JWD-D-20-00181

Molla, W., de Jong, M. C. M., & Frankena, K. (2017): Temporal and spatial distribution of lumpy skin disease outbreaks in Ethiopia in the period 2000 to 2015. BMC veterinary research, 13(1), 1-9.https://doi.org/10.1186/s12917-017-1247-5

Molla, W., Frankena, K., & De Jong, M. C. M. (2017): Transmission dynamics of lumpy skin disease in Ethiopia. Epidemiology & Infection, 145(13), 2856-2863.https://doi.org/10.1017/S0950268817001637

Molla, W., Frankena, K., Gari, G., & de Jong, M. C. (2017): Field study on the use of vaccination to control the occurrence of lumpy skin disease in Ethiopian cattle. Preventive veterinary medicine, 147, 34-41. https://doi.org/10.1016/j.prevetmed.2017.08.019

Moss, B. (2006): Poxvirus entry and membrane fusion. Virology, 344(1), 48 54. https://doi.org/10.1016/j.virol.2005.09.037

Moss**,** B. 2006. *Poxviridae*: the viruses and their replication, p. 2849–2883. *In* D. M. Knipe, P. Howley, D. Giriffin, and R. Lamb (ed.), Fundamental virology, 5 ed. Lippincott Williams & Wilkins, Hagerstown, MD.

Namazi, F., & Khodakaram Tafti, A. (2021): Lumpy skin disease, an emerging transboundary viral disease: A review. Veterinary Medicine and Science, 7(3), 888-896. https://doi.org/10.1002/vms3.434

OIE, 2017. Lumpy Skin Disease, Aetiology Epidemiology Diagnosis Prevention and Control References. OIE Man. Diagnostic Tests Vaccines Terr. Anim. under Head. “B. Diagnostic Tech. 1–8.

OIE, 2021. Lumpy skin disease, OIE (2017): Manual of diagnostic tests and vaccines for terrestrial animals 151, 10–17.

Orynbayev,M.B., Nissanova, R.K., Khairullin, B.M., Issimov,A., Zakarya, K.D.,Sultankulova, K.T., Kutumbetov,..Kock, R.A., (2021): Lumpy skin disease in Kazakhstan. Trop. Anim. Health Prod. 53, 1–7. https://doi.org/10.1007/s11250-021-02613-6

Pandey, N., Hopker, A., Prajapati, G., Rahangdale, N., Gore, K., & Sargison, N. (2022): Observations on presumptive lumpy skin disease in native cattle and Asian water buffaloes around the tiger reserves of the central Indian highlands. New Zealand Veterinary Journal, 70(2), 101-108. https://doi.org/10.1080/00480169.2021.1984335

Payne, S., (2017): Methods to Study Viruses. Viruses 37–52. https://doi.org/10.1016/b978-0-12-803109-4.00004-0

Ramıirez, J.C., E. Tapia and M. Esteban. 2002. Administration to mice of a monoclonal antibody that neutralizes the intracellular mature virus form of vaccinia virus limits virus replication efficiently under prophylactic and therapeutic conditions. J. Gen. Virol. 83(5): 1059-1067. https://doi.org/10.1099/0022- 1317-83-5-1059

Roche, X., Rozstalnyy, A., TagoPacheco, D., Pittiglio, C., Kamata, A., Beltran Alcrudo, D., ... & Sumption, K. (2021): Introduction and spread of lumpy skin disease in South, East and Southeast Asia: qualitative risk assessment and management. Food & Agriculture Org. https://doi.org/10.4060/cb1892en

Rodriguez, J.F., Paez, E. and Esteban, M., 1987. A 14,000-Mr envelope protein of vaccinia virus is involved in cell fusion and forms covalently linked trimers. J. Virol. 61(2): 395-404.

Rouby, S. R., Safwat, N. M., Hussein, K. H., Abdel-Ra’ouf, A. M., Madkour, B. S., Abdel-Moneim, A. S., & Hosein, H. I. (2021): Lumpy skin disease outbreaks in Egypt during 2017-2018 among sheeppox vaccinated cattle: Epidemiological, pathological, and molecular findings. PloS one, 16(10),e0258755. https://doi.org/10.1371/journal.pone.025 8755

Rouby, S. R., Shehata, O., Abdel-Moneim, A. S., Hussein, K. H., & Mahmoud, M. M. (2021): Lumpy skin disease in calves: The association between clinical signs and biochemical alterations. Adv. Anim. Vet. Sci, 9(11), 1863-1868.

Salib, F. A., & Osman, A. H. (2011): Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate,Egypt. Veterinary world, 4(4).https://doi.org/10.5455/vetworld.2011

Saltykov, Y. V., Kolosova, A. A., Filonova, N. N., Chichkin, A. N., & Feodorova, V. A. (2021:. Genetic Evidence of Multiple Introductions of Lumpy Skin Disease Virus into Saratov Region, Russia. Pathogens, 10(6), 716.

