ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF VETERINARY MEDICINE

EPIDEMIOLOGICAL ASPECTS AND ECONOMIC IMPACTS OF LUMPY SKIN DISEASE IN SELECTED DISTRICTS OF ETHIOPIA

BY

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Masters of Veterinary Science in Tropical Veterinary Epidemiology

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TABLE OF CONTENTS **Pages**

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LIST OF TABLES **Pages**

LIST OF FIGURE

Pages

ABBREVIATIONS

AGID	Agargel immunodiffusion
BSA	Bovine serum albumin
CI	Confidence interval
CIRAD	Center of International Research for Animal Disease
CPE	Cytopathic effect
CSAE	Central statistics agency of Ethiopia
DNA	Deoxy ribos nuclic acid
EDTA	Ethlene diamine tetra- acetic acid
ELISA	Enzyme linked Immunosorbent Assay
FAO	Food and Agricultural Organization
GDP	Gross domestic product
GIS	Geographical information system
GMEM	Glasgow's Modified Eagle's Medium
GPV	Goat pox Virus
H and E	Haematoxylin and Eosin
IFAT	Indirect Fluorescent Antibody Test
IU	International Unit
KBP	Killo Base Pair
KSI	Kenya Sheep pox 1
LSD	Lumpy skin Disease
LSDV	Lumpy skin Disease Virus
LT	Lamb Testis
M.A.S.l.	Meters above sea level.
MABS	Monoclonal antibodies
Mbs	Monoclonal antibodies
MOARD	Minister of Agriculture and Rural Development
MRNA	Messenger RNA
NCM	Nitrocellulose membrane
OIE	Office International des Epizooties.

PA	Peasant association
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RPM	Revolution Per minute
SDS	Sodium dodecyl sulphate
SHPV	Sheep pox virus
$TCID_{50}$	5% tissue culture infective dose

LIST OF ANNEXES **Pages**

ABSTRACT

A cross-sectional study and retrospective questionnaire survey were conducted from October 2009 to April 2010 in six selected districts of Ethiopia; three districts for serological study were from Amhara region and the other three districts for retrospective survey in Oromia region. This study aimed at determining the prevalence and associated monetary losses in intensive small scale dairy farms and in extensively managed farms. A total of 263 cattle blood samples were collected from 7 PAs in 3 districts. All the sera were tested using IFAT and VNT. Out of 263 sera, 110 and 85 were positive by IFAT and VNT respectively, giving sroprevalences of IFAT of 41.8% and VNT of 32.3%. The retrospective survey was carried on 355 herds having 2954 cattle. In the latter, 102 herds were intensively managed and had 718 cattle while, 253 herds were extensively managed and had a total of 2236. The relevant data were collected from the herd owners that dwelt in 25 PAs in 3 selected districts with the objectives of estimating animal-level prevalence, mortality and associated monetary losses due to outbreak of LSD. The computed animal-level prevalences of LSD outbreak were 13.8% (95% CI: 12.4 – 15.3) and 31.8% (95% CI 28.3 – 35.8) in extensively and intensively managed farms, respectively with an overall animal-level prevalence of 18.2% (95% CI: 16.8 – 19.6). The computed percent mortality was significantly higher in intensively managed farms with 7.24% (95% CI: 5.3 – 9.1) than the percent mortality in extensively managed farms with 1.25% (95% CI: 0.8 -1.7). The overall observed mortality was 2.7% (95% CI: 1.3 – 4.1). The animal-level prevalence's by age-groups were 12% (95% CI: 8.3 – 17), 17.4% (95% CI: 13.7 – 21.4) and 19.9% (95% CI: 16.2 – 23.6) in calves, yearlings and adults respectively. The percent mortality of 4.5% (95% CI: 3 – 8.9) in calves were significantly higher than in yearlings. of 2.4% (95% CI: 1.2 – 3. 6) and of 2.4% (95% CI: 1.7 – 3.1) in adults The total financial losses due to LSD outbreak were estimated using economic models for livestock both in intensively and extensively managed farms and the results were 28.5 million birr and 5 billion birr respectively. Thus, these findings highlight the importance and negative impacts of LSD in Ethiopia although the actual financial losses were not calculated for the complex impacts that could not be estimated in monetary terms for example abortions, increases in calving intervals, and poor growth rates.

