Molecular characterisation of lumpy skin disease virus in Mahikeng local municipality

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Dissertation submitted in fulfilment of the requirements for the degree Master of Science in Animal Health at the North West University

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Graduation ceremony: July 2020

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DECLARATION

I declare that the dissertation entitled: **"Molecular characterization of lumpy skin disease in Mahikeng Local Municipality"**, is my original work. It is submitted for the degree of Master of Science in Animal Health to the Faculty of Natural and Agricultural Sciences, Northwest University. This work has been done under the supervision and approval of Dr. Lubanza Ngoma and Professor Mulunda Mwanza. This dissertation has not been submitted for any degree or examination at any other university.

Student: _____

Signature: _____

Date: _____

ACKNOWLEDGEMENTS

I would like to express my great appreciation to Dr. Lubanza Ngoma my research supervisor and professor Mulunda Mwanza my co-supervisor for their patient, guidance, encouragement and taking their time in helping me with this research

I would also like to extend my thanks to the principal of Animal Health Laboratory Dr. Mpho Tsheole, for allowing me to work in the laboratory and assist me with the necessary equipment. Also, thanking the ARC Onderstepoort for assisting with the lab work and helping in analysing the samples.

I am grateful to those with whom I have had the pleasure to work with during this research project. Members of my dissertation committee have provided me extensive personal and professional guidance and taught me a great deal about both scientific and research and life in general

The work wouldn't be possible without the financial support of NRF and NWU. Your help is highly appreciated, and may you continue with the great work you're doing.

Lastly, I would like to thank my family members, my parents, for their love and guidance in whatever I pursue, not forgetting my big brother and uncle for always supporting me throughout the research. All my friends and CLC family, I love you so much.

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LIST OF ABBREVIATIONS

BCS:	Body condition score
BLAST:	Basic Local Alignment Search Tool
CaPV:	Capripoxvirus
CBD:	Central business district
CMI:	Cell intervened insusceptibility
DNA:	Deoxyribonucleic acid
dsDNA:	double stranded Deoxyribonucleic acid
DVTD:	Department of veterinary tropical disease
EDTA:	Ethylene diamine tetraacetic acid
FAO:	Food Agriculture Organization
GDP:	Gross domestic products
ITR:	Inverted Terminal repeat
LSD:	Lumpy skin disease
LSDV:	Lumpy skin disease virus
MLM:	Mahikeng Local Municipality
NCBI:	National Center for Biotechnology Information
RNA:	Ribonucleic acid
RT-PCR:	Real time polymerase chain reaction
SAVC:	South Africa Veterinary Council
SNT:	Serum neutralization test
SPS:	Sanitary photosanitary
TAD:	Transboundary animal disease
qPCR:	Quantitative polymerase chain reaction
WTO:	World Trade Organisation

ABSTRACT

Lumpy skin disease (LSD) is an infectious viral disease well-known to cause economic loss and reduced productivity in cattle. Several studies have addressed lumpy skin disease virus (LSDV) seroprevalence in cattle worldwide, including South Africa. Nevertheless, the prevalence of LSDV in Mahikeng Local Municipality (MLM) cattle, North West Province, is unknown. The study aimed to detect and identify LSDV from suspected cattle and those showing clinical symptoms. Approximately 200 samples (100 blood and 100 skin nodules biopsy) were collected from the animal with clinical manifestations with LSD. The serum neutralization test (SNT) method was used to detect antibodies of LSDV in the clinical samples. In addition, a Real-Time polymerase chain reaction high-resolution melt assay (RT-PCR)/ quantitative polymerase chain reaction (qPCR) and conventional polymerase chain reaction (PCR) was performed to genotype the LSDV strains. SNT results showed that out of 100 serum samples analyzed, 67% were positive for LSDV antibodies, while 33% were negative. The highest incidence occurred in Masutlhe (95%), followed by Tswaing (74%) and Meetmekaar (58%), respectively, whereas 50% of the positive samples were recorded in Lokaleng and 29% was reported in Six Hundred. Pearson Chi-Square revealed that there was a significant difference between the prevalence of LSDV in the villages of MLM ($P<0.05$). Out of 100 skin nodules collected 16 samples showed a sufficient amount of DNA material. RT-PCR assay showed that all the 16 samples tested positive for LSDV. Conventional PCR assay resulted in amplification of the DNA samples showed bands at 1203 bp PCR product of G-protein-coupled chemokine receptor gene (GPCR), LSDV-022 gene at 237 bp and (thymidine kinase) TK gene (LSDV-066) at 400 bp. Nevertheless, sequencing results showed six samples (LSD-2-RSA-2018, LSD-3-RSA-2018, LSD-5-RSA-2018, LSD-9-RSA-2018, LSD-13-RSA-2018, and LSD-15-RSA- 2018) were positive to LSDV. Phylogenetic analyses for isolates were done using MEGA 7 and showed that the LSDV were firmly related to FJ869377 (Egypt-Isamalia 18/1989, KR024780 Turkey-02/2015, KY829023 Evros/GR/15, FJ 869375 RSA/06-D 19353-16, MH893760 LSDV Russian Dagestan 2015, KX894508 155920-Israel 2012, KX683219KSGP-0240 Kenya 1974 and AF 409137 NW-LW Warmbaths RSA 1999 strains. This study provided information on LSDV, which is in circulation in MLM, and this finding may help in developing effective prophylactic strategies in the villages affected by the virus.

Keywords: Cattle, LSD, MLM, SNT, Real-time PCR/ qPCR, Conventional PCR.

CHAPTER ONE

INTRODUCTION

1.1 Background

The agriculture field is a key sector in the economies of most countries in Africa (Blench et al. , 2003). Delgado et al. (1999) show that the productivity and demand for meat from livestock are likely to increase from 233 to 300 million tons by 2020. It has been reported that food such as eggs, milk, and meat contribute, on average, about 30% towards agricultural Gross Domestic Product in developing countries (Morgan and Tallard, 2007). Most of the people living in rural areas in Africa depend on their animals (cattle, sheep, and goats) for income generation and as a source of protein (Morgan and Tallard, 2007). In South Africa, gross farming income earned from all agricultural products for the year ended 31 December 2018 increased by 1,2% to R281 835 million, as opposed to R278 531 million of the previous year (DAFF, 2018b). Livestock activity is among a key enterprise in the economies of South Africa and several nations in the world, however, the sector remains exposed to different diseases. The diseases occasionally result in outbreaks that negatively impact the productive capacity, thus resulting in a subsequent decrease in the production of meats and meat products (Pritchett et al., 2005). If not controlled, the outbreaks can affect food security, which could have severe consequences through the inter-connected sectors in the economy (Rich and Wanyoike, 2010).

Lumpy skin disease (LSD) is among the most important infections which affect the productivity of livestock (Coetzer, 2004; Kasem et al., 2018; Katsoulos et al., 2018). Cattle of different ages and breeds are vulnerable to the virus, except animals that were recently recovered from the infection (Coetzer, 2004; Ntombimbini and Klein, 2015). The disease is

initiated trough a virus that seems to be transferred by blood-feeding arthropods and insects (vector) like mosquitoes along with other flies (Magori-Cohen et al., 2012). This virus is well known as a member of the Poxviridae family and genus of Capripoxvirus (Babiuk et al., 2008a). It has a linear, dsDNA genome of about 151kb, flanked by inverted terminal repeat (ITR) sequences which are covalently closed at their extremities. The surface membrane shows tubules or filaments surface (Tuppurainen and Oura, 2012). The replication of LSDV is uncommon as a double-stranded genomic DNA because it takes place in the cytoplasm. The virus encrypts its machinery for genome transcription, a DNA dependent RNA polymerase, which facilitates the replication in the cytoplasm (Fields et al., 2007). The most common symptoms of this infection branded by the presence of circumscribed skin nodules, severe firm, fever, and necrotic plaques in the mucous skins, swelling of different outlying lymph nodes, orchitis, and mastitis. The decrease in milk production, damaged skin, permanent or temporary infertility, and death of cattle may be observed (Alemayehu et al., 2013). The disease and mortality rates vary from 2% to 12% and are fundamentally dependent on the type of cattle (Gari et al., 2010; Salib and Osman, 2011). Furthermore, the morbidity rate is often higher during an outbreak, which can be as high as 85%) (Stram et al., 2008).

It has been noted that LSDV is highly resistant to chemicals as well as to physical means of sterilization, and the virus may survive up to 33 days in the necrotic tissues. The LSDV may survive in lesions for a minimum of eighteen days at room temperature. It estimated that at -80°C, the LSDV could remain viable in skin nodules for 10 years and at 4°C for six months in contaminated fluid culture or lesions (Vorster and Mapham, 2008).

Laboratory diagnosis of the viral infections can be carried out following clinical signs; however, minor and subclinical infection cases might cause problems for their detection. Therefore, rapid diagnostic runs are crucial for diagnosis, which can be done either by

screening methods and characterization of the causative agent or by detection of antibody using different serological assessments (Tuppurainen et al., 2005).

Several studies have addressed LSD seroprevalence in cattle worldwide, including South Africa. Nevertheless, the LSDV strain in cattle in the Mahikeng Municipality North West Province is unknown.

Therefore, there is a need to investigate and identify the infectious pathogen of LSD at the strain level in MLM.

1.2 History of lumpy skin disease

Lumpy skin disease is generally confined to Africa with the potential of spreading to Asian countries, as indicated in Figure 1.1. Initially, an outbreak of LSD was described in Zambia (formerly Northern Rhodesia) in 1929, and it was considered to be the consequence either of poisoning or a hypersensitivity reaction of cattle to insect bites (Hunter and Wallace, 2001). Between 1943 and 1945, several cases of LSD were described in South Africa, Zimbabwe and Botswana, where the infectious nature of the disease was recognized (Davies, 1991b, Hailu et al., 2015).

According to the study conducted by Hunter and Wallace (2001), in several southern African countries, it was revealed that the disease kept on fanning out and occurred as a pan-zoonotic that carried on for years affecting many cattle (Hunter and Wallace, 2001). The first cases in South Africa were reported in the Northwest Province (previously called Marico district of the Western Transvaal) (Hunter and Wallace, 2001; Thomas and Mare, 1945). The disease was named ‘knopvelsiekte’ (Afrikaans for lumpy skin disease). It was also noted that the transport or movement of cattle increased the dissemination of the causative agent of LSD (Hunter and Wallace, 2001). Therefore, it was discovered that the LSD was disseminated from the previous Orange Free State, Natal, Western Cape, and Transkei. Through this period of increased movement, an estimated eight million cattle were killed and subsequently suffered substantial

economic losses (Diesel, 1949; Hunter and Wallace, 2001; Thomas and Mare, 1945). In 1953-1954 there was a severe outbreak of LSD that occurred in the Eastern Transvaal, and the outbreak continued until 1962 (Hunter and Wallace, 2001). In 1957 the disease was diagnosed in Kenya, then in Sudan in 1972, followed by West Africa in 1974 (Davies, 1991b). While LSD was spreading into Somalia in 1983 (Davies 1991a and b). In 2001, LSD occurred in Mauritius, Mozambique, and Senegal. According to Tuppurainen et al. (2005), during the period between 1981 and 1986, the mortality rate in infected cattle was evaluated toward 20% in countries such as Kenya, Tanzania, Zimbabwe, Cameroon and Somalia (Tuppurainen et al., 2005). In March 2004, LSD was noticed in parts of the Southern District, around Moshupa, Sesung, Tsoonyane, Mosepele and Moitchinyi of Botswana (Abera et al., 2015a). In 2001, LSD clinical signs were reported in Mauritius, Mozambique, and Senegal.

In the Middle East, between 1984 and 2009, an outbreak of LSD was also reported in Oman (Kumar, 2011). In 1989 some livestock were culled in a village of Oman where it was suspected to be LSD. Kuwait in 1986 and 1991, Egypt in and 2006 (Ali and Amina 2013; Fayez and Ahmed 2011). The virus was suspected of having arrived in Egypt by way of wind-borne arthropod vectors (Klausner et al., 2017). In 1992 LSD infection was detected in Saudi Arabia and, the outbreak reappeared in 2006 after the importation of infected beef from affected African countries (Tageldin et al., 2014; Tuppurainen and Oura, 2012). Unfortunately, accurate statistics from 1984 until 2009 on these epidemics are limited (Kumar, 2011; Tageldin et al., 2014).

From the study conducted by Coetzer, only four countries on the African continent, namely Tunisia, Libya, Morocco, and Algeria, have never reported outbreaks of LSD (Coetzer, 2004). On the other hand, several researchers have revealed that LSD was observed in different countries in the Middle East, and it is extremely probable that it is autochthonous in the region. Recent occurrences of LSD outside Africa have been reported in 2012 (Israel), 2013 (West

Bank), (2013) Lebanon, 2013 (Jordan), 2013 (Turkey) and in 2013 (Iraq). The Israeli outbreak have been linked to infected *Stomoxys calcitrans* insects whose existence runs on the wind from Ismailia in Egypt (Health and Welfare, 2015). The virus was introduced again in Egypt through the importation of infected animals coming from other African countries in the year 2006 (Abdulqa et al., 2016). According to the investigation conducted by Wainwright et al. (2013), the Syrian Arab Republic was involved in the introduction of LSDV into Turkey (Wainwright et al., 2013).

In 2015, LSD clinical signs were observed for the first time in the European Union from Greece; it possible that the infection originated in Turkey. The Greek outbreak occurred in two beef herds situated in the Evros River Delta from August 2015 to December 2015 (EFSA, 2018). In 2016, LSD spread to the following countries Bulgaria, Republic of Macedonia, Serbia, Montenegro, Kosovo, and Albania. In the same year, LSD occurred again in Iran and Iraq as well as Azerbaijan (Zeynalova., 2016).

It is also known that the disease remains under-reported in Syria because of the civil war (Alkhamis and VanderWaal, 2016). This condition brought about a lot of worries in the intercontinental community, as can be disseminated into different LSD-free European member countries using Turkey as a portal of the entrance (Tuppurainen and Oura, 2014).

According to different studies, it was revealed that there is a high probability for LSD to be disseminated and it poses a risk to countries such as Greece, Bulgaria, and the Caucasus region, as well as Iran and Syria (APHIS, 2006; Tageldin et al., 2014; Tuppurainen and Oura, 2012; Salib and Osman, 2011). Lumpy skin disease virus spread throughout Turkey between 2013 and 2015, to the extent that the disease may now become endemic in that country (Tuppurainen et al., 2017b). Recently, in February (2018), the South Africa Veterinary Council and World Health Organization for Animal Health reported an eruption of LSD in South Africa (Mercier et al., 2018).

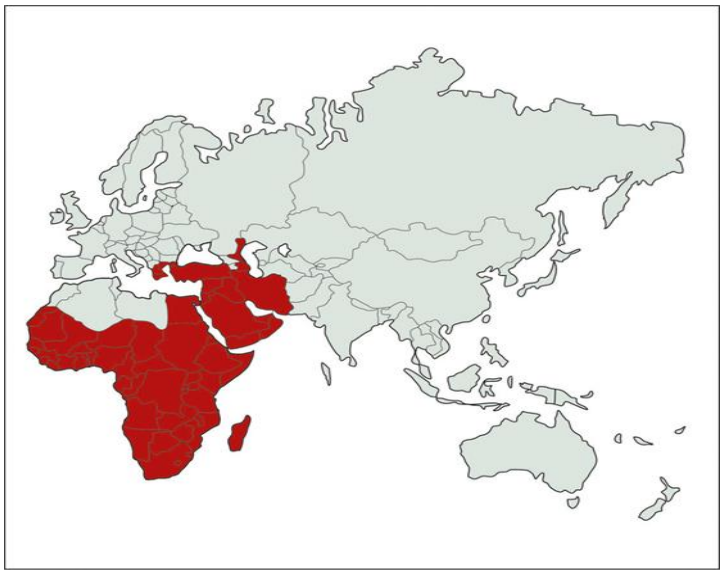

Figure 1.1: Geographical distribution of lumpy skin disease, a map showing endemic zones (in red), which are generally confined to Africa content with the potential of spreading to some Asian countries (Tuppurainen et al., 2015).

1.2.1 Aetiology and symptoms of LSD

All cattle breeds all over the world, including South Africa, can be affected by LSD. The disease usually occurs throughout the rainy summer and autumn months, once arthropod flies and insect populations are in abundance (APHIS, 2006).

For the study conducted in 2012 by Tuppurainen and Oura, the researchers found that the disease is mainly transmitted by blood biting insects and arthropod flies. The virus, which is closely connected to the family of poxviruses of sheep and goats, causes the eruption of skin (nodular skin lesions) on the animal's torso. Lumpy skin disease usually occurs on the Africa continent; however, an outbreak of the disease has occurred in portions of the Middle East (Tuppurainen and Oura, 2012). According to Tuppurainen and Oura (2012), the disease has different symptoms such as fever, nodular lesions on the skin and mucosal surfaces, lymph node enlargement, inflammatory and oedematous swelling of the legs and lameness as well as lachrymation. The development of skin nodules usually appears within 48 hours before the beginning of the fever. According to Molla et al. (2017), the sites of the lumps are the skin of

the head, neck, perineum, genitalia, udder, and limbs. Consistently, affected cattle will develop subcutaneous oedema of the ventral parts of the body, including the dewlap, brisket, limbs, udder, scrotum, and vulva. Oedematous and necrotic tissues in the udder may cause mastitis. In some animals, necrotic lesions located in the trachea and lungs may cause pneumonia (Molla et al., 2017).

1.2.2 Susceptible hosts to the disease

Lumpy skin disease is host specific and causes infection only in cattle (*Bos taurus* and *Bos indicus*), but several cases were also reported in Asian water buffalo (*Bubalus bubalis*) (Gari et al., 2011; Gumbe, 2018). A study conducted in Ethiopia on the susceptibility of different breeds (Holstein Friesian or crossbred cattle) to LSD, shows high morbidity and mortality once comparing to local zebu cattle.

It is also known that LSD is not a zoonotic, and many researchers have revealed that all age groups of animals (male and female) are at risk of getting the infection. The severity of the disease can be affected by the dose and route of virus inoculation (Gari et al., 2011). Actually, it is well-known that any genetic factor influencing the infection is not well documented, but *Bos taurus* breed is more susceptible to the diseases (Gari et al., 2011; Molla et al., 2018). It was also observed by Tageldin et al. (2014) that cows such as Holstein–Friesian, which is considered as high dairy producers, displayed many plain skin nodular lacerations when compared with indigenous breeds. The factors that influence the severity of the disease are not well understood (Tageldin et al., 2014). However, new-born calves, lactating cattle, and cattle suffering from starvation are susceptible to the disease because of weak immunity (Hunter and Wallace, 2001; Tuppurainen et al., 2013) High environment temperatures, together with farm management systems increase the production of milk, which could also influence on the severity of the infection in Holstein–Friesian cow (Molla et al., 2018).