Samojlović, M., Polaček, V., Gurjanov, V., Lupulović, D., Lazić, G., Petrović, T., & Lazić, S. (2019): Detection of antibodies against lumpy skin disease virus by virus neutralization test and ELISA methods. https://doi.org/doi:10.2478/acve-2019-0003

Shahein, M. A. (2021): Diagnosis of naturally occurring lumpy skin disease virus infection in cattle using virological, molecular, and immunohistopathological assays. Vete rinary World, 14(8), 2230. https://doi.org/10.14202/vetworld.2021.2230-2237

Springer, Cham.

Sprygin, A., Pestova, Y., Bjadovskaya, O., Prutnikov, P., Zinyakov, N., Kononova, S., ... & Kononov, A. (2020): Evidence of recombination of vaccine strains of lumpy skin disease virus with field strains, causing disease. PLoS One, 15(5),e0232584. https://doi.org/10.1371/journal.pone.0232584

Sprygin, A., Pestova, Y., Prutnikov, P., & Kononov, A. (2018): Detection of vaccine-like lumpy skin disease virus in cattle and Musca domestica L. flies in an outbreak of lumpy skin disease in Russia in 2017. Transboundary and Emerging Diseases, 65(5), 1137- 1144.https://doi.org/10.1111/tbed.12897

Sprygin, A.; Pestova, Y.; Prutnikov, P.; Kononov, A. Detection of vaccine-like lumpy skin disease virus in cattle and Musca domestica L. flies in an outbreak of lumpy skin disease in Russia in 2017. Transbound. Emerg. Dis. 2018, 65, 1137–1144. [CrossRef]

Sudhakar, S. B., Mishra, N., Kalaiyarasu, S., Jhade, S. K., Hemadri, D., Sood, R., ... & Singh,

Tadesse Degu, B. M., & Fesseha, H. (2020): Epidemiological status and economic impact of lumpy skin disease-review. Int J Recent Biotechnol, 8, 1-15.

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011): MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular biology and evolution, 28(10), 2731-2739.https://doi.org/10.1093/molbev/msr121

Tassew, A., Assefa, A., Gelaye, E., Bayisa, B., & Ftiwi, M. (2018): Identification and molecular characterization of lumpy skin disease virus in East Hararghe and East Shoa

Zone, Oromia Regional State. ARC Journal of Animal and veterinary sceinceJ. Anim. Vet. Sci, 4(3), 1-16.

Tsouloufi, T. K., Donnison, L. M., Smyth, K. L., & Peters, A. R. (2020): Development of a systematic mapping review protocol for the most recent evidence on ruminant infectious disease frequency and disease-associated mortality: Ethiopia as a case study. Animal Health Research Reviews, 21(1), 96-102.https://doi.org/10.1017/S1466252319000203

Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Kutish, G. F., & Rock, D. L. (2001): Genome of lumpy skin disease virus. Journal of virology, 75(15), 7122-7130. https://doi.org/10.1128/jvi.75.15.7122-7130.2001

Tuppurainen, E. S. M., Antoniou, S. E., Tsiamadis, E., Topkaridou, M., Labus, T., Debeljak, Z., ... & Broglia, A. (2020). Field observations and experiences gained from the implementation of control measures against lumpy skin disease in South-East Europe between 2015 and 2017. Preventive Veterinary Medicine, 181,104600. doi.org/10.1016/j.prevetmed.2018.12.006

Tuppurainen, E. S., Lamien, C. E., & Diallo, A. (2021). Capripox (Lumpy Skin Disease, Sheep Pox, and Goat Pox). Veterinary Vaccines: Principles and Applications, 383 397.https://doi.org/10.1002/9781119506287.ch28

Tuppurainen, E. S., Venter, E. H., & Coetzer, J. A. W. (2005): The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. Onderstepoort Journal of Veterinary Research, 72(2), 153-164.

Tuppurainen, E., Alexandrov, T. & Beltrán-Alcrudo, D. (2017): Lumpy skin disease field manual – A manual for veterinarians. FAO Animal Production and Health Manual No.

Tuppurainen, E., Alexandrov, T., & Beltrán-Alcrudo, D. J. F. A. P. (2017): Lumpy skin disease-a manual for veterinarians. FAO Animal Production and Health Manual, (20).

Tuppurainen, E., Dietze, K., Wolff, J., Bergmann, H., Beltran-Alcrudo, D., Fahrion, A., ... & Knauf, S. (2021): Vaccines and Vaccination against LumpySkinDisease. Vaccines, 9(10), 1136.