Key words: Ethiopia, Economic model, IFAT, LSD, Prevalence, Risk factors VNT

1. INTRODUCTION

Ethiopia has the most abundant livestock population in Africa (FAO, 2005) and the cattle population is estimated to be 41.5 million (CSAE, 2006). The livestock sub sector accounts for 40% of the agricultural gross domestic product (GDP) and 20% of the total GDP without considering other contributions, e.g. traction power, fertilization and transportation (Aklilu, 2002). In 2004 the livestock sector contributed around 12% of total foreign currency earnings (Anon, 2009). About 99% of cattle populations are of local zebu breed (CSAE, 2006). Genetically and geographically the main breed classifications in Ethiopia are Arsi, Fogera, Horo, Borana, Nuwer, Sheko and Afar breeds. The remaining 1% of exotic breeds are kept mainly for dairy production in and around urban areas. Traditional cattle management in the rural part of the country is extensive. Animals are free-ranging in communal grazing fields and different age groups are herded together. Natural grass post-harvest crop residuals and straw are the main source of feed. Concentrate feeds and feed additives are seldom used (Alemayehu, 2006).

Lumpy skin disease (LSD) is one of the most serious poxvirus diseases of cattle caused by lumpy skin disease virus (LSDV) within the genus *Capri poxvirus*. The prototype strain is nettling virus. It causes acute to sub acute systemic disease characterized by mild to severe symptoms including fever, nodules on the skin, in the mucous membranes and in the internal organs, skin edema, lymphadenitis and occasionally death (Alexander *et al.*, 1957; Davies, 1982). The skin nodules are painful and have congestion, hemorrhage, edema, and vasculities with consequent necrosis and involve all layers of the epidermis, dermis, subcutaneous tissue, and often adjacent musculature. Lymph nodes draining affected areas are enlarged up to ten folds from normal size with extensive lymphoid proliferation, edema, congestion and hemorrhage (Burdin, 1959; Prozesky and Barnard, 1982).

LSD is one of cattle disease which causes high economic loss due to chronic debility in infected cattle, poor growth, infertility, abortion and sometimes death of the animal. Milk production ceases and pneumonia is a common sequele in animals with lesions in the mouth and respiratory tract (Davies, 1991; OIE, 2008). Severe and permanent damage to hides from the skin lesions could also bring a considerable loss to the tannery industries and loss of foreign currency since

hides are one of the export commodities in Ethiopia (Bayou *et al.*, 1998; Anonymous, 2008). High susceptibility of exotic breeds to LSD would pose a considerable concern to the development of small-scale dairy industry in many parts of the country. Moreover, it is a disease that hinders improvement of the livestock development and also international livestock trade movement due to phyto-sanitary regulations by many live livestock and livestock products importing countries. (Gari *et al.*, 2008).

The laboratory diagnosis to confirm the disease can be made either by the isolation and identification of the virus, or by using serological tests such as the virus neutralization test and indirect fluorescent antibody test (IFAT) (Gari *et al.*, 2008). All the viruses in the capripoxvirus genus share a common major antigen. It is not possible to distinguish the strains of capripoxvirus from either cattle, sheep or goats using serological techniques (Kitcing *et al.*, 1986).

Under experimental infection, the incubation period is 6-10 days. Clinically sick animals shed the virus through saliva, nasal and lachrymal discharges, blood and semen. Skin lesions have high viral concentrations within 7-18 days post-infection. However, the virus can persist in skin plugs for about 42 days (Babiuk *et al.*, 2008).

Morbidity and mortality of the disease vary considerably depending on the breed of cattle, susceptibility levels of the population, insect vectors involved in the transmission and isolates of the virus. In endemic areas, morbidity is usually around 10% and mortality ranges between 1% and 3% (Davies, 1991; Babiuk *et al.*, 2008). The mechanical transmission by the biting flies is the most effective method (Chihota *et al.*, 2001). The incidence of LSD occurrence is high during wet seasons when biting-fly populations are abundant and it declines or ceases during the dry season (Anon, 2009). The LSD has been endemic in Africa for more than 70 years occurring in a wide range of ecotypes. The disease was first described in Northern Rhodesia in 1929 (Morris, 1931). Since then, the disease has spread over most of Africa in a series of epizootics (House, 1990; Davies, 1991). The more recently affected countries include Kuwait in 1986 – 88 (Anonymous, 1988) and Egypt in 1988 (Salem, 1989). An outbreak of LSD occurred in Israel in 1989 (Shimshony, 1989). For the first time, the disease was eradicated by slaughter of all infected

and contact animals and vaccination (Yeruham *et al.*, 1995), and the other most recently in Egypt 2006 (Brenner *et al.*, 2006).