Small ruminants (goats and sheep) seem to be relatively unsusceptible to infections even when they are in promiscuity with cattle during outbreaks. The LSDV DNA was detected in unspecified skin lesions from springbok (*Antidorcas marsupialis*), while wild animals such as Impala (*Aepyceros melampus*), giraffe (*Giraffa camelopardalis*) and Thomson's gazelle (*Eudorcas thomsonii*) developed symptoms of LSD after experimental inoculation. However, there are no reports of disease in these species during outbreaks in cattle. Anti-LSDV antibodies were detected in wildebeest (*Connochaetes* spp.), springbok, eland (*Taurotragus oryx*), impala, African buffalo (*Syncerus caffer*), giraffe, and other species (Gumbe, 2018).

1.3 Research problem

Agriculture is of extreme importance to the North West province. It contributes about 2,6% to the total GDPR and approximately 7% of national agriculture (North West province profile 2017). The most significant percentage of grazing land and cattle herds is concentrated in Vryburg and Mahikeng. In these districts, a wide range of livestock farming, which includes cattle, sheep, goats, and chicken farming, is practiced. This kind of farming contributes a substantial percentage to the economic growth of the area. However, several diseases have emerged, and there is difficulty in diagnosis as all these diseases are caused by numerous infectious agents. They cause inapparent or sub-clinical diseases whose effects are less visible, and they may only show by a reduction in the overall productivity of the cattle (Maropofela and Oladele, 2012). Lumpy skin disease is one of many infections known to cause economic loss and reduced productivity in livestock. This is due to decreased weight gain and permanent damage to hides (Hunter and Wallace, 2001; Coetzer and Tustin, 2004). The virus-induced condition is a notifiable one by the South African state and the World Health Organization for Animal Health (OIE, 2014). It has been revealed that the first cases of LSD in South Africa were reported in 1944 in the Marico District of the Western Transvaal, currently known as

North West Province (Hunter and Wallace, 2001). In 2000 and 2010 outbreak of LSD was recorded in Mahikeng with several attendant consequences (Maropofela and Oladele, 2012). Many cattle were adversely affected, and most of the farmers affected complained of low productivity as a result of the disease outbreak. Recently, from January to December 2018, 476 cases of LSD were reported in cattle reared in the MLM, 1578 cases were recorded in Ratlou and 10 cases were recorded in Ramotshere which gives a total of 2064 cases of LSD recorded in the Ngaka Modiri Molema District Municipality (DAFF, 2018a). However, little information exists on the identification of the virus using a molecular approach. Continuous movement, together with the introduction of cattle in the herd, may be considered as significant sources of the introduction of new strains of LSDV across villages. Small scale farmers in rural communities know little about the impact of the virus on animal health.

Without understanding the real impact of these diseases, it will be very challenging to explain skillfully and effectively the policies for their prevention and control. Irrespective of the awareness related to the occurrence of LSD, which impacts farmers negatively, information regarding the virus strain in the MLM is limited. This study, therefore, undertook to establish and generate an epidemiological understanding of the disease, which will contribute to the development of effective control measures. Also, information on the strain will be used to assess animal health and secure proper vaccine formulation.

1.4 Justification

Considering the importance of cattle in livestock as a major source of income, especially for rural communities, and its contribution to the national development plan against poverty, it is significant to identify the virus strain as well as to investigate the source of the disease. Such information is critical to validate the effectiveness of policies related to disease prevention, management as well as control. The virus strain causing lumpy skin disease in Mahikeng has

not been characterised. Also, there is not enough evidence on whether there is a mutation in the strain involved in the epidemiology of the disease. Therefore, there is a need to identify LSDV at the strain level in the MLM.

1.5 Aim

The study aimed was for the detection of LSDV from suspected infected cattle in selected villages within Mafikeng using serum-neutralization test, and also to confirm the presence of LSDV using RT-PCR and conventional PCR from skin biopsies.

1.6 Objectives

The specific objectives of this study were:

- To investigate LSD in MLM; and
- To characterize LSDV responsible for the disease

1.7 Research questions

- Which is the most common strain of LSDV in the MLM?

1.8 Hypothesis

Knowing that LSD is CaPV affecting cattle, this disease impacts negatively on livestock, causing severe economic losses, affecting as well the productivities of farms located in rural areas where access to veterinary services is scares. Furthermore, it is also acknowledged that the spread of LSD is predominantly associated with the increase of insect vectors and the movement of cattle. Therefore, it is predicted that LSDV may be circulating in livestock held by small scale farmers in rural areas within Mafikeng due to the exchange of bulls within the communities and the abundance of insect vectors.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

According to Chihota et al. (2001), LSD is a severe disease of cattle with substantial economic importance and endemic to Africa and the Middle East. The occurrence of the disease seems to be high during the rainy season, coinciding with times of biting flies abundance and wanes with the start of the dehydrated season (Chihota et al., 2001). In the 1959 Kenyan outbreak of LSD, there were reports of abundant populations of vectors such as *Aedes natronius* and *Culex miricus* (Burdin and Prydie, 1959; Ochwo et al., 2018). Similarly, the1989 Israeli outbreak of LSD is believed to have been the result of infected *Stomoxys calcitrans* being carried in the wind from Ismailiya in Egypt (Abdulqa et al., 2016; Yeruham et al., 1995). It was also shown that *Stomoxys calcitrans*, could mechanically transmit the virus between sheep in the laboratory (Baldacchino et al., 2013; Kononov et., 2019). It is also known that several poxviruses are mechanically transmitted through the bite of arthropods flies, including myxoma virus, whereby *Aedes aegypti* mosquitoes have been identified as a significant vector capable of transmitting LSD to susceptible cattle (Fenner et al., 1952; Sprygin et al., 2019). Mosquitoes have also been shown to mechanically transmit the Shope fibroma virus and fowlpox virus (Chihota et al., 2001).

According to Tuppurainen et al. (2015) taxonomically, members of LSDV are divided into two subfamilies: Entomopoxvirinae: poxviruses affecting insects and vertebrates and Chordopoxvirinae affecting several genera. Within the Chordopoxvirinae, the genus Capripoxvirus (CaPV), comprises LSDV, sheep pox virus (SPPV), and goat pox virus (GTPV). Lumpy skin virus disease virus thus belongs to the family Poxviridae, subfamily Chordopoxvirinae, genus CaPV (Tuppurainen et al., 2015). The subfamily Chordopoxvirinae

is additionally subdivided into ten genera that incorporate infections that cause havoc in domestic and laboratory animals. A substantial and increasing number of CaPV await precise taxonomic assignment and are presently not classified as indicated in Table 2.1.

Table 2.1: Poxvirus: geographical distribution of CaPV family (MacLachlan and Dubovi, 2010).

Genus	Agent	Host	Host Range	Geographical dissemination
Orthopoxvirus	Variola (smallpox)virus	Humans	Slim	Eradicated globally
	Vaccine virus	Swine, rabbits, Humans, Buffalo, Cattle	wide	Globally
	Cowpox virus	Domestic cats and large felids, cattle, rodents, humans, okapi, elephants, rhinoceros, mongoose, alpaca	wide	Europe and Asian countries
	Camelpox virus	Camels	Slim	African countries
	Ectromelia virus	Voles and mice	Slim	European countries
	Monkeypox virus	Squirrels, Humans, anteaters, great apes, monkeys	Wide	Central Africa and Westhern African countries
	Uasin gishu disease virus	Horse	-	Easthern African countries
	Tatera poxvirus	Gerbils (*Tatera kempi)*	-	Westhern African countries
	Raccoon poxvirus	Raccoons	wide	Northern America
	Volepox virus	Voles (*Microtus californicus)*	-	USA
	Skunkpox virus	Skunks (*Mephitis mephitis)*	-	Northern America
Capripoxvirus	Sheeppox virus	Goat and Sheep	Slim	Asia and Africa
	Goatpox virus	Sheep and Goats	Slim	Asia Africa
Cervidpoxvirus	Lumpy skin disease virus	Buffalo and Cattle	Slim	African countries
	Deerpox virus	Gazelle, Deer as well as reindeer,	wide	Northern American countries
Suipoxvirus	Swinepox virus	Swine	Slime	Globally
Leporipoxvirus	Myxoma virus, rabbit fibroma virus	Rabbits	Slime	Europe, Australia, America
	Hare fibroma virus	European hare	Slim	European Countries
	Squirrel fibroma virus	Eastern gray squirrel (*Sciurus carolinensis*)	Slim	Northen America
Molluscipoxvirus	Molluscum contagiosum virus	kangaroos, dogs and equids, nonhumans, primates, birds and Humans	Wide	Globally
Yatapoxvirus	Tanapox virus and Yabapox virus.	Humans and Monkeys	Slim	Westhern African countries

Avipoxvirus	Turkeypox viruses canarypox, crowpox, sparrowpox, starlingpox, Fowlpox virus juncopox, mynahpox, pigeonpox, psittacinepox, quailpox,	Different bird, turkeys and Chickens	Slim	Globally
Crocodylidpoxvirus	Crocodilepox virus	Crocodiles	Slim	African countries
Parapoxvirus	Orf virus	Humans, sheep as well as goat	wide	Globally
	Pseudo cowpox virus	Humans, Cattles	Slim	Globally
	Bovine popular stomatitis virus	Human, Cattles	Slim	Globally
	Ausdyk virus	Camels	Slim	Asian countrie and Africa
	Parapoxvirus of red deer Sealpox virus	Seals, humans and Red deer,	Slim Slim	Globally New Zealand
Presently unclassiffied	Carp edema virus	Common and koi carp (*Cyprinus carpio*)	Slim	European countries and Japean
	Salmonid gill poxvirus	Atlantic salmon S*salmo salar*)	Slim	Norway
	Squirrel poxvirus	gray squirrels, Red	Slim	North America . Europe

2.2 Morphology of LSDV

Pox virion appears by electron microscopy to be brick or oval-shaped with an estimated average length of 294±20 nm and width 262±22 nm, Figure 2.1 (Kitching and Smale, 1986). Based on the study conducted by Fenner et al., (2011) the virion encompasses more than a hundred polypeptides arranged in a core, two lateral bodies, an envelope as well as an outer membrane. In addition, the core of the virus is dumbbell-shaped, and the nature of lateral bodies is unknown. The core has proteins that include transcriptase and other enzymes (Fenner et al., 2011). Early electron microphotographs of the poxvirus (Figure 2.1) revealed that the viruses exist in the intracellular space, with or without an envelope and they are enveloped in the extracellular space (Fenner et al., 2011). Both forms are infectious and have the same core and genetic material. “Mature virions” (MV), also named “intracellular mature virions”, are bounded by one lipid membrane asymmetrically arranged with tubular proteins on the surface (Fenner et al., 2011). These forms of poxviruses are believed to be responsible for host-to-host

spread, intracellular enveloped virions (IEV) (Fenner et al., 2011). More recently referred to the intracellular enveloped virions "wrapped virions" develop from MV, surrounded by two additional layers of membrane, originating from the trans-Golgi apparatus or endoplasmic network (Moss, 2006). While budding out, the outmost layer of wrapped virions fuses with the plasma membrane, releasing extracellular enveloped viruses (EV) (Fenner et al., 2011). According to Woodroofe and Fenner (1962), all vertebrate poxviruses share a group-specific antigen (NP antigen) (Tuppurainen et al., 2017b; Woodroofe and Fenner, 1962).

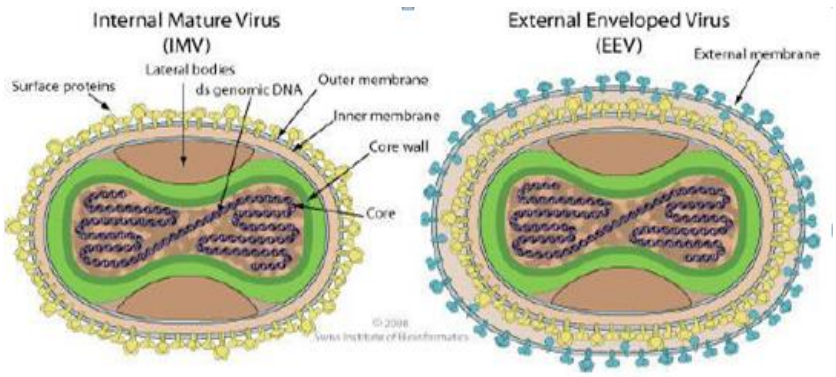

Figure 2.1: Morphological structure of LSDV (Gumbe, 2018).

2.3 Genomic structure of LSDV

According to King et al. (2012), the family of Poxviridae is characterized by substantial double - stranded DNA containing virions that are re-assembled in the cytoplasm of contaminated cells (cell cytoplasm). Virions are enormous (220-450 nm × 140-260 nm) and more often than not block moulded with the outside surface film containing lipid and showing rounded or globular protein structures (Health and Welfare, 2015; King et al., 2012). Each single virion holds a solitary straight genome that shifts in size (130-360 Kb) in light of the infection strain. The genomes are conservative, with open reading frames (ORFs) being firmly separated and non-covering with no proof-reading of mRNA grafting. Albeit individual strains may contain more

than 200 ORFs, just 50 are thought to encode proteins fundamental for viral interpretation, DNA replication, or the arrangement of new virions. The ORFs are clustered in the focal area of the genome and are very much monitored in grouping and position crosswise over various species. The remaining ORFs are conveyed more towards the terminal parts of the bargains that encode components and give harmfulness, tissue tropism, or serve to grow host extend.

According to the study conducted by Nelson et al. (2015), the poxviruses captured host genes during their evolution to evade immune detection and elimination. Furthermore, poxviruses adapt to changes in host defense by altering their existing repertoire of factors through the accumulation of point mutations, the occurrence of unequal crossovers giving rise to chimeric factors, or transient genomic expansions that increase the number of targets available for the mutation (Nelson et al., 2015). Based on the same study, the poxvirus family genomes are modified in response to evolutionary pressure; numerous poxvirus family's express signs of ORF duplication and divergence. These include the ankyrin-repeat proteins, the serpin family, the C7L family, the kelch-like proteins, and the Bcl-2-like proteins (Nelson et al., 2015).

In the study conducted by Tulman et al. (2001), it was found that LSDV contains a 151-kbp genome, which contains a coding region bounded by identical 2.4 kbp-inverted terminal repeats and contains 156 putative genes. When comparing chordopoxviruses of other genera to LSDV, it has been found that LSDV has 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. In addition, it was found that in the central genomic region, LSDV genes share a high degree of similarity and amino acid identity (average of 65%) with the genes of other poxviruses such as leporipoxvirus suipoxvirus and yatapoxvirus (Tulman et al., 2001). In contrast, in the terminal regions, the similarity is absent or share a lower percentage of amino acid identity (average of 43%) (Tulman et al., 2001). According to the finding of Tulman and other researchers (2001),

these differences include specific genes that seemed to be associated with viral virulence and host range (Tulman et al., 2001; Tulman and Rock, 2001). In terms of gene content and organization, LSDV shares some similarity with leporipoxviruses but it also contains homologues of interleukin-10 (IL-10), IL-1 binding proteins, G protein-coupled CC chemokine receptor, and epidermal growth factor-like protein, which are found in other poxvirus genera (Tulman et al., 2002). Based on Mesay (2018) and according to Tuppurainen et al. (2014), they demonstrate that LSDV is also very similar to SPPV and GTPV, sharing 96% nucleotide identity within the genus CaPV (Stram et al., 2008; Tulman et al., 2002). However, molecular studies have demonstrated that LSDV, SPPV, and GTPV are phylogenetically distinct (Mesay, 2018; Tuppurainen et al., 2014).

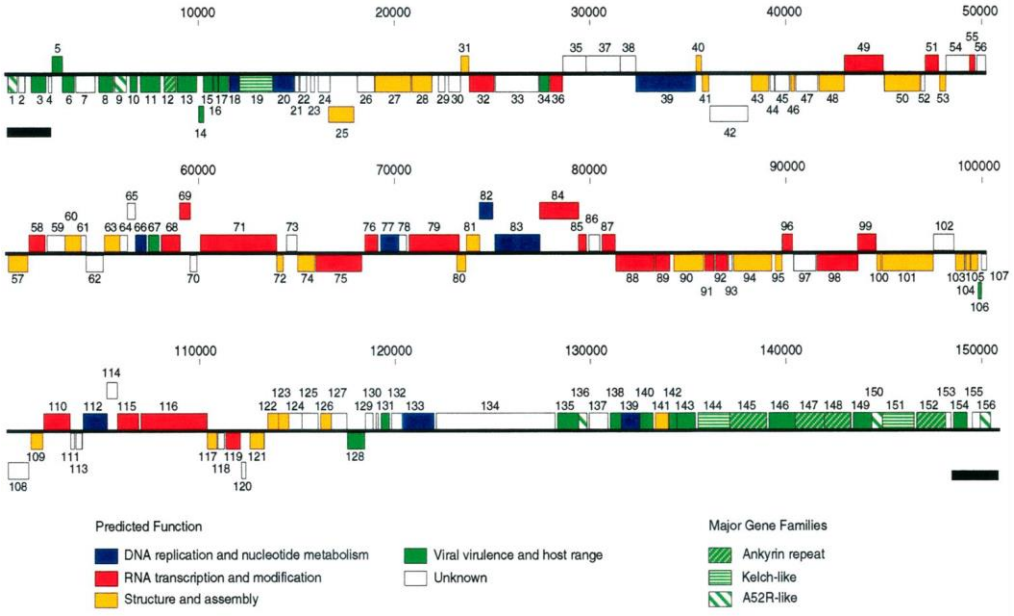

Figure 2.2: Linear map of the LSDV genome (Tulman et al., 2001).

The characteristics of the LSDV and its main effects are:

- Cytopathic effects and the presence of intracytoplasmic bodies in cell cultures (Tuppurainen, 2015; Weiss, 1968).
- Presence of visible lesions (pocks) in the chorioallantoic membrane of embryonated chicken eggs (Ali and Obeid, 1977; El-Kholy et al., 2008).

- Development of generalised nodular skin lesions in rabbits (Felsenstein, 2001).
- Morphological and antigenic resemblance with sheep and goat pox virus (Kitching and Smale, 1986).
- The presence of dsDNA (Weiss, 1968).

2.4 Environmental factors

The impacts of climatic factors are that cattle use common grazing areas and watering points, as well as the free movement of cattle from infected to uninfected areas or villages during the rainfall period, are some of the risk factors. The dissemination of LSD in different agro-climatic territories, along with the introduction of new cattle into the built-up herd and the nearness characteristic or fake water bodies, are among the other hazard factors that could encourage the spread of outbreaks in different villages. According to Hunter and Wallace (2001), It has been noted that the incidence of LSD is high during the rainfall period (summer/autumn) when insect activities are abundant, with a high impact and activity throughout summer/autumn, and it decreases or ceases during mild winter/winter (Hunter and Wallace, 2001).