V. P. (2020): Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: Epidemiological features and molecular studies. Transboundary and Emerging Diseases, 67(6), 2408-2422.https://doi.org/10.1111/tbed.13579

Van Schalkwyk, A., Kara, P., Ebersohn, K., Mather, A., Annandale, C. H., Venter, E. H., & Wallace, D. B. (2020). Potential link of single nucleotide polymorphisms to virulence of vaccine-associated field strains of lumpy skin disease virus in South Africa. Transboundary and Emerging Diseases, 67(6), 2946-2960.

Vidanovic, D.; Sekler, M.; Petrovic, T.; Debeljak, Z.; Vaskovic, N.; Matovic, K.; Hoffmann, B. Real-Time Pcr Assays for the Specific Detection of Field Balkan Strains of Lumpy

Skin Disease Virus. Acta Vet. 2016, 66, 444–454. [CrossRef]

Von Backstrom, U. (1945): Ngamiland cattle disease: preliminary report on a new disease, the etiological agent being probably of an infectious nature. Journal of the South African Veterinary Association, 16(1), 29-35.

Wang, Y., Zhao, L., Yang, J., Shi, M., Nie, F., Liu, S., ... & Li, Y. (2021): Analysis of vaccine-like lumpy skin disease virus from flies near the western border of China. Transboundary and Emerging Diseases.https://doi.org/10.1111/tbed.14159

Wassie Mollaa,b, Klaas Frankenaa, Getachew Garic, Mart C.M. de Jonga.,2017. Field study on the use of vaccination to control the occurrence of lumpy skin disease in Ethiopian cattle. *Preventive Veterinary Medicine 147 : 34–41*

Weiss, K.E. (1968): Lumpy skin disease. In: Virology Monographs. Vienna-New York, Springer Verlag, 111-131.

Wolff, J., Tuppurainen, E., Adedeji, A., Meseko, C., Asala, O., Adole, J., ... & Hoffmann, B. (2021). Characterization of a Nigerian Lumpy Skin Disease Virus Isolate after Experimental Infection of Cattle. Pathogens, 11(1),16.https://doi.org/10.3390/pathogens1 1010016

Wolff, J.; Krstevski, K.; Beer, M.; Hoffmann, B. Minimum Infective Dose of a Lumpy Skin Disease Virus Field Strain from North Macedonia. Viruses 2020, 12, 768

Wolff, J.; Moritz, T.; Schlottau, K.; Hoffmann, D.; Beer, M.; Hoffmann, B. Development of a Safe and Highly Efficient Inactivated Vaccine Candidate against Lumpy Skin Disease Virus. Vaccines 2021, 9, 4.

Wolff, E.J., S. Vijaya and B. Moss. 1995. Amyristylated membrane protein encoded by the vaccinia virus L1R open reading frame is the target of potent neutralizing monoclonal antibodies. Virology. 211(1): 53-63. https://doi. org/10.1006/viro.1995.1378.

Zahra Bamouh, a, b Siham Fellahi, b Slimane Khayi, c Jihane Hamdi, a, b Khalid Omari Tadlaoui, a Ouafaa Fassi-Fihri, b M.E., AResearch, 2021. Draft Genome Sequence of the Capripoxvirus Vaccine Strain 10, 29–30.

Zewdie, G., Mammo, B., Gelaye, E., Getachew, B., Bayssa, B., (2019): Isolation, Molecular

8. ANNEXES

Annex 1: Primary Lamb kidney cell culture preparation

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 sterilemortar 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 0.4 ml filtered supernatant onto a monolayer of PLK cells in a6 well plate.
5. Incubate at 37°C for an hour for adsorption, and then add 9 ml Dulbecco's Modified Eagle Medium (DMEM, 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: Virus Neutralization test procedure

Principle: to detect the presence of serum specific antibodies against Lumpy skin disease virus by *in vitro* neutralization of the viral cytopathic effect on cell culture. This method is based on a reaction between the virus and specific antibody in the test serum. un-neutralized virus is detected by cytopathic effect. A loss of infectivity of the virus is caused by interference by the bound antibody with any of the steps leading to the release of the viral genome from the host cells including attachment, infection, or viral release.

Procedures

- Trypsinize the lamb kidney cells and prepare suspension of $4X10^5$ cells per ml in complete MEM medium. The cells are then kept appropriately diluted at 37^0C in the incubator.
- Dilute the viral suspension to be titrated in sterile tubes 10 fold from 10-1 to 10-7 (0.5ml viral suspension in 4.5 ml of MEM without serum) and place it in ice rack.
- With multichannel pipette, dispense 100µl of this cell suspension in all the wells ($4x10^5$ cells/well)
- In the wells of the last row, which serve as a negative control, dispense 100µl of the MEM without serum
- Dispense there after different viral dilutions on to cells in a way to have ten replicates for each dilution in 100µl volumes.
- Fill the periphery wells with 200µl media so as to make barrier against desiccation
- Incubate at 37^0C under 5% CO2 and humidity. In case of no CO2 supply, it is possible to wrap the cells in paraffin (making an impermeable envelope). The humidity supplied by the water tank/rack is a requirement against desiccation.
- The test serum from -80 is thawed by incubating 56^0c for 30 minutes
- A working record format is prepared in a layout of the 96 plate according to the samples to be tested, each sample must be recorded

individually, the stock virus, the sera dilution(inverse log), and all the information required