LSD was first observed in the western part of Ethiopia (southwest of lake Tana) in 1983 (Mebratu *et al.*, 1984). It has now spread to almost all the regions and agro ecological zones (MOARD, 2000 – 2007). Vaccination is classically used to control outbreaks when ever they occur. Few epidemiological studies have been carried out since the disease has become established in the country, with limited scope in terms of the diverse agro-ecological and producing systems. Studies based on clinical disease observation around Nekemt town have reported a prevalence of 7.02% (Regassa, 2003). Another study based on seroprevalence in Southern Ethiopia reported a prevalence of 6% (Gari *et al.*, 2008). Targeted sampling from outbreak areas around southern range-lands, Wolliso town and North Ethiopia reported prevalence's of 11.6%, 27.9% and 28%, respectively (Beshahwured, 1991; Asegid, 1991; Gari *et al.*, 2008).

Taking into an account the country wide distribution of the infection and the size and structure of the cattle population in Ethiopia it is likely that LSD is one of the most economically important livestock diseases in the country (Gari *et al.*, 2008). Since there is evidence of the movement of strains between species and recombination between strains in the field (Tulman *et al.*, 2001), it is desirable to conduct in-depth investigation into the epidemiology and economics of LSD so as to take the necessary control measures.

Thus, the present study was conceived with the following objectives:

- To determine the prevalence of LSD in the populations of the study areas
- To assess the financial losses due to LSD in the intensively and extensively managed farms. Based on the perspectives of livestock owners.

2. LITERATURE REVIEW

2.1. Etiology

2.1.1. Classification and taxonomy

Capri pox viruses represent one of the eight genera within the chordo pox virus subfamily of the *Poxviridae*. The Capripox virus genus is currently comprised of lumpy skin disease virus (LSDV), sheep pox virus (ShPV), and goat pox virus (GPV). These viruses are responsible for some of the most economically significant disease of domestic ruminants in Africa and Asia (Fenner, 1996). Capripox virus infections are generally host specific and they have specific geographic distribution (Davies, 1991; Carn, 1993; Coetzer *et al.*, 1994). Capripox viruses are however, serologically indistinguishable from each other. They are able to induce hetrologus cross-protection, and in some instances they are experimentally able in some to cross-infect susceptible hosts (Capstick, 1959; Davies, 1982; Davies, 1991; Carn, 1993). The restriction fragment analysis and limited DNA sequencing data support this close relationship between capripox viruses (Black *et al.*, 1986). However, the molecular analysis of capripox viruses to determine the specificity of host range and virulence remain requires elucidation (Davies, 1982).

2.1.2. Morphology

The capripox virion is brick shaped, covered in short tubular elements and measures approximately 290 × 270 nm. A host-cell-derived membrane may surround some of the virions, and as many as possible should be examined to confirm their appearance (Kitching and Smale, 1986).

The virions of capripox virus are indistinguishable from those of orthopox virus, but, apart from vaccinia virus and cowpox virus, which are both uncommon in cattle and do not cause generalised infection, no other orthopox: virus causes lesions in cattle. However, vaccinia virus may cause generalised infection in young immuno-compromised calves. In contrast, orthopox viruses commonly cause buffalo pox in domestic buffalo. This disease usually manifests itself as

pock lesions on the teats, but may also cause skin lesions at other sites such as the perineum, the medial aspects of the thighs and the head. Orthopox viruses that cause buffalo pox cannot be distinguished from capripox virus by electron microscopy. The virions of parapox virus that cause bovine papular stomatitis and pseudocowpox are smaller, oval in shape and each is covered in a single continuous tubular element that appears as striations over the virion. The capripox virus is also distinct from the herpesvirus that causes pseudo-LSD (Allerton – herpes mammillitis (Gershon and Black, 1987).