2.5 Pathogen risk factor

Generally, it is well known that LSDV is resistant to dryness, survives cold and thawing environments. The virus is inactivated at 55°C after two hours and at 65°C after 30 mins. According to a study conducted by Gumbe (2018), it has been found that LSDV can be recouped from skin lumps kept at -80°C for a long time and tainted tissue culture liquid put away at 4°C for a half year. The infection is likewise present in nasal, lachrymal and pharyngeal emissions, semen, milk, and blood, and it might continue in spit for as long as 11 days and in semen for 22 days (Gumbe, 2018). It was also found that the virus is susceptible to alkaline, as well as to formalin (1%), chloroform (20%) substances, and some other disinfectants, such as sodium dodecyl sulphate. In addition, it shows susceptibility to phenol (2%) for 15 mins,

sodium hypochlorite (2–3%), iodine compounds (1:33 dilution), Virkon® (2%) and quaternary ammonium compounds (0.5%). According to Şevik and Doğan (2017), the virus remains inactive to sunlight and liquid containing lipid solvents, however, in a wet environment, it can remain viable for a long time (Annandale et al., 2014; Şevik and Doğan, 2017).

2.6 Immunity

Klimpel (1996) stated that the pathogenesis of the viral disease is brought about by explicit and vague systems. The study also found out that the actuation of various invulnerable capacities and the length and size of the safe reaction relies upon how the infection connects with host cells and on how the infection spreads (Klimpel, 1996).

Carn (1993), found out that the immunity to CaPV infections is mainly based on cell-mediated immunity still, according to Kitching and Smale (1986), the term cell-interceded resistance refers to the acknowledgment as well as executing of infection and infection tainted cells by leukocytes and the creation of various solvent elements (cytokines) by these cells when animated by infection or infection contaminated cells (Kitching and Smale, 1986).

According to Appleyard and Boulter (1973), they stated that antigens are presented to the organism's cells, fluids, and structures of resistance to infection, and the process are coordinated by the intracellular processing of the infectious agent by the cells of immunity. Based on the study conducted by Appleyard and Boulter (1973), the result showed that most of the subsequent infections stay within contaminated cells except for wrapped infections, which are discharged straight away into the circulatory system (Appleyard and Boulter, 1973). By spreading, starting with one cell, then onto the next cell, the irresistible infection is far from circling antibodies. Circling antibodies against CaPV can constrain the spread of the infection in experimental organisms. However, they do not obstruct its cooperation with host cells at the site of disease (Kitching and Smale, 1986). Three immunoglobulin classes (IgG, IgM, and IgA) have antiviral activity. It is also known that the activity of viruses may be neutralized by these

antibodies (IgG, IgM, and IgA) by producing aggregation, consequently preventing adsorption of infection to cells and decreasing the chances of infecting new cells (Gari et al., 2010).

Animals that recover from a CaPV disease develop long-lasting resistance that protects them from ensuing reinfection with any CaPV (Milovanović et al., 2019; Varshovi et al., 2018). Cattle that were inoculated utilizing indigenous infectious weakened strains by the sequential subculture of infection in tissue culture usually are sheltered and give long haul security (Roitt and Delves, 1992). The immune status of a recently tainted or inoculated animal cannot be determined with serum levels of antibodies (Kitching and Smale, 1986).

According to the study conducted by Al-Salihi (2014), young calves receive maternal antibody through colostrum that confers on them immunity and makes them resistant to the infection for six months (Al-Salihi, 2014). Always, cattle infected with lumpy skin virus clear the infection, and there is no carrier state for the disease (Tuppurainen et al., 2017b). It was observed during a study that LSD leads to 40–50% of the infected cattle that developed nodular skin lesions all over their body. The remaining animals either created confined and constrained agonizing growing at the immunization site of LSD or demonstrated no clinical indications separated from a fever response (Weiss, 1968).

According to Varshovi et al. (2018), numerous vaccines for CaPV are applied for the control and prevention measures of LSVD (Varshovi et al., 2018). These injections are live attenuated CaPV strains which include: Neethling strain of LSDV, Kenyan sheep and goat poxvirus (KSGPV), theYugoslavian strain of sheep poxvirus (YSPV), Romanian strain of sheeppoxvirus (SPV) and Gorgan strain of goatpox virus (GPV) (Kitching, 2003; Gari et al., 2015).

2.7 Transmission

An outbreak of LSD is most likely associated with a high insect vector population and with the upcoming rainy season (Magori-Cohen et al., 2012). Watering points and communal grazing

areas have been pointed out to be linked with the incidence of LSD (Gari et al., 2012; Seyoum and Teshome, 2018). The transmission of the disease can either be direct or mechanical:

Direct - occur in the cutaneous lesion, saliva, milk, respiratory fluids, and semen following infection.

Mechanical- Blood feeding by arthropods has been acknowledged as one of the major modes of transmission of the diseases (Carn and Kitching, 1985; Molla et al., 2017). Three blood-sucking arthropods, *Aedes aegypti*, mosquitoes, and *Stomoxys calcitrans* flies, have been involved in the spread of the LSDV from infected to susceptible cattle (Gumbe, 2018). This happens after the bite of mosquitoes that had fed earlier (2 to 6 days) on an infected lesion (Chihota et al., 2003).

According to Lubinga (2014), some specific ticks, such as *Rhicephalus appendicularatus* (brown ear tick), *Amblyoma hebraeum* (blont tick), and *Rhicephalus decoloratus* (blue tick) are considered as vectors of viruses (Lubinga, 2014). His study showed that the virus could be determined in these ectoparasites in the middle of epidemic periods (Lubinga, 2014). Lumpy skin virus was originated from the biting organs of ticks, and it could probably overwinter in ticks (Lubinga, 2014).

2.8 Risk factor associated with the occurrence of LSD

The study conducted by Rehman et al. (2017) shows that the incidence of LSD varies in diverse agro-ecological zones, depending on the modifications in the husbandry system and the size of the herd. The results of the study were in agreement with those of Ayre-Smith (1960) and (Brenner et al., 2006) as stated by Gari et al. (2010) and (Hailu et al. (2014) they observed that the incidence of LSD in Ethiopia was high in the midland and lowland agro-climates as compare to highland region agro-climates. In that study, it was also found that the occurrence of LSD was linked to the presence of anthropods, herd density at watering points and grazing,

wet seasons, farm management systems, agro-ecologic conditions, and introduction of new animals to an area that was not tested.

2.9 Pathogenesis

There has been limited research investigating the pathogenesis of LSD in cattle (El-Kenawy and El-Tholoth, 2011). Intradermal inoculation of cattle with LSDV results in the development of a localized swelling at the site of inoculation after four to seven days, followed by an enlargement of the regional lymph nodes (Mulatu and Feyisa, 2018). The eruption of skin nodules with congestion, haemorrhage, oedema, and necrosis frequently appears 7 to 19 days after inoculation (Mulatu and Feyisa, 2018). According to Abera et al. (2015b) and Gumbe (2018), it has been observed that the skin nodules may exude serum primarily, but the development of secondary bacterial infections is common within the necrotic tissue (Abera et al., 2015b; Gumbe, 2018). In the generalized form, there is initial febrile reaction and viremia, and these signs occur after two weeks (Vorster and Mapham, 2008). Viral replication in pericytes, endothelial, cells and probably, other cells in the blood and lymph vessel walls causes vasculitis and lymphangitis in some vessels, and infarction may result in severe cases (Coetzer and Tustin, 2004).

2.10 Clinical signs

The characteristics of symptoms have been defined in detail by numerous researchers (Coetzer, 2004; Babiuk et al., 2008a). Concisely, in most cases, the first indication of infection is lachrymation and fever (40-41°C), but in some cases, fever is not-febrile. Shortly after the beginning of the fever, skin nodules (1-5cm in diameter) become more apparent, in varying numbers, from only a few to multiple lesions covering the entire organism, as shown in Figure 2.4. Following skin nodule appearance, sub-scapular and precrural lymph nodes become noticeably enlarged (Tuppurainen and Oura, 2012). In strictly infected cattle, ulcerative lesions

appear in the mucous membranes of the oral and nasal cavities as well as eyes, causing extreme lachrymation, nasal discharge, and salivation. Usually, these secretions carry the virus (Babiuk et al., 2008a; Babiuk et al., 2008b). In most cases, clinical signs are skin nodules on the whole body and swollen superficial lymph nodes, especially subscapular (Just cranial to the point of the shoulder) and precrural lymph nodes (situated in front of the leg).

According to Wainwright et al. (2013), these skin nodules can also affect the genital mucosa, and their numbers may range from a few to several hundred. The virus can also affect the oral, ocular, and nasal areas of the organism. Lesions on the skin might resolve fast or may indurate and persist and become hard lumps or become sequestrated to leave deep ulcers partly filled with granulation tissue, which frequently suppurates (Wainwright et al., 2013). The papules are most seen in hairless areas of the udder, inner ear, perineum, eyelids, and muzzle (Babiuk et al., 2008a). According to the finding of CFSPH (2008), the papules may lead to the growth of ulcerative lesion with extreme lacrimation, nasal discharge, and salvation, which may contain the virus. Cows at the peak of lactation experience a decrease in milk production due to high fever (40-41°C) and secondary bacterial infection causing mastitis. Necrosis can occur in the upper respiratory tract of the cattle, and the debris may be inhaled, causing pneumonia. Stenosis of the trachea may occur following the healing of a lesion with scar tissue formation a few weeks or even months after the disease (CFSPH, 2008).

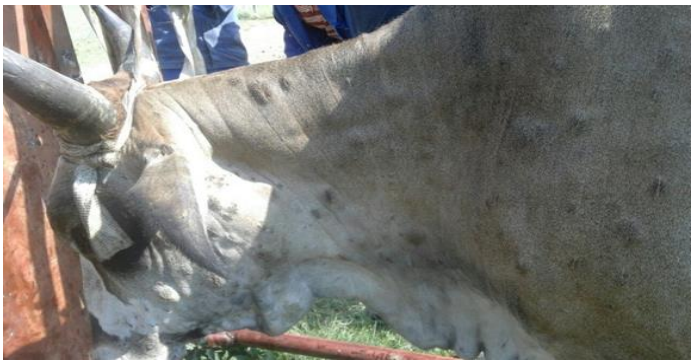

Figure 2.3: Typical clinical symptoms of LSD. The picture shows nodules along the neck on the cow (Northwest University clinic, 2018).

2.11 Diagnosis

Currently, there are no diagnostic test kits commercially available for the LSD virus. The diagnostic methods that exist are a tentative diagnosis of the disease based on typical clinical and differential diagnosis along with laboratory validation of the presence of the virus or antigen (OIE, 2010). The gold standard method for the detection of lumpy skin antigen and antibody are virus neutralization tests and electron microscopy examination, respectively (Tuppurainen et al., 2011).

Serological screening might not be very complex to detect mild and long-standing lumpy skin antibodies in immunized cattle. ELISA has been implemented with limited success (Tuppurainen et al., 2011). An indirect fluorescent antibody test (IFAT) can also be used as a diagnostic method for LSD. However, the test requires long procedures and may be more costly as compared to the ELISA technique (Gari et al., 2008). Lumpy skin disease can be confirmed using conventional PCR or RT-PCR/qPCR methods (Mafirakureva et al., 2017; Radostits and Gay, 2007). An experimental study conducted in the evaluation of different methods of diagnostic of LSD revealed that PCR more reliable in demonstrating viral DNA from a skin biopsy and in blood. But it does not consume time when using it compared to other time-consuming methods (Tuppurainen et al., 2005).

- **Different diagnosis**

Although skin diseases are characteristic of LSD, they also occur in other diseases of cattle. The disease can be confused and misdiagnosed with cowpox virus infection, dermatophilosis, pseudo cowpox, vaccinia virus or besnoitiosis, rinderpest, demodicosis, *Hypoderma bovis* infection, photosensitisation, urticaria, insect or tick bites, bovine herpes virus, bovine papular stomatitis cutaneous tuberculosis and onchocercosis (Abdulqa et al., 2016).

Epidemiological data of the areas affected can assist in differentiating LSD from other skin lesions (Tuppurainen et al., 2017a). A final analysis may only be established by the identification of the virus from samples of skin lesions.

2.12 Laboratory confirmation

Early detection of the virus is crucial to start appropriate control measures. Several conventional PCR, as well as RT-PCR techniques, are accessible for the detection of viruses (OIE, 2010). In addition, techniques such as direct immunofluorescence, virus neutralization, or ELISA and PCR assays can be used to confirm the presence of the LSDV.

Serological findings might, from time to time, be hard to interpret due to small antibody titres in vaccinated animals during mild infection. Although the virus neutralization test is not appropriate for large-scale testing, numerous ELISA methods for screening of lumpy skin antigen or antibody were published, but currently, none of them is commercially available on indirect antibody ELISA based on inactivity (OIE, 2010).

According to EL-Kenawy and EL-Tholoth (2011), electron microscopic demonstration of virus in negatively stained preparation of biopsy specimens taken from affected skin/ mucous membrane tissue sections can also be used to demonstrate the activities of the virus in acute and chronic skin lesions.

2.13 Histopathology

Based on the finding of Ahmed and Zaher (2008), histopathology might be considered as an important tool used to exclude viral, bacterial, or fungal causes of nodular development in cases of lumpy skin disease and characteristic cytopathic effects.

Skin nodules are round and raised; some are blended to form a large irregular and circumscribed plaque. The intersection of the nodules shows reddish-grey surface and contain

serous fluid in the subcutis layer where oedema develops. Sit fasts develop when resolved lesion appears. The necrotic alimentary lesion may be seen on the body of the animal (Davies, 1991a). The nodules are about 10-30 mm diameter in the kidney and 10-20 mm diameter in the lungs. Strictly, infected cattle may present secondary bacterial pneumonia (Davies and Otema, 1981; El-Neweshy et al., 2012; Kumar, 2011).

- **Histological findings**

According to EL-Neweshy et al. (2012), histologic modifications in all severe cases consist of acute ballooning degeneration of the epidermis, furunculosis, lymphoplasmacytic dermatitis, with severe vasculitis touching the dermal capillaries, venules, and arterioles. In the area around the nodular lesions, cells are inflamed and infiltrated by macrophages, lymphocytes, and eosinophils. Large epithelioid and macrophage type cells are seen on the epidermis and dermis of infected animals. In early lesions and older lesions, plasma cells and lymphocytes are present, and red cells are predominated by the polymorph nuclear leucocytes (El-Neweshy et al., 2012).

2.14 Treatment

It is well known that up to now, there is no appropriate treatment against LSD. Infected animals are removed from the group, and supportive care (antimicrobial therapy) is given to prevent secondary bacterial infection. In addition, insecticide sprays and wound dressings have been used to reduce flystrike from attacking the wound (Abutarbush et al., 2013; Abutarbush, 2017; Agianniotaki et al., 2017a). However, the treatment of LSD does not ensure full recovery of the animal suffering of lumpy skin disease; consequently prevention is likely to be significant to prevent considerable economic loss linked to skin deteriorations, decreasing of milk production due to mastitis and loss of animal product due to death (Mulatu and Feyisa, 2018).

2.15 Control/Prevention

Vaccination of healthy animals is reported to be the most effective means of prevention of LSD in both endemic and non-endemic areas (Ayelet et al., 2014). Currently, only live vaccines are commercially available against LSD, with different vaccines licensed for use in different countries (Klement et al., 2018). Capripoxvirus vaccine strains including Kenyan sheep and goat pox virus (KSGPV) O-240 and O-180 strains, Romanian SPP, and Gorgan goat pox (GTP) strains Yugoslavian RM65 sheep pox (SPP) strain, and LSDV Neethling strain, are commercially available (Abutarbush, 2017). The Neethling strain was reported to offer cross-protection (Mulatu and Feyisa, 2018). However, experiences during the outbreaks in 1990/91 have challenged the affirmation that the Neethling strain confers life immunity to LSD. Vaccination and an extra administration of a vaccine after an earlier dose (booster) over a period of two to three years will significantly decrease the chances and occurrence of clinical disease. According to OIE, two distinct vaccines have been extensively and successfully applied for the prevention of LSD in Africa (Hunter and Wallace, 2001).

- Heterologous live attenuated virus vaccine (sheep or goat pox vaccine). This vaccine can occasionally cause some severe reactions to the cattle.
- Homologous live attenuated virus vaccine (Neethling strain). Two Neethling virus-based vaccines, Bovivax LSD-N, and Lumpyvax™, are licensed for use in the country.

2.16 Sanitary prophylaxis

According to Brown and Torres (2008) and Kahn (2005), the spread of disease can be controlled by the restriction of movement of infected livestock, carcasses, skin, and semen. Lumpy skin disease can also be controlled by strict quarantines of infected animals, elimination of infected and exposed cattle, proper discarding of carcasses (incineration), as well as washing and disinfection of the sites. The use of insecticides, together with repellents, can aid in the control of infected vectors.

2.17 Biosecurity

Considering how LSD spreads and the risk of contamination of the environment, it is important that improved biosecurity measures be put in place. This includes clinical surveillance implemented to detect cattle presenting with symptoms of LSD. Control measures should also be followed and maintained at the abattoir. Early detection of LSD, good biosecurity, and prompt reporting are important aspects in controlling the spread of the disease. Furthermore, any concerns about potential LSD in the animal herd, or carcass should be reported as soon as possible to the closest State veterinary services (Abera et al., 2015a; Davies, 1991b). Assessments of the environment, carcass or live animal will be used to advise government on the risk level of LSD in the country and also inform the consideration of preventative controls (Rweyemamu et al., 2000). Should there be the risk of the spread of LSD into the country increase, the Government would inform stakeholder organisations to allow them to consider appropriate preventative measures (Abera et al., 2015a; Rweyemamu et al., 2000).

2.18 Economic impact of LSD

Lumpy skin virus is responsible for some of the most financially critical ailments of local ruminants in Africa and Asia, and the OIE classifies LSD on the A-List of diseases. The disease displays wide variations in clinical representation that range from sub-clinical infection to death (Elhaig et al., 2017). Clinical indications are presented as skin knobs covering the neck, back, tail, perineum, rear legs, and genital organs; fever is also present in most of the cases. In a few animals, superficial lymph node enlargement may be observed with lameness and can also be present as well as oedema of the limbs. Abortions and temporary or permanent infertility occur among affected cattle. Emaciation and a long convalescence period can significantly decrease the growth rate in beef cattle (Weiss, 1968).

Morbidity may range from 2% to as high as 85%. Herd mortality is, however, low, ranging from 1% - 5%. However, mortality of 40% was recorded in some cases (Davies, 1991a).

Cattle are particularly susceptible to LSD during the peak lactation period, which affects milk production. Mastitis, together with high fever, usually affects the productivity of dairy cows. According to Weiss (1968), severe orchitis might result in brief or changeless fruitlessness among contaminated creatures. Anorexia, can likewise essentially diminish the body weight in beef cattle (Weiss, 1968). Abortion may follow infection in approximately 10 percent of pregnant cows. Moreover, nodular skin sores leave lasting scars, which lead to a reduction of the market estimation of skins and covers in the cowhide business (Tuppurainen, 2015).

In the Middle East, direct financial effects brought about by LSD in dairy cattle farming were estimated to be between 45 and 65% (Kumar, 2011).