- Prepare a sufficient volume of medium: MEM + 2% antibiotic/antimycotic + 1% L- Glutamine of 200 mM with and without serum.
- Take out the viral suspension from -80 right before use.
- The virus stock with known titer should be diluted to give a 10^3 $TCID_{50}$ /ml. Use MEMwithout serum but with 2% antibiotics & antimycotics and 1% glutamine.
- 100 □l (10^2 $TCID_{50}$) of this viral dilution will be added to each well, therefore 10ml ofthe viral suspension is required for one plate.
  - Addition of medium: 100 µl of medium without serum is distributed in all wells.
  - Take a 96 well tissue culture plate. One plate allows testing 8 sera in duplicate inserial dilutions (1:5).

25 µl of test sample 1-16, added in well A1-H1 and A7-H725 µl of test sample 1-16, added in well A2-H2 and A8-H8

With the multichannel pipette set at 25 µl, perform <u>5 fold serial dilutions</u> (from colomn1-6, and Colomn7-12) with initial dilution 1/5 and discard 25 µl of suspension from the end point dilution 1/15625 i.e. from column 6 and 12.

- Addition of virus: Add 100µl of 1000 TCID50/ml viral suspension to each wells.
- **Control plate**: The plate should contain positive and negative controls and prepareseparately

Negative control: 6 wells in the last row are without virus.

Positive control: 6 wells in each 4 rows are filled with different dilutions of LSDV

Viruses (100$TCID_{50}$, 10$TCID_{50}$, 1$TCID_{50}$ and 0.1$TCID_{50}$ in 100 µl suspension) thenincubate in 37°c for 1 hour.

- Addition of cells: After 1 hour add in each well of the plate 50 µl of lamb kidney cellssuspension of ($4x10^5$ cell/ml**).** Then the plates are incubated at 37°c with 5% CO_2.

- Microscope reading: Plate reading to monitor for CPE formation at 8,10 and 14 days
- The test result is valid only if CPE does not occur in the Negative control wells and
- For the positive controls the CPE formation should be according to the virus titer in eachfour rows and expected standard protocol would be (++++, +++, +- +-, ---).

100TCID$_{50}$/100µl: + + + + +

10TCID$_{50}$/100µl: + + + + +

1TCID$_{50}$/100µl: + - + - +

0.1TCID$_{50}$/100µl: - - - - -

Annex 3: DNA extraction (Qiagen)

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 brieflycentrifuge 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 AW1andcentrifuge it at 8000rpm for 1 min.

8. Discard the collection tube, transfer the spin column in to new 2 ml collection tube and add500µlbuffer 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.

Collect the extracted DNA and store at -20°C until use

Anex 4 : 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

4.1.Occurrence

First time yes ☐ _ No ☐ _

Commonly occurred yes ☐ _ No ☐ _

4.2. Season of occurrence

Dry season yes ☐ _ No ☐ _

Rainy season yes ☐ _ No ☐ _

Any season yes ☐ _ No ☐ _

4.3. The last outbreak of LSD in the village occurred in (For Vet. Clinician).

Before 2017 yes ☐ _ No ☐ _

2018-2021 yes ☐ _ No ☐ _

In 2021-2023 yes ☐ _ No ☐ _

None yes ☐ _ No ☐ _

5. Animals at risk

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

6. Animals affected

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

7. Total number of animal dead

Breed		sex	Age group			Total
Local	Cross		<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 ☐

Part II:LSD vaccine and vaccination status

Vaccination status

1. Have you vaccinated your animals against LSD? Yes ☐ ☐

   No

   When the vaccination performed? ______________

   Who gave the vaccine? ____________________

   Where is the source of the vaccine?_________________

2. How do you plan delivery of LSD vaccine? periodically every year , a☐ outbreak reports ☐

   - If it is following outbreak, how do you know you are not vaccinating of already infected animals?________________________________

3. Do you think vaccine transport and storage is to the standard? yes ☐ ☐

   What are the major challenges in vaccine transport?

   ____

   ____

   ____

   ____

   What are major challenges on vaccine storage in the clinics?

   ____

   ____

   ____

4. Do you think the current LSD vaccine effective? ☐ , No ☐

   - If not effective , why do you think ?_____________________
   - Have you seen any vaccine-associated side effects after vaccination?

     ____

5. Any other things to be added as a challenge related to vaccination of LSDV?

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