2.1.3. Structure and Composition

Lumpy skin disease virus (LSDV) is a member of the capripox virus of the *Poxviridae*. The 151-kbp LSDV genome consists of a central coding region bounded by identical 2.4 kbp-inverted terminal repeats and contains 156 putative genes. Comparison of LSDV with chordopox viruses of other genera reveals 146 genes which encode proteins involved in transcription and mRNA biogenesis, nucleotide metabolism, DNA replication, protein processing, virion sturucture and assembly, and viral virulence and host range. In the central genomic region, LSDV genes share a high degree of colinearity and amino acid identity (average of 65%) with genes of other known mammalian pox viruses, particularly suipox virus and leporipox viruses. In the terminal regions, colinearity is disrupted and pox virus homologues are either absent or share a lower percentage of amino acid identity (average of 43%). Most of these differences involve genes and gene families with similar functions involving viral virulence and host range (Black *et al.*, 1986).

2.2. Epidemiology

2.2.1. Host range

The LSDV contains a number of potential host range genes with equivalent functions of either modulating or evasion of the host immune responses, in modulating or inhibiting of host cell apoptosis, and in aspects of cell and/or tissue tropism (Fleming *et al.*, 2000). Many potential LSDV host range genes are similar in sequence and in terminal genomic location to genes present

in other pox viruses. However, LSDV encodes unique complement genes which dictate its specificity of host range properties (Moore *et al.,* 1993).

Lumpy skin disease is a disorder of cattle. There is inconclusive evidence regarding the infection of water buffalo (*Bubalus*) with LSDV. The African Cape buffalo *(Synercus caffer)* and other wild ungulates have not been infected during epidemics of LSD in Africa. However, experimental infection of some species is possible (Davies, 1991).

2.2.2. Source of infection and mode of transmission

The LSDV appears to be mainly transmitted and spread mechanically by biting insects (Mellor *et al.,* 1987; Chihota *et al.,* 2001, 2003). The direct transmissions by contacts between animals are insignificant. The occurrences of epidemics of LSD are associated with rainy seasons. The disease spreads along river basins and areas conducive to insect multiplication (Davies, 1982; Salem, 1989). Experimentally, the *Stomoxys calcitrans* transmitted LSDV but, neither biting lice (*Mallophaga spp.*), sucking lice (*Damalinia spp.*), nor *culicoides nubeculosis* did not (Kitching and Mellor, 1986).

Under experimental conditions, it was demonstrated that no LSDV was transmitted and nor immunity was generated in naive animals that were housed with infected animals in the absence of suitable vector insects (Carn and Kitching, 1995b). Animals that did not show clinical signs and did not shed virus in oral, nasal nor viruses were found on by swabbing conjunctiva (Caran and Kitching, 1995a). The LSDV can be isolated from the semen of infected animals (Irons *et al.,* 2005; Osuagwuh *et al.,* 2007), and thus, transmission may occur by this route. The transmission of LSDV between cattle is inefficient, and arthropod-vector transmission is significant during epidemics (outbreaks) and can spread the LSD into non-endemic regions (Davies, 1991). The spread can also occur through trade of infected animals and animal products such as wool and hides, as well as through the movements of potential insect vectors (Babiuk *et al.,* 2008).

2.2.3. Risk factors

The important risk factors in the occurrence of LSD are virus isolates, the breed of cattle, the immunological status of the population, the vector insects involved in the transmission, communal grazing and watering points, cattle movement, agro-climate, the hosts reaction to the piercing pain from the fly's and introduction of new animals to the herd (Gari *et al.,* 2008). Certain breeds of cattle are more susceptible than others, especially those that are thin-skinned such as Jersey and Guernsey breeds and African Sanga cattle such as the fogera in Ethiopia (Davies, 1991).

An additional outbreak of LSD occurred in Egypt in 2006, having been introduced with foot and mouth disease by cattle imported form Ethiopia, and spread to Israel (World Animal Health Information data base, OIE) creating a real risk of LSDV establishing itself in the middle east and spreading into Asia and Europe (Kitching and Carn, 2004).

The impact of global climate change on insect vectors, establish or a route of transmission for LSD and speculated as possible for sheeppox and goatpox viruses (because of very high viral loads in the skin), suggest that there are real risks of further spread of this disease in to other geographic regions (Yeruham *et al.,* 1995).

The warm and humid climate in midland and lowland agro-climates has been considered as more favorable environment for the occurrence of large populations of biting flies than the cool temperature in the highlands (Gari *et al.,* 2008).

Introduction of new cattle was associated with an increase in the risk of disease introduction to the herd, as already reported for infectious diseases such as tuberculosis and paratuberculosis (Tiwari *et al.,* 2009).