Based on a survey conducted by Mdlulwa and Klein (2015) along with Ntombimbini and Klein (2015) in 12 villages in Limpopo province in Marble Hall the Ephraim Mogale District, in South Africa on the incidence of LSD between 2010 and 2012, the studies revealed that the mortality cases based on diagnosis were 19 cattle valued at R123 500. However, the survey respondents reported that 68 cows were lost due to LSD, resulting in a revenue loss of R442 000 (Mdlulwa and Klein, 2015; Ntombimbini and Klein, 2015).

The study also revealed that the mortality rate was always low (1-3%) but may occasionally reach 40% (Tuppurainen and Oura, 2012). It was pointed out that treatment and vaccination costs of livestock may also lead to financial loss (Hailu et al., 2014). If the disease is not properly controlled in the endemic areas, it affects the income in the farming business. According to Miller et al. (2014), the mortality and morbidity of the disease rely upon the type of cattle, the immunological status of the populace, and bug vectors engaged with the transmission.

The study conducted by Alemayehu et al. (2013) and Rich and Perry (2011) showed that the probability of the acquisition of LSD in the market chain through exchanged livestock is always high. In Ethiopia, the financial analysis showed that the total mortality and morbidity due to LSD at the animal level were between 4.5% and 21.2% and at herd level were 24.3% and 82.3%, respectively. A large proportion (92.2%) of the animal owners indicated that LSD affected the cattle business. A median loss of USD 375 (local Zebu; USD 325 and Holstein-Friesian local Zebu cross cattle; USD 1250 was estimated per dead animal. Furthermore, a median loss per affected lactating cow was found to be USD 141 (local Zebu cows; USD 63 and Holstein-Friesian local Zebu cross cows; USD 216 (Rich and Perry, 2011). According to Al-Salihi (2014) and Abutarbush et al. (2015), the economic losses are because of starvation, diminished or suspension of milk creation, low weight gain, premature birth, myiasis and lasting harm which causes to decrease the activities of the business (Abera et al., 2015b; Abutarbush et al., 2015; Al-Salihi, 2014).

The costs of cattle illness can be grouped into direct costs, which include the death of animals due to the disease, and indirect costs, which consist of prevention costs, losses in the market, and other revenues (Oxford-Analytica, 2012; Rushton, 2009). Understanding the effect of animal infection and assessing its losses may help policymakers and farmers to weigh the losses against the costs of disease control each at their level (Pritchett et al., 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

More than 60 villages surround the MLM. Villages were grouped into clusters. A cross-sectional study was conducted in different villages within the Mahikeng municipality from October 2017 to April 2018 to assess the prevalence of LSD in cattle. Due to the huge size of areas of assessment, the assortment of villages was applied through random sampling to decrease sampling errors that frequently occur on or after intrinsic unpredictability among samples drawn from a large population. In total, four clusters were used to accomplish the set level of precision for the estimation of the prevalence of LSD. Their orientations were dependent on cardinal directions from Mafikeng town; East-West, North-South.

Herd selection was based on a purposive sampling method. In each cluster, 25 cattle showing symptoms of LSD, such as fever, the eruption of skin nodules, superficial lymph node enlargement, oedema of the limbs, and brisket together with lameness, suspected animals were also targeted for sample collection. Samples were collected on the various breed (Brahman, Bonsmara, and indigenous breed) during community outreach trips organized by the Animal Health Department, North West University.

Furthermore, sampling was done during the rainy season because of the high incidences reported. During this period the biting-fly populations were substantial and decreased during the dry season (Gari et al., 2010).

3.2 Research area

The research was conducted in rural areas of the Mahikeng Local Municipality, home to Mahikeng, the capital city of the province. The MLM is one of the six Local Municipalities of

the Ngaka Modiri Molema District in the North West province. The area is located close to South Africa's border with Botswana, with coordinates of 25.8560° S, 25.6403° E, temperature 24°C, Wind N at 8 km/h and 48% humidity (Marks, 2017). The vegetation type in all the areas resembles that of a semi-arid. The temperature ranges from 3℃ to 39℃ and can drop to -2℃ during winter nights (Bureau, 1986). The annual rainfall ranged from 300 mm to 700 mm from November to March (Snyman, 1998).

3.3 Selection of villages

The department of Statistics' list of all villages in MLM was used to determine the number of villages used to form a cluster for sampling. From a total of 62 villages found in Mafikeng, five villages were randomly selected for laboratory data collection. The selected villages are located in the North, South, West, and East relative to Mahikeng (the exact villages were as follows: Masuthle 25˚48'16" S, 25˚25'07" E; Meetmekaarr (bethel) 26°03'34" S, 25°39'22" E; Tswaing 26° 37'29" S, 25°45'11" E; Six hundred 25˚42'32" S, 25˚37'04" E; Lokaleng 25˚80'69" S, 25˚59'77''E.

3.4 Sample collection

Based on the statistic of DAFF (2018a), the total population of the communal cattle in the MLM was estimated to be 3 486. The sample size was obtained after consultation with the State Veterinary Services on the population size of bovine in communal areas of Mafikeng. A statistician was consulted, and the formula below was used:

$$n= z^2 \times p\,(1-p)/\varepsilon^2$$

Where:

z =Z score
ε = error margin
n= population
p= population preparation

A maximum, estimated number of animals to be sampled was 100. A total of 200 samples (100 blood samples and 100 skin biopsies) were collected from different villages shown in Table

3.1. Blood samples were collected from all the 100 (n=100) cattle that showed clinical signs of LSD infection. Skin biopsies (n=100) were taken randomly from cattle.

Table 3.1: Sampling areas and number of samples.

Municipality	**Sampling areas**	**Nature and number of samples**
MLM	Masuthle	SN= 20; BS=20
	Meetmekaarr	SN= 20; BS=20
	Tswaing	SN= 20; BS=20
	Six Hundred	SN= 20; BS=20
	Lokaleng	SN= 20; BS=20
Total	5	SN= 100; BS=100

SN: Skin nodule; BS: Blood sample

In the current study, in some samples, there was a challenge of extracting a high concentration of DNA. This made it impossible to obtain or detect a sufficient amount of DNA material from some skin biopsy samples. However, high-quality DNA concentration was obtained in 16 samples.

The sample collected in this study was small due to the reason mentioned hereunder:

- Most of the participant was not willing to agree to the biopsy of skin nodules. Secondly,
- In some cattle, the size of the nodules was slightly raised above the surrounding normal skin. This made the farmers reluctant about the procedure (skin nodule biopsies).

The procedures were safe and straightforward to perform; occasionally, veterinarians come across complications such as wound infection and bleeding. All the samples were aseptically collected and immediately placed in a cooler box and transported to the Microbiology laboratory at the Animal Health Department, Mafikeng Campus, North-West University, for further analysis.

3.5 Ethical consideration

An ethical clearance application was submitted and approved by the Animal Ethics Committee, North West University. All the farmers involved in the study signed a consent form following a sufficient explanation of the project.

The information obtained from this study were stored as confidential for the researcher and remained the property of the North West University. Only authorized persons such as management or supervisors may access the data.

3.6 Cattle handling

During outreach clinics, the community members would bring their cattle in the morning (7h:00-9h:00). The primary concern was the safety of the cows and the researcher. Animals were handled with patience and tolerance with due allowance for their natural behaviour. Young calves were carried, and they were penned with adults. Upon arrival, cattle were housed in the Department of Agriculture, forestry, and fisheries (DAFF) kraals. In the communities where the kraal and crush pens do not exist, communal kraals and crush pens were used for animal handling.

3.7 Medical history and clinical examination of animals

A veterinarian undertook sample collection, and the following information was recorded before sample collection: visible skin lesions, size of the lymph nodes and fever, farming style, condition score (BCS), drugs, and vaccine used.

3.8 Sampling

- **Blood**

Blood was collected from the jugular or tail (caudal) vein using an 18 to 20-gauge sterile needle, and blood collection tubes (10 ml) with or without anticoagulant (EDTA) were used to store it. For each animal, different needles and different labelled tubes were used.

- **Skin nodule biopsy**

Skin nodule samples were taken from every animal from which blood samples were previously collected, using 2% Lignocaine as local anaesthesia to reduce pain. On the other hand, any animal presenting signs of discomfort during incision were subjected to a sedative such as Domosedam. The incision of the nodule was done using a sterile surgical scalpel blade, scissors, as well as forceps and biopsy samples, were taken aseptically. Following incision, the wound was sutured with 2-0 Ethicon Vicryl, and the area around the wound was sterilized. Furthermore, wound spray was used to help prevent the contamination of the wound by flies. Skin biopsy was put in cryo-tube (1.8 ml) and kept at -80 °C for future analysis.

3.9 Transport and storage

Samples were placed in a cooler box filled with an ice block and transported to North West University, Animal Health Laboratory for further analysis. Blood samples without anticoagulant were centrifuged at 3000 rpm for 10 min for serum separation; the aliquots were transferred into 1.5 µL sterile Eppendorf tubes and were kept at cool temperatures (below 4^{o}C) to prevent spoilage. For long term storage, the blood was aliquoted, and the nodular lesion samples were stored at -80^{o}C.

3.10 Serological Diagnosis

- **Serum neutralization test (SNT)**

The virus neutralization test is an accurate gold standard method and is considered the most specific test for the detection of antibodies against LSD (Samojlović et al., 2019). The protocol includes the use of a known dilution of specific neutralizing antiserum mixed with various dilutions of the homologous and suspect virus and subsequent observation of the ability of the virus to produce a cytopathic effect when inoculated onto a sensitive cell line (Tuppurainen, 2017).

The test is relatively low-cost using standard laboratory equipment; however, the assay is not sensitive enough to identify each animal that was in contact with the virus, and it cannot be used in national reference laboratories operating in low-level biocontainment facilities. In addition, it requires live virus and cell cultures, more time and labor, and skills (Samojlović et al., 2019; Tuppurainen, 2017).

This test was performed following the protocol of the Agriculture Research Council (ARC) Onderstepoort (Department of Veterinary Tropical Diseases) (Park et al., 1999). Briefly, 0.5 mL of blood was inoculated into confluent monolayer containing 96-well, flat-bottomed cell culture microtitre plate. Test sera were diluted 1:5 in MEM containing 5% foetal calf serum and gentamycin (0,05 mg/ml) and inactivated at 56^{o}C for 30 min. A series of two-fold dilutions of the inactivated test serum were prepared, and a 100 µL of serum was added to the wells. The titre of the LSDV to be used was determined, and 100 µL of a 100 TCID50 was added to each well. For the control, 200 µL of MEM was added to 12 wells. For the antigen control, three ten-fold dilutions of antigen (100 TCID50) were made, and 100 µL of each dilution was added to 100 µL of MEM in each well. The microtitre plate was incubated at 37 °C for 1 hour. Following incubation, 80 µL of bovine dermis cells at a concentration of 480 000 cells/ml were

added to the wells. The microtitre plates were then placed in an anaerobic incubator containing 5% CO_2 at 37°C. Using an inverted microscope, the monolayers were examined daily for evidence of cytopathic effect (CPE). The cell control indicated how long the cells remained viable and for how long it was possible to read the test before cell degeneration. The results were interpreted accordingly.

3.11 Molecular Identification

3.11.1 Genomic DNA Extraction

Skin nodule tissue was used in this study for DNA. The extraction of genomic DNA was performed using Qiagen DNA Blood and Tissue kit according to the manufacturer's instructions. Skin nodule tissue was grounded to a fine powder by using a chilled mortar and pestle. Then the crushed tissues were put into 15 ml centrifuge tubes. After that, 180 µL of ATL buffer and 20 µL proteinase K were added, mixed using a vortex and incubated at 56℃ until completely lysed. The mixture products were vortexed occasionally during incubation. After the incubation period, the mixture products were vortexed for 30 secs. Thereafter, 200 µL of AL buffer was added, mixed systematically using vortex and followed by the incubation of the samples at 56℃ for 10 min.

Following incubation, 200 µL of ethanol (70%) was added and mixed carefully using a vortex. The products were transferred into a DNeasy Mini Spin column using a pipette and placed inside a collection tube (2 ml). Then the product was centrifuged at 6 000 x g (8 000 rpm) for one min. Thereafter, the flow-through from the collection tube was discarded. The column was placed in a new collection tube (2 ml), and 500 µL of AW1 buffer was added and centrifuged for 1 min at ≥ 6000 x g. After centrifugation, the flow-through was discarded. The spin column was placed in a new collection tube (2 ml), and 500 µL of AW2 buffer was added and centrifuged for 3 min at 20,000 x g (14 000 rpm). The flow-through was discarded. The spin-

column was moved to a new microcentrifuge tube (1.5 ml or 2 ml), and 200 µL of AE Buffer was added to the centre of the spin column membrane to elute the DNA. The microcentrifuge tubes were incubated for one min at room temperature (15-25℃), followed by centrifugation for one min at 6000 x g. The microcentrifuge tubes were kept at -80ºC for additional analysis.

3.11.2 Real-Time PCR/ qPCR Testing

The real-time PCR/qPCR assay was performed as a screening method for the detection of LSDV in the samples. It was performed at the ARC-Onderstepoort. Specific primers of CaPV revere and forward were used as according to Balinsky et al. (2008). Positive control, probe, and primers, as well as other materials used in this study, were provided by ARC (Onderstepoort), as shown in Table 3.2.

Table 3.2: Primers used in RT-PCR/qPCR.

Primer/probe/PTC	Sequence
Forward primer	5'-TCC-GAG-CTC-TTT-CCT-TAC-TAT-'3
Reverse primer	5'-TAT-GGT-ACC-TAA-ATT-ATA-TAC-GTA-AAT-AAC-'3
Probe A	5'6FAM-CAATGGGTAAAAGATTTCTA- MGBNFQ '3
Positive template control	5'ATG GCG ATG TCC ATT CCC TGA CCA ATG GGT AAA AGA TTT CTA TCG TAA CAG ATG AAA GAG CAA GCT ACT ATT CCT CAC GGA AAT GAA ATG CTT C '3

The assay was performed according to Balinsky (2008), a volume of 5 mL of DNA template was added to 15 mL of Master Mix (Thermo Scientific, Johannesburg, South Africa) for a total amount of 20 mL. All qPCR assays were performed using 3 replicates along with appropriate controls, including a positive amplification control (LSDV; type SA-Neethling) and a no-template control TE buffer.

The samples were run on a Thermal cycle (C1000 Touch TM, Bio-Rad, California, USA) using the thermocycler protocol, according to Balinsky et al. (2008). Before sample testing, the positive template control was serially diluted from1pg/mL to 0.001fg/mL and run on the

RAPID PCR instrument (T100TM Thermal cycler, Bio-Rad, California, USA). The resultant cycle threshold vs. log of the positive template control concentration graph gave as the slope of 3.76, corresponding to 92% amplification efficiency; this was enough for going ahead with the screening of samples (Balinsky et al., 2008).

3.11.3 Polymerase chain reaction assay

The CaPV genome was subjected to PCR amplification using six pairs of primers targeting four regions comprise GPCR (LSDV-011), LSDV- 022, LSDV132, and TK (LSDV-066) genes as shown in Table 3.3. The GPCR (LSDV011) and LSDV066 genes are characterized and, at the same time, conserved in the evolutionary line; hence LSDV032 and LSDV022 gene were selected to determine also the similarity of the isolates to LSDV since LSDV032 and LSDV022 genes are found within the genome of the virus.

Table 3.3: Primer set for PCR.

Primer	Annealing	target
GpCrf (5'-TAATGTTCGTATAAATGTAATAAAG- '3) GpCrfr (5'-TTACAAATCCAACAATGAGT-'3)	58°C	GPCR gene (LSDV-011)
GpCf (5'-AAGTAAAGCATAACTCCCACA-3') GpCrfr (5'-TTACAAATCCAACAATGAGT-3')	55°C	GPCR gene (LSDV-011)
LSD022 (5'-CATCATTTGAAACACTGTCGTC- '3) LSD022 (5'-GGAAAAGCAATATGAAAAAGGC-'3)	58°C	LSDV- 022 gene
L132F (5'-CAC TTC CCT CCT TTT AAG C-'3) L132R (5'-CAT TCT ACA ATC TCC ATG CG-'3)	55°C	LSDV gene (LSDV132)
OP3 (5'-CAC CAG AGC CGA TAA C-'3) OP49 (5'-GTG CTA TCT AGT GCA GCT AT-'3)	52°C	TK gene (LSDV-066)

The above-indicated set of primers were designed by the Agricultural Research Council - Onderstepoort, Pretoria. They were synthesized by Inqaba Biotech, Pretoria, South Africa to amplify 1.5kb region of the GPCR, LSDV- 022, LSDV132, and TK (LSDV-066) gene. The

amplification of sequences was done with dideoxy sequencing chemistry and ran on an automated DNA sequencer (Applied Biosystem, Prism 377).

The amplification reaction was applied in a total volume of 25 µL containing: 12.5 µL Terra PCR direct mixes (Clonetech Takara, SA), 6.5 µl DNA free water, 1 µL of 25 Mm of forward and reverse primers and 5 µL of the DNA template. The template control materials (280 and 279) were also included for each reaction, and negative controls comprised of water control. Amplification was performed in a programmable thermocycler (T100TM Thermal cycler, Bio-Rad, California, USA) as follows: One cycle of: 95°C for 4 min followed by 40 cycles of: 95°C for 30 secs, 55°C for 30 secs, 72°C for 30 secs and a final extension at 72°C for 10 min and a holding temperature of 4°C until taken out of the thermocycler.

3.11.4 Agarose gel electrophoresis

The PCR products were analyzed with a 5 kb ladder (Gene-foci, USA) as a molecular marker on 1.5% high-resolution agarose gels for 1 hr. at 100 V. The gel was stained in 1 µg/ml ethidium bromide (Bio-Rad Laboratories, Canada) and the expected positive PCR products of 1540 bp (for pair 1 primers), 1203 bp (for pair 2 primers), 237 bp (for pair 3 primers), 492 bp (for pair 4 primers) and 442 bp (for pair 5 primers) were visualized on a 1.5% agarose gel for all of the 16 samples. The amplicons were visualized using a gel doc (Gel Doc^{TM} XR^{+}, Bio-Rad, California, USA).

3.11.5 DNA Sequencing and phylogenetic analysis

The amplified PCR products were sent to Inqaba Biotech, Pretoria, South Africa, for sequencing. The GPCR (LSDV-011), LSDV-022, LSDV-132, and TK (LSDV-066) sequences from the 16 results from Inqaba Biotech were analyzed with Bio-edit software. Then sequences were aligned with those of reference virulent and vaccine CaPV strains in the GenBank. Multiple alignments of the GPCR (LSDV-011), LSDV-022, LSDV-132, and TK (LSDV-066)

sequences were created using the Clustal W program (Invitrogen) and the Bio-Edit software packages (Altschul et al., 1990; Thomspon, 1994). Nucleotide sequence similarities and dissimilarities were computed using Meg-Align software (DNA STAR® Lasergene® version 7.2, USA). The evolutionary analyse was based on the neighbour-joining method and conducted in the Mega 4 software package (Saitou and Nei, 1987). The genetic distances between the nucleotide sequences were computed using the Kamura-2-parameter model (Kimura, 1980), and the gaps were deleted from the analysis. A bootstrap confidence analysis was performed with 1,000 replicates (Felsenstein, 2001). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The branches corresponding to partitions that reproduced less than 50% bootstrap replicates were deleted.