Communal grazing and watering point utilization were found to be significantly associated with LSD occurrence. Sharing watering points, grazing post-harvest fields would allow contact and

intermingling of different herds that would probably increase the risk of exposure and enhance the virus transmission through speculated mechanical vectors (Chihota *et al.*, 2001).

The host's reaction to the piercing pain from the fly's bite would interrupt the insects feeding which would lead to the flies looking for other nearby hosts to complete their feeding, allowing the transmission of the infection from infected to susceptible animals (Waret-Szkuta *et al.*, 2009). Contamination of the pasture and water could be considered as a potential risk in communal grazing and watering point utilization despite the fact that contagious transmission is considered to be inefficient route of transmission (Davies, 1991; Babiuk *et al.*, 2008).

2.2.4. Occurrence

Lumpy skin disease (LSD) was first seen in Zambia in 1929, spreading into Botswana by 1943 (Morris, 1931), and then into South Africa, where it affected over eight million cattle causing major economic loss. In 1957 it entered Kenya, associated with an outbreak of sheep pox (Weiss, 1968). In 1970 LSD spread north into the Sudan, by 1974 it had spread west as far as Nigeria, and in 1977 was reported from Mauritania, Mali, Ghana and Liberia. Another epizootic of LSD between 1981 and 1986 affected Tanzania, Kenya, Zimbabwe, Somalia and the Cameroon, with reported mortality rates in affected cattle of 20%. However, the true extent of this epizootic was not clear, and it probably affected a considerable area of central Africa. In 2000/2001, another large outbreak spread across sub-Saharan Africa (Coetzer, 2004). In 1988 LSD became established in Egypt, and in 1989 a single outbreak was reported in Israel. LSD must be considered to have the potential to become established outside Africa. The principal method of transmission is mechanical by arthropod vector (Carn and Kitching, 1995b; Chihota *et al.*, 2001).

2.2.5. Course of infection and pathogenesis

In the acutely infected animal, there is initial pyrexia, which may exceed 41°C and persist for 1 week. The incubation period under field conditions has not been reported, but following inoculation is 6–9 days until the onset of fever. A rhinitis and conjunctivitis develop, and in lactating cattle there is a marked reduction in milk yield. Nodules of 2–5 cm in diameter develop

over the body, particularly on the head, neck, udder and perineum between 7 and 19 days after virus inoculation (Coetzer, 2004).

These nodules involve the dermis and epidermis and may initially exude serum, but over the following 2 weeks may become necrotic plugs that penetrate the full thickness of the hide. All the superficial lymph nodes are enlarged, the limbs may be oedematous and the animal is reluctant to move. The nodules on the mucous membranes of the eyes, nose, mouth, rectum, udder and genitalia quickly ulcerate, and by then all secretions contain LSD virus. On the appearance of clinical signs, the discharge from the eyes and nose becomes mucopurulent, and keratitis may develop. Nodules may also develop in the mouth, subcutis and muscle, in the trachea and alimentary tract, particularly the abomasum, and in the lungs, resulting in primary and secondary pneumonia. Pregnant cattle may abort, and there are reports of aborted fetuses being covered in nodules. Bulls may become permanently or temporarily infertile and the virus can be excreted in the semen for prolonged periods (Irons *et al.*, 2005). Recovery from severe infection is slow; the animal is emaciated, may have pneumonia and mastitis, and the necrotic plugs of skin, which may have been subject to fly strike, are shed leaving deep holes in the hide (Prozesky and Barnard, 1982).
LSD virus is not transmissible and infectious to humans (Regnery, 2007)

2.2.6. Immunity

Animals that recover for a virulent capripoxvirus infection generate lifelong immunity consisting of both antibody and cellular immunity, which protects the animals form all capripoxvirus isolates (kitching *et al.*, 1987a). Capripoxvirus infection can be prevented by the administration of anit-capripoxvirus serum (Kitching, *et al.*, 1986), but cell-mediated immunity is likely the most significant component in recovery from infection and in long-term protection, evidenced by the protection afforded by vaccination where the presence of specific antibodies cannot be detected (using existing serological tests) (Roth and Spickler, 2003).

Immunity to LSD infection is predominantly drive by a cell-mediated response. Humoral immunity remains detectable only for 7 months (OIE, 2008). Serological studies with vaccinated

cattle have shown that many animals resist challenge with virulent LSDV while they have no detectable antibody (Davies, 1991). Thus although LSD surveillance based on serological results would probably not have shown the long-standing infections and the true immunological status of the target population (Gari *et al.*, 2008).