3.12 Data analysis

The results were analyzed using SPSS (The Statistical Package for the Social Sciences) version 23.0. Bar charts, pie charts, and histograms were used to summarize the percentage frequencies of Cochran's Q test, which were implemented to determine the differences between the different areas of samples collected and the occurrence of positive and negative cases. To determine the association between variables such as areas of collection and presence of positive cases, Pearson Chi-Square was used.

CHAPTER FOUR

RESULTS

4.1 Village level sero-prevalence

A total of 100 blood samples from cattle were examined to investigate the incidence of LSD in the MLM. These animals showed signs of skin lesions or skin diseases secondary to other systemic diseases such as nasal discharges, depression, and skin nodules in different areas of the animal's body as well as fever (Figure. 4.1). The antibody responses of the infected cattle were assessed using SNT, and the titres were detectable (between 1:4 and 1:32). The test also showed that 67% of samples were positive for LSDV while the rest were negative (33%), as described in Figure 4.2.

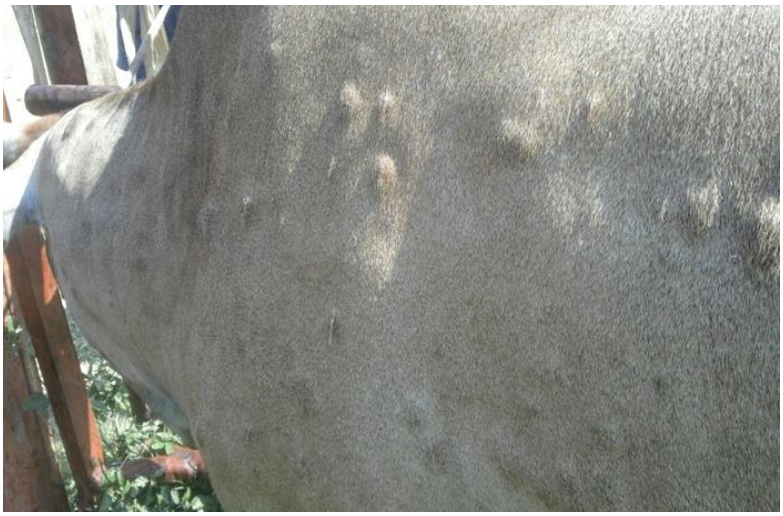

Figure 4.1: Cattle infected with LSD reveals multiples nodules. The picture was taken during the clinical examination of the animal.

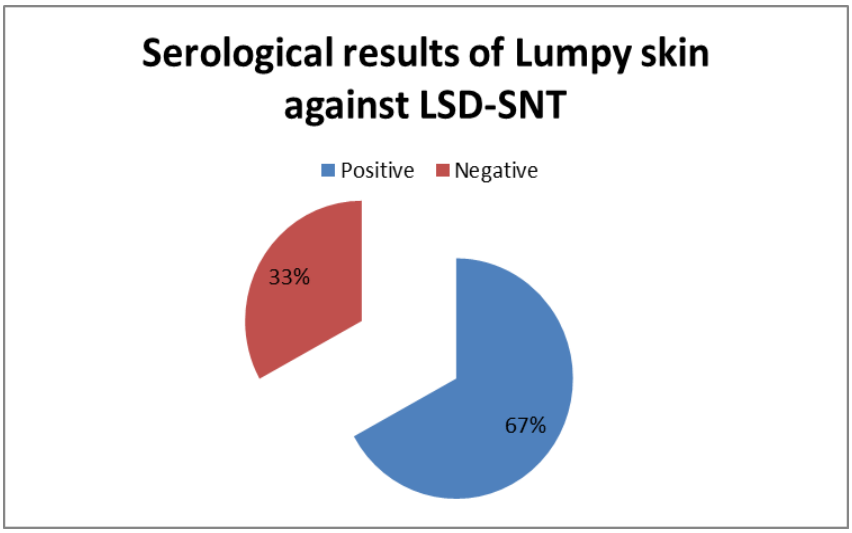

Figure 4.2: Overall representation of LSD in the MLM.

The overall incidence of LSD in each village showed that most positive cases were recorded in Masutlhe (95%, i.e., 19/20) followed by Tswaing (74%; 15/20) and Meetmekaar (58%; 11/20) respectively (Figure 4.3a, b, and c). Fifty percent were recorded in Lokaleng (50%; 10/20) and 29%; 5/20 in Six Hundred (Figure 4.3d and e). The seroprevalence values collected in different villages revealed that more than 50%; 10/20 of the animals (n=20) tested positive to LSD, whereas Lokaleng village showed a low percentage (Figure 4.3d).

Figure 4.3a results showed that 95% (19/20) of cattle from Masutlhe tested positive to LSD while 5% (1/20) tested negative. Furthermore, in Meetmekaar village (Figure 4.3c), the results showed that 58% (11/20) of cattle tested positive to LSD, while 42% (8/20) were found to be negative to the test. The samples collected from the village called Six Hundred were subjected to the SNT test, and the results showed that 29% (5/20) of cattle tested positive to LSD, whereas 71% (14/20) were negative, as shown in Figure 4.3e. Tswaing village shows that 74% (15/20) tested positive for the disease, whereas 26% (5/20) of cases were negative, as presented in Figure 4.3b.

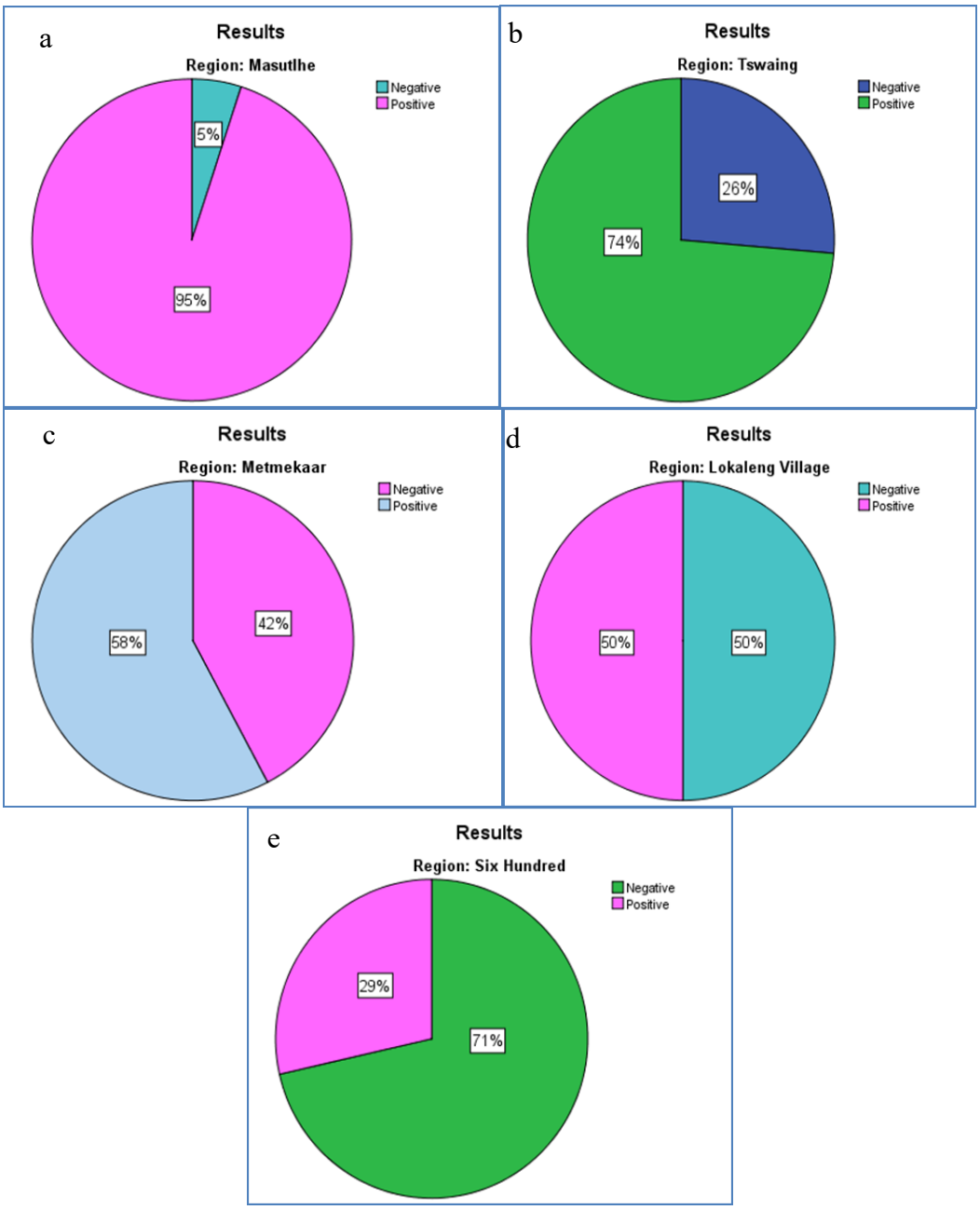

Figure 4.3 (a, b, c, d and **e):** Percent seroprevalence of LSD at the animal level in Lokaleng, Masutlhe, Metmekaar, Six Hundred and Tswaing villages.

Pearson Chi-Square test results between the five villages of the municipality revealed that there was a significant difference between the incidence of LSD in the villages (Table 4.1), whereby P was less than 0.05 ($P<0.05$).

Table 4.1: Comparison of results by area using the Chi-square test of association between the region and results.

Chi-Square Tests			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.862	4	.012

4.2 Molecular assay

4.2.1 Detection of LSDV using RT –PCR/ qPCR assays

A total of 100 samples (skin nodule biopsy) were collected and screened using RT- PCR for the detection of LSDV. Out of 100 samples, 16 samples showed a sufficient amount of DNA material from a skin biopsy. The positive percent of RT-PCR for skin biopsy was 16%; 16/100, as presented in Table 4.2 and Figure 4.4.

Table 4.2: Real-time PCR/qPCR testing results.

Sample references	Sample types	Test	Results
M1F	Skin nodule biopsy	LSDV RT-PCR	+
M4F	Skin nodule biopsy	LSDV RT-PCR	+
Black	Skin nodule biopsy	LSDV RT-PCR	+
S1	Skin nodule biopsy	LSDV RT-PCR	+
Tan	Skin nodule biopsy	LSDV RT-PCR	+
Brown and White	Skin nodule biopsy	LSDV RT-PCR	+
Meetmekaar Leopar	Skin nodule biopsy	LSDV RT-PCR	+
M Black	Skin nodule biopsy	LSDV RT-PCR	+
M2F	Skin nodule biopsy	LSDV RT-PCR	+
MSF	Skin nodule biopsy	LSDV RT-PCR	+
M Brown	Skin nodule biopsy	LSDV RT-PCR	+
Black Tan	Skin nodule biopsy	LSDV RT-PCR	+
Black and Brown	Skin nodule biopsy	LSDV RT-PCR	+
Black White	Skin nodule biopsy	LSDV RT-PCR	+
M2	Skin nodule biopsy	LSDV RT-PCR	+
M1	Skin nodule biopsy	LSDV RT-PCR	+

+ = positive

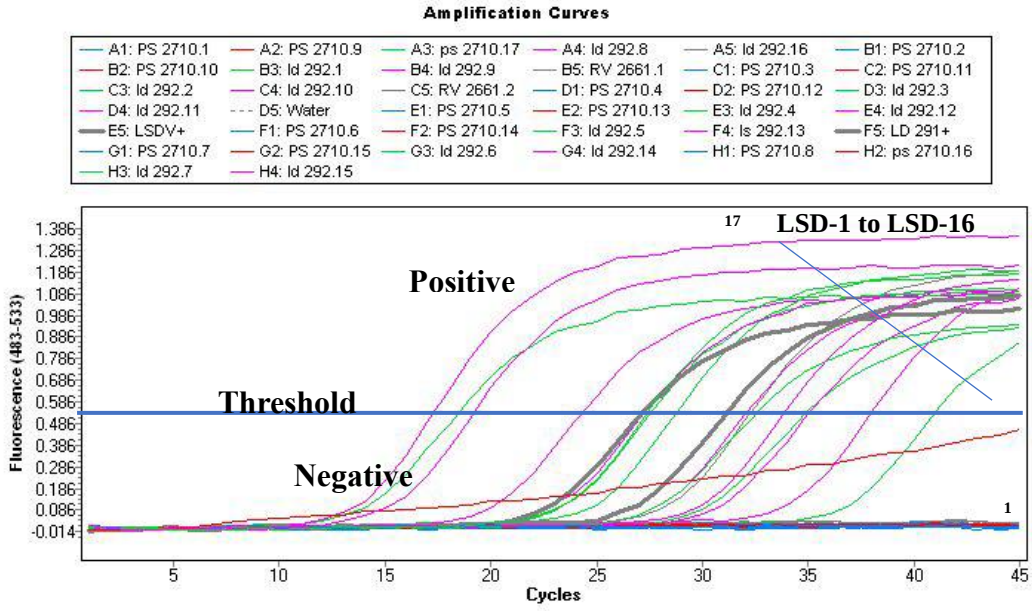

Figure 4.4: RT-PCR/qPCR amplification results.
Keys: LSD-1 to LSD-16 is a positive amplification. Curve 17: Ct of positive control DNA of LSDV. Curve 1: DNA-free sample negative control.

4.2.2 Conventional PCR analysis

Genomic DNA product was amplified a 1.5 kb region of the GPCR (LSDV-011), and samples LSD-2, LSD-3, LSD-5, LSD-9, and LSD-15 were submitted for sequencing with both primers used during the PCR. The expected amplicon size was 1540 bp. Primer CrF-R produced good sequence, but there was a homopolymer with primer CrF-F at position 240 bp. Based on this, a new primer GpCf was designed.

With the newly designed primer, all the samples (LSD-1-16) were positive (showed bands), and the amplicon size was 1203 bp. The PCR products were submitted for sequencing. Unfortunately, samples LSD-1, LSD-2, LSD-3, LSD-4, LSD-6, LSD-7, LSD-8, LSD-10, LSD-11, LSD-12, LSD-14, and LSD-16 were all non-specific sequences for LSDV but contained DNA from cattle genome (Figure 4.5 and Table 4.3).

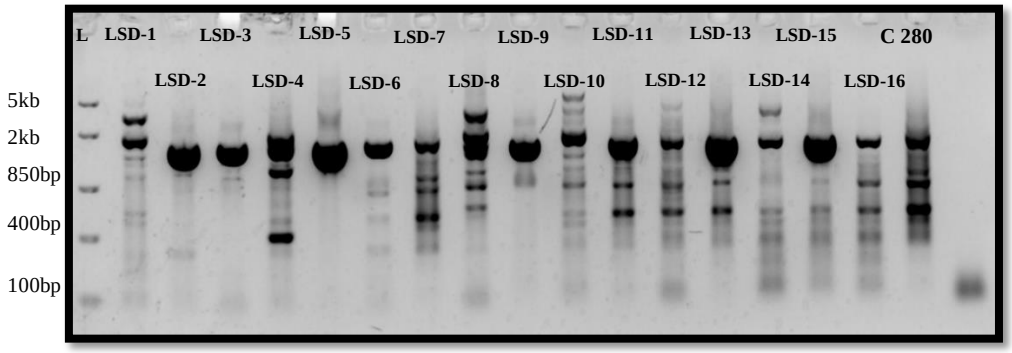

Figure 4.5: Gel electrophoresis pattern of PCR products for LSDV using primer GpCf.
Keys: L represents the 5 kb ladder; C280 represents the control, and numbers LSD-1 - LSD-16 represents different LSDV.

The third primer set LSD022f, and LSD022r targeting LSDV-022 gene, samples LSD-1 to LSD-16 were submitted for sequencing, and the primer produced good sequences. Unfortunately, LSD-1, LSD-4, LSD-6, LSD-7, LSD-8, LSD-10, LSD-11, LSD-12, LSD-14 and, LSD-16 were all non-specific sequences for LSDV but contained DNA from cattle genome (Table 4.3). The amplicon size was 237 bp (Figure 4.6).

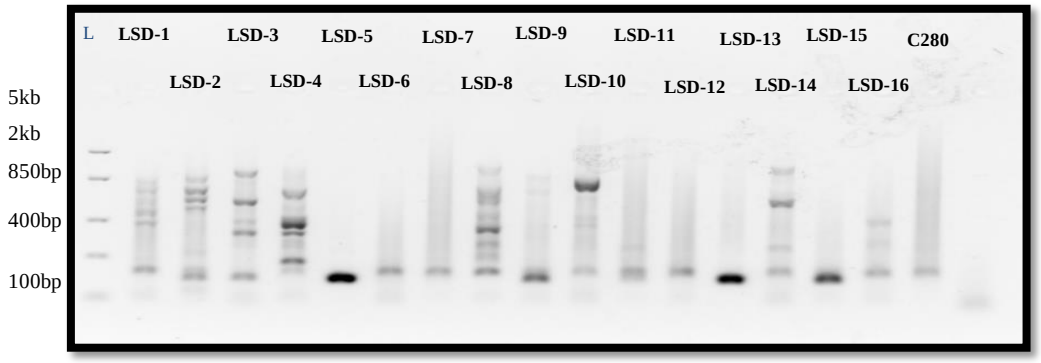

Figure 4.6: Gel electrophoresis pattern of PCR products for LSDV using primer LSD022. Keys: L represents the 5 kb ladder; C280 represents the control, and numbers LSD-1-LSD-16 represents different LSDV.

Using the fourth primer set L132F and L132R, only sample LSD-13 produced an amplicon (492 bp) (Figure 4.7); unfortunately, the PCR product was not sequenced for the following reasons: low throughput of the method used and time gap between amplification and sequencing. These factors contributed to the PCR product to degrade substantially the PCR products could not pass the quality control test.

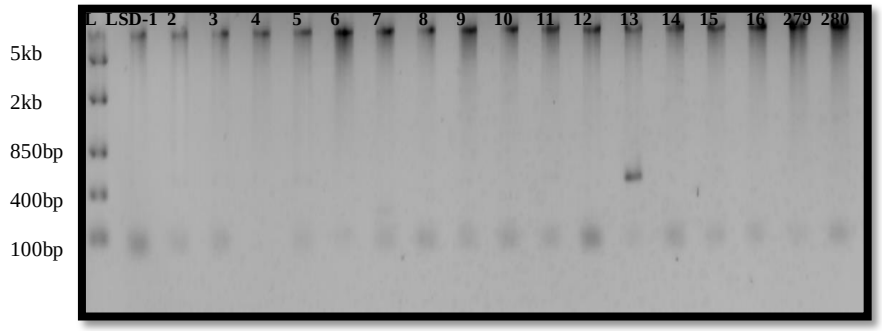

Figure 4.7: Gel electrophoresis pattern of PCR products for LSDV using primer L132.
Keys: L represents the 5 kb ladder; C 279-280 represents the control, while numbers LSD-1-13 represents different LSDV.