2.2.7. Morbidity and mortality

Morbidity and mortality of the disease vary considerably depending on the breed of cattle, the immunological status of the population, insect vectors involved in the transmission and isolates of the virus. In endemic areas morbidity is usually around 10% and mortality ranges between 1% and 3% (Davies, 1991; Babiuk *et al.*, 2008). However, generally morbidity for LSD varies form 3% to 85% (Mac Owen, 1959).

2.3. Clinical signs

Lumpy skin disease virus causes in apparent to severe disease in cattle. All ages of cattle can be affected, but young calves are usually more severely affected .The severity of the disease depends on the dose of the inoculum as well as the susceptibility of the host (*Bos taurus* is more susceptible than *Bos indicus*) and the route of exposure. A fever 104 to 107 ^{o} F (40-41.5 ^{o} C) can occur and can be transitory or last up to 4 weeks. Generally within 2 days after the appearance of the fever, swellings or nodules 1 to 5 cm in diameter appears in the skin and generalization occurs. Depression, anorexia, excessive salivation, oculonasal discharge, agalactia, and emaciation are presented (Carn and Kitching, 1995a). Nodules 1 to 7 cm in diameter may occur anywhere on the body but especially in the skin of the muzzle, nares, back, legs, scrotum, perineum, eyelids, lower ear, nasal and oral mucosa, and tail. The hair stands erect over early skin lesions. The nodules are painful and involve the epidermis, dermis, and subcutaneous tissue and may even involve the musculature. As the disease progresses, the nodules become necrotic, and eventually a deep scab forms; this lesion is called a sitfast. Secondary bacterial infection can complicate healing and recovery. Lesions on the teats can result in severe secondary bacterial infection with loss of the quarter owing to mastitis (Prozesky and Barnard, 1982).

Where extensive generalization occurs, animals can become lame and reluctant to move because of edema. Lameness also may result from inflammation of the tendons, tendon sheaths (tendosynovitis), joints (synovitis), and laminae (laminitis). Severe edema in the brisket and legs can occur. If secondary bacterial infection develops in the tendon sheaths and joints, permanent lameness may result. Superficial lymph nodes such as the mandibular, parotid, prescapular, and prefemoral nodes, draining affected areas of skin become enlarged 4 to 10 times normal size (Prozesky and Barnard, 1982).

Abortion may occur as the result of prolonged fever. Davies has reported intrauterine infection of late-term fetuses in which calves are born with LSD lesions. Temporary or permanent sterility in bulls can result from the fever or lesions of the reproductive organs. Cows may not come into estrus for several months after LSD (Davies, 1991). The lesions may persist in various stages over a course of 4 to 6 weeks. Final resolution of lesions may take 2 to 6 months, and nodules can remain visible 1 to 2 years. Permanent damage to the hide is inevitable in clinical cases. The disease has high economic costs due to production losses and chronic debilitation. Milk production ceases and permanent damage can occur to the hides (Ali *et al.*, 1990).

2.4. Pathology

Skin nodules have congestion, hemorrhage, edema, and vasculitis with consequent necrosis and involve all layers of the epidermis, dermis, subcutaneous tissue, and often adjacent musculature. Lymph nodes draining affected areas are enlarged up to 10 times normal size with extensive lymphoid proliferation, edema, congestion, and hemorrhage (Burdin, 1959).

Mucous membranes of the oral and nasal cavities can have pox lesions that coalesce in severe cases. Pox lesions may occur in the pharynx, epiglottis, and trachea .Pox lesions are not easily visualized in the lungs but appear as focal areas of atelectasis and edema. In severe cases, pleuritis can occur with enlargement of the mediastinal lymph nodes (Prozesky and Barnard, 1982). Synovitis and tendosynovitis with fibrin in the synovial fluid can occur. Pox lesions can be present in the testicles and urinary bladder (Haig, 1957).

2.5. Diagnosis

2.5.1. Field diagnosis

A tentative diagnosis of LSD can be made based upon clinical signs. A contagious disease with generalized skin nodules having a characteristic inverted conical necrosis of skin nodules (sitfast), persistent fever, emaciation, and low mortality suggests LSD (Davies, 1991).