Furthermore, primers OP3f and OP49r targeting the TK gene (LSDV-066) showed that samples LSD-2, LSD-3, LSD-4, LSD-5, LSD-6, LSD-7, LSD-8, LSD-9, LSD-11, LSD-13, LSD-14, and LSD-15 produced bands and the PCR products were submitted for sequencing. The amplicon size was 400 bp, as indicated in Figure 4.8. Unfortunately, samples (LSD-3, 4, 6, 7, 8, 9, 11, 13, and 14 were all non-specific sequences for LSD but contained DNA from cattle genome (Table 4.3). Samples LSD-2, LSD-5, LSD-9, LSD-15 and LSD-280 (control) were included in maximum likelihood phylogenetic tree

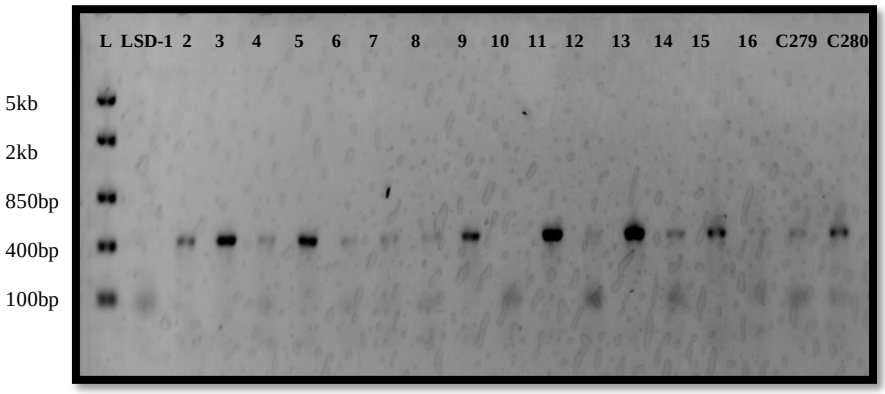

Figure 4.8: Gel electrophoresis pattern of PCR products for LSDV using primer OP
Keys: L represents the 5 kb ladder; C279-280 represents the control, while numbers LSD-1-16 represents different LSDV.

4.2.3 LSDV genotypes

Positive PCR product samples (showing bands) were sent to Inqaba Biotechnology, Pretoria, South Africa, for sequencing. Following the sequencing, the nucleotide sequences were edited, subjected to blast analysis, and the results showed no genetic differences between the isolates, which were sequenced. The sequences were confirmed to be LSDV isolates (Appendix A). However, it was consistently the same samples that were managed to obtain amplicons and sequences. For comparative purposes, more CaPV gene sequences were retrieved from the GenBank and included in the data, as shown in Table 4.3 and Table 4.4.

Table 4.3 Identity of presumptive LSDV strains isolated from skin nodules biopsy of infected cattle in Mahikeng.

S/N	Isolate code of organisms identified	Reference from NCBI database	Percentage similarity (%)	Acc. No. in GenBank (PCR)
1	LSD-1-GPC	Bos taurus isolate CDY472 mitochondrion	99%	MN200938.1
2	LSD-2-GPC	LSDV 155920/2012	89%	KX894508.1
3	LSD-2-OP3	LSDV Kenya	96%	MN072619.1
4	LSD-2-22-LSO22	LSDV 155920/2012	96%	KX894508.1
5	LSD-3-22-LSO22	LSDV 155920/2012	98%	KX894508.1
6	LSD-3-22-LSO22	LSDV 155920/2012	98%	KX894508.1
7	LSD-4-GPCF	Bos taurus isolate CDY341 mitochondrion	99%	MN200889.1
8	LSD-5_GPCF	LSDV/Russia/Dagestan/2015	90%	MH893760.2
9	LSD-5- OP3	LSDV 155920/2012	98%	KX894508.1
10	LSD-5-22-LSO22	LSDV 155920/2012,	99%	KX894508.1
11	LSD-5-GPCF	LSDV 155920/2012	98%	KX894508.1
12	LSD-6-GPCF	Bos taurus CDY472	99%	MN200938.1
13	LSD-7-GPCF	Bos taurus isolate CDY341 mitochondrion	99%	MN200889.1
14	LSD-8-GPCF	Bos taurus isolate c847 mitochondrion	98%	MK028735.1
15	LSD-9-GPCF	LSDV/Russia/Dagestan/2015	94 %	MH893760.2
16	LSD-9-OP3	LSDV 155920/2012	97%	KX894508.1
17	LSD-9-GPCF	LSDV 155920/2012	99%	KX894508.1
18	LSD-9-LSO22	LSDV Sudan/06 Obied	96	FJ869369.1
19	LSD-10-GPCF	Bos taurus c847 mitochondrion	99	MK028735.1
20	LSD-11 -GPCF	Bos taurus c847 mitochondrion	98%	MK028735.1
21	LSD-12-GPCF	Bos taurus isolate CDY472 mitochondrion	99%	MN200938.1
22	LSD-13-LSO22	LSDV 155920/2012	100%	KX894508.1
23	LSD-13-GPCF	LSDV RSA/00 OP126402	92%	FJ869374.1
24	LSD-13-GPCF	LSDV 155920/2012	98%	KX894508.1
25	LSD-14-GPCF	Bos taurus isolate CDY472 mitochondrion	99%	MN200938.1
26	LSD-15-GPCF	LSDV/Russia/Dagestan	93%	MH893760.2
27	LSD-15-OP3	LSDV Kenya	97%	MN072619.1
28	LSD-15-22-LSO22	LSDV 155920/2012	100%	KX894508.1
29	LSD-15-GPCF	LSDV 155920/2012	98%	KX894508.1
30	LSD-16-GPCF	Bos taurus isolate CDY472 mitochondrion	99%	MN200938.1

Table 4.4: Details of the isolated LSDV and CaPV reference strains retrieved from GenBank whose sequence was analyzed and compared in the current study.

Isolates	Country of isolation	Year of isolation	GenBank accession number
LSD-2-RSA-2018	South Africa (Mahikeng)	2018	-
LSD-3-RSA-2018	South Africa (Mahikeng)	2018	-
LSD-5-RSA-2018	South Africa (Mahikeng)	2018	-
LSD-9-RSA-2018	South Africa (Mahikeng)	2018	-
LSD-13-RSA-2018	South Africa (Mahikeng)	2018	-
LSD-15-RSA-2018	South Africa (Mahikeng)	2018	-
LSDV	Russia Dagestan	2015	MH893760
155920	Israel	2012	KX894508
Bujanovac	Serbia	2012	KY702007
Evros	Greece	2015	AF829023
KSGP-0240	Kenya	1974	KX683219
NI-2490	Kenya	1958	AF325528
NW-LW Warmbaths	South Africa	1999	AF409137

4.2.4 Phylogenetic and sequence analysis

Phylogenetic reconstruction of the full-length GPCR gene was done to evaluate the phylogenetic relatedness between new LSDV obtained from this study as well as other CaPV isolates from GenBank, and this was done using MEGA 7. The relatedness was based on more than 60% similarity of the profile. The analysis was of the full-length GPCR (LSDV-011), LSDV-022 gene, LSDV-132 gene and TK gene (LSDV-066) sequences of the 16 study isolates collected from the infected animals categorized under the LSDV as shown in Figure 4.9, 4.10 and 4.11. The evolutionary relationship was evaluated using a maximum-likelihood phylogenetic tree. It is evident that the genomes of these members are highly conserved (63%-89%) sequence identity. Cluster showed two groups, the first composed of vaccine viruses and the second of field isolates (Figure 4.9, 4.10, 4.11).

The following samples were included in the GPCR gene (LSDV-011) tree: LSD-5_RSA-2018, LSD-9_RSA-2018, LSD-13_RSA-2018, and LSD-15_RSA-2018 (Figure 4.9). Sequence analysis of the GPCR region clustered LSDV isolated from this study with the FJ869377

(Egypt-Isamalia 18/1989, KR024780 Turkey-02/2015, KY829023 Evros/GR/15, and FJ 869375 RSA/06-D 19353-16 (Figure 4.9).

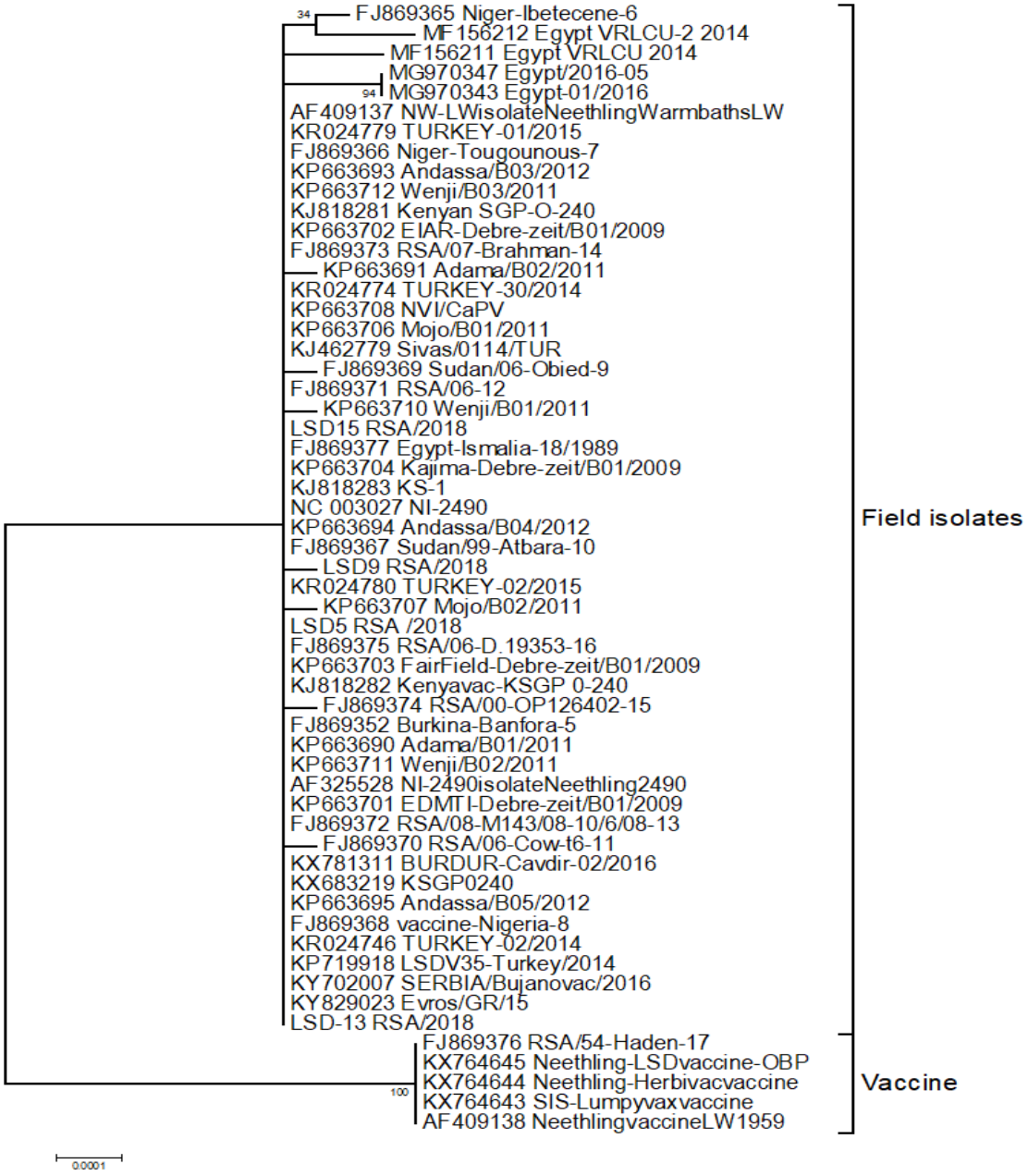

Figure 4.9: Phylogenetic tree based on the GPCR gene (LSDV-011) constructed using Maximum likelihood. General Time Reversible model, Bootstrap: 1000, Gamma Distributed (G):4, 1025-1037 bp in alignment. The analysis involved 53 nucleotide sequences retrieved from the GenBank.

The samples which were included in the second tree (LSDV-022) were as follows: LSD-2_RSA-2018, LSD-3, LSD-5_RSA-2018, LSD-9_RSA-2018, LSD-13_RSA-2018, and LSD-15_RSA-2018 (Figure 4.10). Sequence analysis of LSDV-022 gene clustered LSDV isolated from this study with MH893760 LSDV Russian Dagestan 2015, KX894508 155920-Israel 2012 and AF 409137 NW-LW Warmbaths RSA 1999 (Figure 4.10).

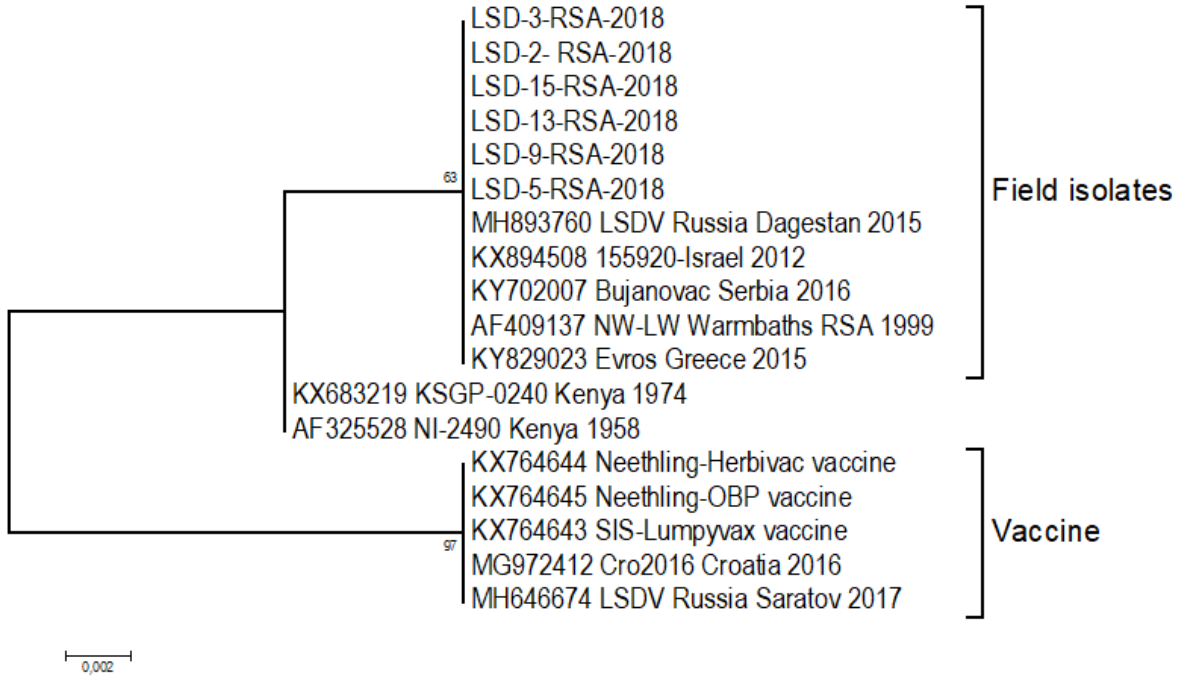

Figure 4.10: Phylogenetic tree constructed based on LSDV-022 gene, using Maximum likelihood. General Time Reversible model, Bootstrap: 1000, Gamma Distributed (G):4, 387-406 bp in alignment. The analysis involved 12 nucleotide sequences retrieved from the GenBank.

The samples which were included in the third phylogenetic tree (TK gene; LSDV-066) were as follows: LSD-2_RSA-2018, LSD-5_RSA-2018, LSD-9_RSA-2018, LSD-15_RSA-2018, and LSD-280_RSA-2018 (control) (Figure 4.11). Sequence analysis of the TK gene (LSDV-066) clustered LSDV isolated from this study with KY829023 Evros/GR/15, KX894508 155920-Israel 2012 and AF 409137 NW-LW Warmbaths RSA 1999 (Figure 4.11).

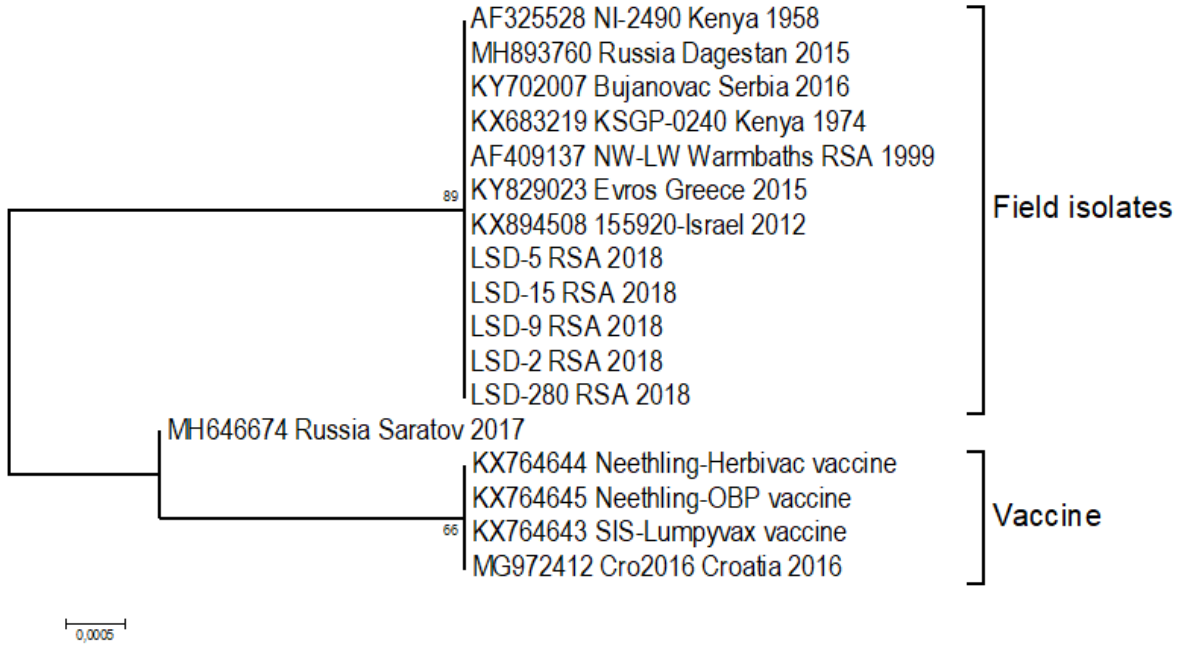

Figure 4.11: Phylogenetic tree constructed based on the TK gene (LSDV-066), using maximum likelihood. General Time Reversible model, Bootstrap: 1000, Gamma Distributed (G):4, 387-390 bp in alignment. The analysis involved 12 nucleotide sequences retrieved from the GenBank.

All these isolates clustered with field isolates, and the low amount of genetic diversity is observed across all the sequences obtained from the National Center for Biotechnology Information NCBI. The only difference is between field isolates and vaccine viruses.

CHAPTER FIVE

DISCUSSION

It is well known that LSD is present in different regions of Africa and causes a lot of economic losses (Elhaig et al., 2017; Tuppurainen et al., 2015). The disease was observed primarily on cattle in South Africa by the end of 1944. The first cases were noted in the Marico District of the Western Transvaal, currently known as North-West Province, where it was identified as 'knopvelsiekte' (Afrikaans for lumpy skin disease) (Thomas and Mare, 1945). Thereafter the disease LSD disseminated to the previous Orange Free State, Natal, Western Cape, and Transkei. Through this time, approximately 8 million cattle were estimated to be affected. In 2000 and 2010, LSD was recorded in Mahikeng with several attendant consequences (Maropofela and Oladele, 2012). Many animals were affected, and the farmers complained of low productivity as a result of the disease outbreak. In 2018, 476 cases of LSD were reported in cattle reared in the MLM, 1578 cases were recorded in Ratlou, and 10 cases were recorded in Ramotshere 2064, which gives a total of 2064 cases reported in the Ngaka Modiri Molema District Municipality (DAFF, 2018a). Considering the previous reports on the incidence of LSD in cattle, it is evident that this disease is a cause of severe economic losses in livestock and continues to cause health problems in the cattle industry in the North West province. However, information on the strain circulating in the mentioned territory is scarce.

The diagnosis of LSD is mostly focused on the symptoms observed like generalised skin nodules, ocular discharge, oedema of the depended on parts as well as lameness (Body et al., 2012; Salib and Osman, 2011). Nevertheless, clinical diagnosis of LSD sometimes presents a few problems, because the primary skin lacerations might be confused with other skin infections of cattle (Maclachlan and Dubovi, 2010).

In this study, the blood and skin nodules were collected in the middle of winter and during the summer season in 2018. This period is linked with the peak period for the development of blood-feeding arthropod populations, which are considered as vectors (Awad et al., 2010).

The overall seroprevalence of LSD in the targeted population was 67%, more than half of the cattle considered for this study. This incidence is near to the 52, 6%, as reported by Molla et al. (2018) and 64% in cattle population, as observed by Gari et al. (2012). The same authors also reported that the high prevalence might be attributed to the increased number of blood feeding arthropods (vectors), high number of cattle around the watering point and communal grazing field, farming systems, rain seasons, agricultural management, and the introduction of new cattle without diagnostic test for LSD (Gari et al., 2010; Tuppurainen and Oura, 2012; Van den Bossche and Coetzer, 2008). In the current study, the SNT result indicated LSD dominance in the study area due to one or more factors that could be attributed to the occurrence of the disease.

Another potential explanation could be due to the fact that MLM is a predominantly rural area where livestock production, integrated with crop production, form the economic backbone of the province. Water supply in these areas is mainly groundwater, the Molopo Rivers starts in the east of Mahikeng and flows westwards for around 1,000 km to link to the Orange River near the south eastern border of Namibia. The most important dams in the river are Disaneng Dam and Setumo Dam; the two of them are near to Mahikeng municipality. In a study conducted by Maropofela and Oladele (2012) on farmer competencies and identification of LSD in Mahikeng municipality showed that approximately 40% of participants stated that they were using water from the rivers for livestock while 33.3% rely on water from the dams and 20% utilised water provided by the government-sponsored windmills. Furthermore, farmers who have intensified their farming system (6.7%) used water from creeps. It was noticed that during the unavailability of water on farms, farmers send their livestock to communal drinking

points (dam and rivers) (Maropofela and Oladele (2012). In addition, Maropofela and Oladele (2012) indicated that 97% of respondents confirmed the presence of mosquitoes to be high around the water points. This report corroborates the high seroprevalence of LSD in the current study. Possibly the high seroprevalence of the virus in animals, determined from this study, might also be linked to the susceptibility of the cattle to the biting-flies' population found at the water point. These flies played an important role in the transmission of the infection, as previously described. The cattle were exposed to the biting flies while drinking water at the dam or rivers. Epidemiological data shows that *Stomoxys calcitrans,* are strictly blood-sucking arthropods (Todd, 1964) horseflies (Tabanidae) (Saegerman et al., 2018), *Aedes aegypti* female mosquitoes (Chihota et al., 2001) and ticks (Tuppurainen et al., 2013) perform an important role in the dissemination of LSD between cattle. The same authors show that these insects may transmit the virus to vulnerable cattle without the manifestation of symptoms in the cattle. The present observation supports previous research showing that irrigated lands and rivers provide conducive environments for arthropod vectors and may increase the spread of LSD (Davies, 1991a; Hunter and Wallace, 2001; Tuppurainen and Oura, 2012; Woods, 1988).

In the current study, samples were collected throughout mild winters and during summer (wet season). The period is linked with the breeding and fast intensification of insect vectors in different villages. This also clarifies the high seroprevalence values found in the current study. These results align with the result found by Maropofela and Oladela (2012), who showed that the most prevalent seasons for LSD in Mahikeng are during the spring and summer. In the rainy season, the presence of floods and irrigation in the villages could contribute to the increase of potential insect vectors, which facilitate the dissemination of the infection (Ali et al., 1990; Davies, 1991a; Tuppurainen and Oura, 2012). Comparable results were also stated by Abera et al. (2015a), that the LSD outbreak was connected with wet and warm periods or conditions because of the significant presence of blood-feeding arthropod populations in the summer

season (Abera et al., 2015a). Recently the disease was observed on a dairy cattle farm in Oman, , and the cause was traced to be a lack of proper management related to the control of insect vectors and ineffectiveness of the vaccination (Kumar, 2011). In the study conducted by Tuppurainen et al. (2011) and Lubinga et al. (2014) reported the potential role of ticks in the transmission of LSD. The report shows the transovarial transmission of LSD by *Rhipicephalus* (*Boophilus*) *decoloratus*, mechanical or interstadial transmission by *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* males, as well as transstadial transmission by *Amblyomma hebraeum*. The survey conducted by Spicket et al. (2011) showed that livestock in the North West province harbour 22 tick species (18 ixodids; 4 argasids), and animals displayed tick-borne symptoms were prevalent in the north-eastern region, which also showed the highest tick species diversity. This also could explain the high seroprevalence values found in the present study.

The study also found out that there was a significantly higher incidence (at 67%) than the seroprevalence of 44% observed by Hailu et al. (2014) in the North-Eastern Ethiopia and the incidence of 6.43 % stated by Abera et al. (2015a) in the specific areas of West Wollega zone, Ethiopia. The research carried out by Elhaig et al. (2017), related to the frequency and occurrence of LSD, revealed a much lower seroprevalence of 17.3% on cattle.

This current research reveals that the incidence of LSD varies in different villages. Masutlhe showed a seroprevalence of 95%, followed by Tswaing (74%) and Meetmekaar (58%). Fifty percent (50%) prevalence was recorded in Lokaleng and 29% in Ramatlabama Six Hundred. This could be due to the high cattle population density, uncontrolled movement of unvaccinated cattle in the villages and introduction of new cattle in the herd without following quarantine methods, animal isolation and vaccination (Gari et al., 2010; Salib and Osman, 2011; Hailu et al., 2014; Tuppurainen and Oura, 2012; Van den Bossche and Coetzer, 2008).

This finding also aligned with the report obtained from the North-West University, Mafikeng Campus, and Animal Health Department 2019. The report showed that during outreach, small-scale farmers from different villages bring their cattle in the morning, and these animals are housed in the DAFF Kraals. In the communities where the kraal and crush pens do not exist, communal kraals and crush pens were used. The movement of unvaccinated cattle to areas of outreach is the major risk for the spread of the disease.

Similar results were obtained by Gari et al. (2010), which stated that new cattle to a farm have a solid link with amplified risk of infection in the herd. In 2014 the same authors reported that a regular cattle herd contact and mixing with other animals' species such as *ovine*, *caprine*, and *equine* at the grazing field and watering places are six times more likely to be infected with LSD than cattle that graze separately. This seems to be quite different from other studies, which stated that cattle with regular contact with other animal species at the collective grazing ground and watering points have two times lesser probabilities of being contaminated with LSD than cattle grazed separately (Masemola et al., 2018). Mola et al. (2018); Randolph and Dobson (2012) and Swaddle and Calos (2008), observed that allowing the cattle (preferred hosts) with other animals such as sheep, goats, donkey, mules, and horses (non-preferred hosts) reduced insect attacks and therefore the transmission to cattle was at low risk (Mola et al. 2018; Randolph and Dobson, 2012; Swaddle and Calos, 2008).

The low prevalence around Ramatlabama and Six Hundred can be linked to the absence of insect vectors or an environment whereby insect's activities were less (Rweyemamu et al., 2000; Troyo et al., 2008). It is also reported that vegetation may allow tick species as well as insects to develop (Tuppurainen et al., 2017a). According to Hunter and Wallace (2001), changes in weather, such as cold, may decrease the insect vector populations, also reduce LSD

transmission. Another possible explanation could be the cattle were vaccinated (Hunter and Wallace, 2001).

A recent study conducted by Maropofela and Oladele (2012) based on farmer's competency and identification of LSD causes, clinical signs and management practices in Mafikeng metropolitan area showed that antibiotics, anti-inflammatory (73.3%) agents as well as vaccination (83.3%) had been applied in the management of the disease, but other small-scale farmers opted for the use of Dettol antiseptic which is a non-veterinary recommended product. They inject it intra-muscularly in infected cattle. This shows that certain farmers in the villages around Mahikeng do not vaccinate their cattle annually. They believe that injecting animals with Dettol is an effective, cheap, and useful means of treating the disease. This is also a possible alternative explanation for the high seroprevalence of the disease in certain villages, as mentioned previously.

Nevertheless, the situation of the inefficacy of the vaccine, together with the re-infection of vaccinated cattle were documented by other studies (Brenner et al., 2009; Kumar, 2011). Eradication of LSD needs fast and sensitive diagnosis technics reinforced by symptoms (Tuppurainen et al., 2005). In the current study, three validated diagnostic tools (conventional PCR, RT-PCR, as well as SNT) were used to detect LSDV rapidly, and the SNT result showed that all the blood samples collected were antibody positive for LSD. The obtained sequence information from six isolates was recognized as LSDV afterward sequential diagnosis by using conventional PCR. Another research conducted by Zeynalova et al. (2016), managed to detect the LSDV in two-thirds of the samples tested by using the same assay.

RT-PCR seems to be a specific, rapid, sensitive technique in the detection of CaPV as well as its identification (Balinsky et al., 2008). This approach was performed to determine CaPV genetic makeup, and it was done based on the amplicon melting temperature. The method helps

to avoid confusion between LSD and sheeppox virus, which may occur, as it is rarely transmitted to cattle producing skin lesions (Burdin and Prydie, 1959; Capstick et al., 1959; Kitching, 2008). The same authors also indicated that an experimental infection of cattle associated with sheep poxvirus could produce identical lesions to LSD. However, the conventional PCR result did not correlate to the field diagnosis. But this method successfully detected six LSDV DNA in the skin nodule samples, and the samples were amplified and sent for sequencing. Results from conventional PCR (six samples) did corroborate with the results obtained from the RT-PCR. All the six sequences obtained from this study were subjected to blast analysis for similarities; after analysis the sequences were found to be LSDV based on their similarities compared to the ones from the GenBank.

To determine if there were any differences between the newly identified six CaPV strains from different sites under the study were subjected to multiple sequence alignment and the phylogenetic tree was constructed. Analysis of the three genes GPCR (LSDV-011), LSDV-022 gene, and TK gene (LSDV-066) suggests that there was similarity with LSDV isolated in this research.

In the present study, all the isolates clustered with field isolates, and the consensus tree showed 2 major genetic clusters: Cluster I had a maximum of the field strain, and cluster II had the vaccine strains. Based on the phylogenetic analysis, it was noticed that all six isolates from this study grouped into one cluster, and they also clustered with other field isolates from Africa, Europe, and the Middle East. The phylogenetic tree analysis showed that the isolates from the current study were identical to each other and they were similar to FJ869377 (Egypt-Isamalia 18/1989, KR024780 Turkey-02/2015, KY829023 Evros/GR/15, FJ 869375 RSA/06-D 19353-16, MH893760 LSDV Russian Dagestan 2015, KX894508 155920-Israel 2012, KX683219KSGP-0240 Kenya 1974 and AF 409137 NW-LW Warmbaths RSA 1999 strains.

This clustering was important as it revealed the probability of the homology strain of the virus, which might be responsible for different cases of the infection (LSDV) circulating in the country. Close homology was established based on the degree of similarity among the genes. This evidence suggests that there is only one serotype of LSDV.

The present finding also supports previous studies conducted by Coetzer et al. (2018), Prydie and Coackley (1959), and Weiss (1968). The report stated that LSDV collected from outbreaks occurred in South Africa, Kenya and Malawi, showed reciprocal cross-neutralization with the prototype Neethling strain (Davies et al., 1971; De Boom, 1948; Coetzer et al., 2018; Kitching and Taylor, 1985). In addition, a whole-genome sequencing of SERBIA (Bujanovac/2016) and Greece (Evros/GR/15) showed 99.5% and 99.8% homology, respectively with the LSDV Neethling Warmbaths LW isolated in South Africa in 2000. The result indicates the genetic stability of LSDV and also provides genetic evidence in support of a single serotype (Agianniotaki et al., 2017b; Toplak, 2017).

Based on the edge size of the branches in the phylogenetic tree, all the LSD RSA 2018 seemed to be closely correlated to each other, when comparing different strains from other countries. The clusters also show that the field isolates were different from the vaccine strain. However, three of the LSDV isolates retrieved from the GenBank (KX 683219 KSGP-0240 Kenya 1974, AF 325528 NI- 2490 Kenya 1958 and MH 646674 Russia Saratov 2017), were outliers and did not cluster with the other LSD RSA 2018 isolates (LSD 022 and 066).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The current study showed that LSD is widespread across villages in MLM, and this disease is associated with the wet period (rainfall) and, it is linked to the activities of insect and tick populations. This study also showed that the seroprevalence of LSD in the MLM was greater than the disease incidence observed in Ethiopia and in some African countries whereby the disease is endemic (Davies, 1991a; Gari et al., 2010). In addition cattle management practices such as the introduction of new animals into a farm without screening for LSD, mixing of cattle at the grazing field and uncontrolled access to the water point, uncontrolled movement of cattle to different villages and regular contact of cattle with wild animals were incriminated to promote the LSD (Abera et al., 2015a). These risk factors may intensify the spread of LSD into new villages that were previously considered as areas free of the infection. The findings of this research also make available the primary data on the identification of LSD strain circulating in the MLM, and its distribution in the study area.

Molecular assays showed that there was no important nucleotide difference between the current and previous field isolates of CaPV strains in the present research. Nevertheless, the vaccine strains were genetically different from the field strains. Molecular and SNT are very useful for specific detection of CaPV strains and could also help to understand the disease epidemiology in different study areas. Hence, the genotyping, as well as sequencing of the current isolates, is suggested to determine the antigenic variation of circulating field strains with the vaccine strain.

6.2 RECOMMENDATION

Prevention is more helpful than its treatment; hence, to prevent the negative impact of LSDV on cattle, it is critical to educate farmers of LSD by promoting mass vaccination strategies using effective vaccines (attenuated Neethling strain vaccine). Also, quarantine animals before entry into a new area for a while, restriction of cattle movement during the active period of insect movement, eradication of vectors, and depopulation of infected and exposed cattle should be ensured. The implementation of biosecurity measures and farm management could also help in reducing the occurrence of LSD in the affected villages (Gumbe, 2018; Mulatu and Feyisa, 2018). Therefore the collaboration of South Africa with neighbouring countries (Botswana) should be promoted to avoid the spread of the disease across national borders. Due to a limited study period, additional studies are needed to evaluate the status of the disease in other municipalities of the Ngaka Modiri Molema to suggest the implementation of appropriate control and prevention methods in the areas.

6.3 LIMITATIONS

First level samples collected in this study were small because most of the participants were not willing to agree on the biopsy of skin nodules. Secondly, two years of research was not long enough to assess LSD in other municipalities of the Ngaka Modiri Molema, and thirdly budget was not enough to cover all the areas of the District.

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Appendix A: Nucleotide sequences of an LSDV strain isolated from the skin of infected cattle from MLM

1) LSD-1-GPC

Tgaaggtcaggactaattatttcaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactatttc
tcaataatatttatcccagtagcactattcgtcacatgatctattatagaattctcaatatgatatatatactcagaccccaatatta
ataaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccagctattcattggctg
agaaggcgtcggaatcatatcattcctactcatcggatgatgatacggacgagcagatgcaaacacagcagccctacaag
caatcttatataaccgcatcggcgacattggtttcattttagcaatagcatggttcctaacaaatctcaatacctgagacctcca
acagatcttcatactaaacccaagcgactcaracatacccttgattggactagcattagctgcaaccggaaaatccgcccaat
ttggcctccacccgtgacttccctctgcaatagaaggcccaactcccgtctcagcactactccattcaagcacaatagtggta
gcaggtatcttcctactaatccgtttctatcccctcacagaaaacaataaatacatccaatctattacattatgcttaggagccat
taccacactatttacagcaatatgcgccctcacccaaaatgacattaaaaaaatcatcgccttctccacatccagtcaactgg
gccttataatagtaactattggcattaaccaaccttacctagctttcctccacatctgtacccacgccttttcraagctatactatt
catatgctccggttccattattcacagcctaaacgacgaaaaagatattcgaaaataggaggcctatttaaagccatgccattc
accacacagcactcattgtggaatttgtaaa

2) LSD-2_GPC

Tggcagttatttttgcaaaagattttgataataataatattgtatagttatattttgtttcattctgataaaaagatatttgattatct
aactagtcttggtatcgatatggatgaaactaatacgtcaggttacaattgtaaggatttaattaaatttagaaatctcacctttttt
gacaaacaataaaaagtttttaaaaaaaataaatgatgatacaaatactgtgtttttattaaaaaaaaaaaaaaaaaaaaaag
gggatgattaaaaaaagcataactcccacaaaaaggaattatactcttaaaacagttagaaccccacccttgtttaatagtag
cagaaattttccccctttagctcctacaattattaaaacaatttcaacaaatcaaaataatgttacacccccccccaacttatgaaa
tccaacaacgatatctaattatacaccgcatataaacaacttatttagcgataatatgatgatatggagggacctagccatttcc
ccttggtgtgagggtgggaacaacagttggacggttacttatatccactattctttctggatattggaaataatgggtactgtct
cgtattagataacatcggttgttgctattgcctgcgatattcggtggtcttatacaagatcctacatgatagatgtgtaattaagc
atgtacttgttgaagcatgcataactgatggatgtagatctactgtatacgtatcatgctacgcacgattgatgactagatggtg
agtacattgagctcatagattagcagacgat

3) LSD-2_OP3

Cattcatttttataaaatatcattatttgttgttattatttttttattttttatccaatgctaatactaccagcactactggtgctacgcaa
tcgtaaaagctttttagtaaattctctactacaaaacgcataaattagtggattgataaaacaatgacatagagacacaatttca
gctacatgaactgcaaggttgacaaatcttaacgccatacatccactaaaaacatttaacaaatacaacgatgaaacaaatac
agttacactaaatgggagtaaaaacaatactgaacagataacaatcaaaaacaccatctttatggctttcttattctttgtttgtg
atgtttttaaagtatttaagattttataataacaatatagcaaaatagttagcggtataatcattccaaatatgtttatttcaaaatttat
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gaaaggattcaatagttgagacaatccaaaccaccatactaagtacaattccatatcgttttgtccttatcggcattgattttact
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gctttaaatttacacaaacaatctcctaaactccattgtttagcgatcctatcgtataaattaaaaggaaacaccaacacgaaaa
ttaaatcagacagtgtcaaattaagcaaaaacatatcctgtattgtttttatcttatatttacgaagaacagttaacacaattatattt
ccaaataatccaagaaaagaatatagtcgaatataagtaatcagtccaaaactttgtagtatccacaccatcatcacatgtgg
gatatcgactatgctccactcatatcatcatatcatcgctataatagtggatatatgcgatgaaattagatatcgtgtgattcata
gtgagcgtgtacgtagtcgattgctggcactgtactaatagtggtagctagct

4) LSD-2-22_LSO22
Aggttttagtgaaggtggtcgggtggtttcggcccgtagacttgcagaagggatgtatgtagtggagggtcctcagtactaa
cggtattactatcactatcactattactattactatcattagcaccatcaccatcaccatcaccatctaacaatgtaacacgttcaa
tagatattgccttttttcgtattgcttgtgcaccgg

5) LSD-3-22_LSO22
ccgttttatgaggtgtcttgctttttgtaaatttatacttgtagttataattatatttagtatcattatcctcactactaacggtattacta
tcactatcactattactattactatcattagcaccatcaccatcaccatcaccatctaacaatgtaacacgttcaatagatattgcc
tttttcatattgcttttccaga

6) LSD-3-22_LSO22
Tcgtyttatgaggtgtctttctttttgtaatttatacttgtagttataattatatttagtatcattatcctcactactaacggtattactat
cactatcactattactattactatcattagcaccatcaccatcaccatcaccatctaacaatgtaacacgttcaatagatattgcct
ttttcatattgcttttccaga

7) LSD-4-GP_GPCF-AR
ctcagggcaagactaattatttcaaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactattt
ctcaataatatttatcccagtagcactattcgtcacatgatctattatagaattctcaatatgatatatatactcagaccccaatatt
aacaaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccaactattcattggct
gagaaggcgtcggaatcatatcatttctactcatcggatgatgatacggacgagcagatgcaaacacagcagccctacaag
caatcttatataaccgcatcggcgacattggtttcattttagcaatagcatggttcctaacaaatctcaatacctgagacctcca
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gccttataatagtaactattggcattaaccaaccttacctagctttcctccacatctgtacccacgccttttcaagctatactattc
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caccacaacagcactcattgttgggattttgtaaaa

8) LSD-5_GPCF
gagcggagttatttttgcaaaagattttgataataataatattgtatagttatattttgtttcattctgataaaaagatatttgatta
tctaactagtcttggtatcgatatggatgaaactaatacgtcaggttacaattgtaaggatttaattaaatttagaaatctcacctt
tttgacaaacaataaaaagttttttaaaaaaaataaatgatgatacaaatactgtgtttttattaaaaaaaaaaaaataaaataaa
agggtatgattaagtaaagcataactccaacaaaaatgaattatactcttaaaacagttagtagcgcaaccttgtataatagta
gcagtaatattccccctatagctactacaattattagaacaatttcaacaaatcaaaataatgttacaaccccttcaacttatgaa
aatacaacaacgatttctaattatacaaccgcatataaaacaacttattatagcgatgattatgatgattatgaaggggcatagc
caaatcccccattgtgatgatggtgtggatactacagtttggactgatactttatattcactatattcttctggatattggaatataa
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atcgtaacatgagtagaatgttggtaatttaagctgttacttgtgttacatgcatgcattaactgatgaatgagatctagctgagt
ccagtaatcatgccgaagcacgatgatgactagatggagatgtcacgtgactcaggtattagacagatgatcgatgcagtat
taacgaaagcagtgagatatatgataca

9) LSD-5- OP3

tctttcatttttataaaatatcattatttgttgttattatttttttattttttatccaatgctaatactaccagcactactggtgctacgcaat
cgtaaaagctttttagtaaattctctactacaaaacgcataaattagtggattgataaaacaatgacatagagacacaatttcag
ctacatgaactgcaaggttgacaaatcttaacgccatacatccactaaaaacatttaacaaatacaacgatgaaacaaataca
gttacactaaatgggagtaaaaacaatactgaacagataacaatcaaaaacaccatctttatggctttcttattctttgtttgtgat
gtttttaaagtatttaagattttataataacaatatagcaaaatagttagcggtataatcattccaaatatgtttatttcaaaatttata
aataatttccaaatttttgcattatcgttataaaatacatgacaatacgttattccatatacttttttgtttcataaaataacattattgg
aaaggattcaatagttgagacaatccaaaccaccatactaagtacaattccatatcgttttgtccttatcggcattgattttactg
ggtgaactacagctaggtatctatcaatactcatcaatgttataaatgacatgctattgtaaaaaccaacaaagtaaaacatag
ctttaaatttacacaaacaatctcctaaactccattgtttagcgatactatcgtataaattaaaaggaaacaccaacacgaaaatt
aaatcagacagtgtcaaattaagcaaaaacatatcctgtattgtttttatcttatatttacgaagaacagttaacacaattatatttc
caaataatccaagaaagaatatagtcgaatataaagtaatcagtccaaaacttgtagtatccacaccatcatcacaatgtggg
atatcgactatgctcacttcataatcatcataatcatcgctataataagtgtatatatgcggttgtatatagatatcgttgtgaatttc
atagtgaggcgtgacatattgatgtgaatggtactaataattgtag

10) LSD-5-22_LSO22

Tgattactttatgaggtgtctttctttttgtaatttatacttgtagttataattatatttagtatcattatcctcactactaacggtattact
atcactatcactattactattactatcattagcaccatcaccatcaccatcaccatctaacaatgtaacacgttcaatagatattgc
ctttttcatattgcttttccagaa

11) LSD-5-GP_GPCF

atcgttagatcagttagtagcgcaaccatgtataatagtagcagtaatattaccactatagctactacaattattagtacaatttc
aacaaatcaaaataatgttacaacgccttcaacttatgaaaatacaacaacgatatctaattatacaaccgcatataatacaact
tattatagcgatgattatgatgattatgaagtgagcatagtcgatatcccacattgtgatgatggtgtggatactacaagttttgg
actgattactttatattcgactatattctttcttggattatttggaaatataattgtgttaactgttcttcgtaaatataagataaaaaca
atacaggatatgtttttgcttaatttgacactgtctgatttaattttcgtgttggtgtttccttttaatttatacgatagtatcgctaaac
aatggagtttaggagattgtttgtgtaaatttaaagctatgttttactttgttggtttttacaatagcatgtcatttataacattgatga
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tggtttggattgtctcaactattgaatcctttccaataatgttattttatgaaacaaaaaaagtatatggaataacgtattgtcatgt
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tttgtcaacccttgcagttcatgtagctgaaattgtgtctctatgtcattgtttatcatccactaatttatgcgtttgtagtagagatta
ctaaaaagctttacgatgcgtagcaccagtagtgcttgttagattagcattggcataaattaaaataatacarcaatratgaaatt
taat

12) LSD-6-GP_GPCF-AR

tcagggccagactaattatttcaaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactatttct
caataatatttatcccagtagcactattcgtcacatgatctattatagaattctcaatatgatatatatactcagaccccaatattaa
caaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccagctattcattggctga
gaaggcgtcggaatcatatcatttctactcatcggatgatgatacggacgagcagatgcaaacacagcagccctacaagca
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ggcctccacccgtgacttccctctgcaatagaaggcccaactcccgtctcagcactactccattcaagcacaatagtggtag
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tatgctccggttccattattcacagcctaacgacgaacaagatattcgaaaaaataggaggcctatttaaagccatgccattca
ccacaacagcactcattgtggggatttgtaaa

13) LSD-7-GPCF

tagggtcagactaattatttcatactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactatttctc
aataatatttatcccagtagcactattcgtcacatgatctattatagaattctcaatatgatatatatactcagaccccaatattaac
aaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccagctattcattggctgag
aaggcgtcggaatcatatcatttctactcatcggatgatgatacggacgagcagatgcaaacacagcagccctacaagcaa
tcttatataaccgcatcggcgacattggtttcattttagcaatagcatggttcctaacaaatctcaatacctgagacctccaaca
gatcttcatactaaacccaagcgactcaaacatacccttgattggactagcattagctgcaaccggaaaatccgcccaatttg
gcctccacccgtgacttccctctgcaatagaaggcccaactcccgtctcagcactactccattcaagcacaatagtggtagc
aggtatcttcctactaatccgtttctatcccctcacagaaaacaataaatacatccaatctattacattatgcttaggagccattac
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ttataatagtaactattggcattaaccaaccttacctagctttcctccacatctgtacccacgccttttcaaagctatactattcat
atgctccggttccattattcacagcctaacgacgaacaagatattcgaaaatagggaggcctatttaaaaccatgccattcac
cacaacagcactcattgtggratttgtaaaa

14) LSD-8-GPCF-AR

ctcaggtcagactaattatttcaaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactatttct
caataatatttatcccagtagcactattcgtcacatgatctattatagaattctcaatatgatatatatactcagaccccaatattaa
caaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccagctattcattggctga
gaaggcgtcggaatcatatcatttctactcatcggatgatgatacggacgagcagatgcaaacacagcagccstacaagca
atcttatataaccgcatcggcgacattggtttcattttagcaatagcatggttcctaacaaatctcaatacctgagacctccaac
agatcttcatactaaacccaagcgactcaaacatacccttgattggactagcattagctgcaaccggaaaatccgcccaattt
ggcctccacccgtgacttccctctgcaatagaaggcccaactcccgtctcagcactactccattcaagcacaatagtggtag
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ccacactatttacagcaatatgcgccctcacccaaaatgacattaaaaaaatcatcgccttctccacatccagtcaactaggc
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tatgctccggttccattattcacagcctaacgacgaacagatattcgaaaataggaggcctatttaaagccatgccattcacca
caacagcactcattgtgggatttgtaaa

15) LSD-9-GPCF

aagctggagttatttttgcaaaagattttgataataataatatattgtatagttatattttgtttcattctgataaaaagatatttgatta
tctaactagtcttggtatcgatatggatgaaactaatacgtcaggttacaattgtaaggatttaattaaatttagaaatctcacctttt
tttgacaaacaataaaaagttttttaaaaaaaataaatgatgatacaaatactgtgtttttattaaaaaaaaaaaaaaaaaaaaaa
gggtatgattaagtaaagcataactcccacaaaaatgaattatactcttaraacagttagaagcgcaaccatgtttaatagtag
cagtaatattacccctatagctactacaattattaaaacaatttcaacaaatcaaaaaaatgttacaaccccttcaacttatgaaa
atacaacaacgatttctaattatacaaccccatataaaacaacttattatagcgatgattatgatgattatgaagtgagcatagc
aaatcccccattgtgatgatgggtggatacacagtttggcctgaatacttatattcactatttttctgatattggaaatatgtgtact
gtctcgaattagataaacatcaggattgtttgctattgcctgctgattattcggtggttccttattacgagtcgtacatgattagaa
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atgactagatggtgagccaattgactcgaggtattagcaagatgatacgatgcagatcacgaatgcagtgaga

16) LSD-9-OP3

cattcatttttataaaatatcattatttgttgttattatttttttatttttttatccaatgctaatactaccagcactactggtgctacgcaat
cgtaaaagctttttagtaaattctctactacaaaacgcataaattagtggattgataaaacaatgacatagagacacaatttcag
ctacatgaactgcaaggttgacaaatcttaacgccatacatccactaaaaacatttaacaaatacaacgatgaaacaaatacа
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tgtatttttcataagtggacggcgttgtaacatatttgattgtgaagtctactaataattgtagtaagccta

17) LSD-9-GPCF

tacctttatgaggtgtctttctttttgtaatttatacttgtagttataattatatttagtatcattatcctcactactaacggtattactatc
actatcactattactattactatcattagcaccatcaccatcaccatcaccatctaacaatgtaacacgttcaatagatattgcctt
tttcatattgcttttccaga

18) LSD-9-LSO22

cttggacccatcaatgatgagtgcgtccgtgagtaatagtaccagtattattaccactatagctactacaattattagtacaattt
caacaaatcaaaataatgttacaacgccttcaacttatgaaaatacaacaacgatgtctaattatacaaccgcatataatacaa
cttattatagcgatgattatgatgattatgaagtgagcatagtcgatatcccacattgtgatgatggtgtggatactacaagtttt
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cctgcagtcatgcagctggaattgggtctctatgcattgggttatcatcscactaatttatgggtttggagaaaaattactaaaa
agctttaccaattgcgtacccccag

19) LSD-10-GPCF

ctcaggggtcaaggaactaattatttcaaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagac
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attcaccacaacagcactcattgttggatttggtaaa

20) LSD-11-GPCF

ctcaggggtcaaggaactaattatttcaaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagac
tatttctcaataatatttatcccagtagcactattcgtcacatgatctattatagaattctcaatatgatatatatactcagaccccaa
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attcaccacaacagcactcattgttggatttggtaaa

21) LSD-12-GP-GPCF

tcagggccagactaattatttcaaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactatttct
caataatatttatcccagtagcactattcgtcacatgatctattatagaattctcaatatgatatatatactcagaccccaatattaa
caaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccagctattcattggctga
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cttataatagtaactattggcattaaccaaccttacctagctttcctccacatctgtacccacgccttttcaaagctatactattca
tatgctccggttccattattcacagcctaacgacgaacaagatattcgaaaaaataggaggcctatttaaagccatgccattca
ccacaacagcactcattgtggggatttgtaaa

22) LSD-13-LSO22

ccctttatgaggtgtctttctttttgtaaatttatacttgtagttataattatatttagtatcattatcctcactactaacggtattactatc
actatcactattactattactatcattagcaccatcaccatcaccatcaccatctaacaatgtaacacgttcaatagatattgcctt
tttcatattgcttttccaga

23) LSD-13-GPCF

atctttagtcagttagtagcgcaaccatggataatagtagcagtaatattaccactatagctactacaattattagtacaatttca
acaaatcaaaataatgttacaacgccttcaacttatgaaaatacaacaacgatatctaattatacaaccgcatataatacaactt
attatagcgatgattatgatgattatgaagtgagcatagtcgatatcccacattgtgatgatggtgtggatactacaagttttgga
ctgattactttatattcsactatattctttcttggattatttggaaatataattgtgttaactgttcttcgtaaatataagataaaaacaa
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ataacgataatgcgacattttgaaaatcatttataacttttgaaataaacatatgtggaatgattataccgctatctatttttgctatct
tgttatatcaattcttaaatactttacaaacatctcaaaccaagaatactaatccataaacagggtcctttatttttgatcctctgtca
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atttgtcaccctggcagatcaggtaactaaagtgggtctcttgtcatggtttatcagtccctatttatgccgttgtgaagtaaaatt
tactaaaagcttttaccattgctaaccccatagggccgtgtagtat

24) LSD-13-GPCF

gccattagatcagttagtagcgcaaccatgtataatagtagcagtaatattaccactatagctactacaattattagtacaatttc
aacaaatcaaaataatgttacaacgccttcaacttatgaaaatacaacaacgatatctaattatacaaccgcatataatacaact
tattatagcgatgattatgatgattatgaagtgagcatagtcgatatcccacattgtgatgatggtgtggatactacaagttttgg
actgattactttatattcgactatattctttcttggattatttggaaatataattgtgttaactgttcttcgtaaatataagataaaaaca
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tggtttggattgtctcaactattgaatcctttccaataatgttattttatgaaacaaaaaaagtatatggaataacgtattgtcatgt
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atattgttattataaaatcttaaatactttaaaaacatcacaaacaaagaataagaaagccataaagatggtgtttttgattgttat
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gaatttactaaaaagctttacgatggcgtagcaacagtatgctgtagattagccattgggaaacaaaattaaaaactaattaa
ccacaaagataggtgat

25) LSD-14-GP_GPCF-AR

ctcagggcaggactaattatttcaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactatttc
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acaaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccagctattcattggctg
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cagatcttcatactaaacccaagcgactcaaacatacccttgattggactagcattagctgcaaccggaaaatccgcccaatt
tggcctccacccgtgacttccctctgcaatagaaggcccaactcccgtctcagcactactccattcaagcacaatagtggta
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acaacagcactcattgtggsatttgtaaac

26) LSD-15-GPCF

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acaaacaataaaaagttttttaaaaaaaataaatgatgatacaaatactgtgtttttattaaaaaaaaaaaaaaaaawaaaagg
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tgatgatgggtggatacacagtttgactgatacttttcactattctttctgatattgaaatatgggtactgtctcgtattagataac
atcagatgtttgctaatgactgcgataattcggtggttccttaataacgagatctacatgatagaatgtgtaattaagctgtactt
ggttcaagcagcataactgtgaatgtagatctacttagtacagtaatcatgcgatagcaacgatatgatgactagatcgtatga
tgatccagtgactcatagtattagacaagat

27) LSD-15-OP3

ccttcatttttataaatatcattatttgttgttattatttttttattttttatccaatgctaatactaccagcactactggtgctacgcaatc
gtaaaagctttttagtaaattctctactacaaaacgcataaattagtggattgataaaacaatgacatagagacacaatttcagc
tacatgaactgcaaggttgacaaatcttaacgccatacatccactaaaaacatttaacaaatacaacgatgaaacaaatacag
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aaggattcaatagttgaracaatccaaaccaccatactaagtacaattccatatcgttttgtccttatcggcattgattttactggg
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aaatttacacaaacaatctcctaaactccattgtttagcgatactatcgtataaattaaaaggaatcaccaacacgaaaattaaa
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aataatccaagaaaagaatgatagtcgaatataaaggtaatcagtccaaacttgtagtatccacaccatcatcacatgtggat
atcgactatgctcactcatatcatcatatcatcgctataatagttgatatatgcgatgatattagattcgtgtgatttcatagtgagc
tgaacttatcgatgtgagctgactatataaagtagctaa

28) LSD-15-22_LSO22

tcctttatgaggtgtctttctttttgtaaatttatacttgtagttataattatatttagtatcattatcctcactactaacggtattactatc
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tttcatattgcttttccaga

29) LSD-15-GPCF

cttcggaaacagacatggtatcgcaaccgtgtataatagtagcagtactattaccactatagctactacaattattagtacaattt
caacaaatcaaaataatgttacaacgccttcaacttatgaaaatacaacaacgatatctaattatacaaccgcatataatacaa
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tggaatagtgt

30) LSD-16-GPCF

ctcagggcaggactaattatttcaactgacactgactaaccatccaaactcttaaattatccctcagctttaaaatagactatttc
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acaaattcttcaaatatctactcctattcctcattactatgctcatccttgtaaccgcaaacaacctcttccagctattcattggctg
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acaacagcactcattgtggsatttgtaaac