2.5.2. Specimens for laboratory

Sample collection, submission and preparation

Material for virus isolation and antigen detection should be collected by biopsy or at post-mortem from skin nodules, lung lesions or lymph nodes. Samples for virus isolation and antigen-detection enzyme-linked immunosorbent assay (ELISA) should be collected within the first week of the occurrence of clinical signs, before the development of neutralising antibodies (Davies, 1991). Samples for genome detection by polymerase chain reaction (PCR) may be collected when neutralising antibody is present. Following the first appearance of the skin lesions, the virus can be isolated for up to 35 days and viral nucleic acid can be demonstrated by PCR for up to 3 months. Buffy coat from blood collected into heparin or EDTA (ethylene diamine tetra-acetic acid) during the viraemic stage of LSD (before generalisation of lesions or within 4 days of generalisation), can also be used for virus isolation. Samples for histology should include tissue from the surrounding area and should be placed immediately following collection into ten times the sample volume of 10% formalin (Tuppurainen *et al.*, 2005).

Tissues in formalin have no special transportation requirements. Blood samples with anticoagulant for virus isolation from the buffy coat should be placed immediately on ice and processed as soon as possible. In practice, the samples may be kept at 4°C for up to 2 days prior to processing, but should not be frozen or kept at ambient temperatures. Tissues for virus isolation and antigen detection should be kept at 4°C, on ice or at -20°C. If it is necessary to transport samples over long distances without refrigeration, the medium should contain 10%

glycerol; the samples should be of sufficient size that the transport medium does not penetrate the central part of the biopsy, which should be used for virus isolation (Walace and Viljoen, 2005).

Material for histology should be prepared by standard techniques and stained with haematoxylin and eosin (H&E) (Burdin, 1959). Lesion material for virus isolation and antigen detection is minced using sterile scissors and forceps and then ground in a sterile pestle and mortar with sterile sand and an equal volume of sterile phosphate buffered saline (PBS) containing sodium penicillin (1000 international units [IU]/ml), streptomycin sulphate (1 mg/ml), mycostatin (100 IU/ml) or fungizone (2.5 μg/ml) and neomycin (200 IU/ml). The suspension is freeze–thawed three times and then partially clarified by centrifugation using a bench centrifuge at 600 ***g*** for 10 minutes. Buffy coats may be prepared from unclotted blood by centrifugation at 600 ***g*** for 15 minutes, and the buffy coat carefully removed into 5 ml of cold double-distilled water using a sterile Pasteur pipette. After 30 seconds, 5 ml of cold double-strength growth medium is added and mixed. The mixture is centrifuged at 600 ***g*** for 15 minutes, the supernatant is discarded and the cell pellet is suspended in 5 ml growth medium, such as Glasgow's modified Eagle's medium (GMEM). After centrifugation at 600 ***g*** for a further 15 minutes, the resulting pellet is suspended in 5 ml of fresh GMEM. Alternatively, the buffy coat may be separated from a heparinised sample by using a Ficoll gradient (Davies *et al.*, 1971)

2.5.3. Laboratory diagnosis

a*) Culture*

LSD virus will grow in tissue culture of bovine, ovine or caprine origin, although primary or secondary culture of lamb testis (LT) cells are considered to be the most susceptible, particularly those derived from a breed of wool sheep. Sample material prepared as above, i.e. 1 ml of clarified supernatant or buffy coat, is inoculated on to a 25 cm^2 culture flask at 37°C and allowed to absorb for 1 hour. The culture is then washed with warm PBS and covered with 10 ml of a suitable medium, such as GMEM, containing antibiotics and 2% fetal calf serum. If available, tissue culture tubes containing LT cells and a flying cover-slip, or tissue culture microscope slides, are also infected (Tuppurainen *et al.*, 2005).

The flasks are examined daily for 14 days for evidence of cytopathic effect (CPE) and the medium is replaced if it appears to be cloudy. Infected cells develop a characteristic CPE consisting of retraction of the cell membrane from surrounding cells, and eventually rounding of cells and margination of the nuclear chromatin. At first only small areas of CPE can be seen, sometimes as soon as 2 days after infection; over the following 4–6 days these expand to involve the whole cell sheet. If no CPE is apparent by day 14, the culture should be freeze–thawed three times, and clarified supernatant inoculated on to fresh LT culture. At the first sign of CPE in the flasks, or earlier if a number of infected cover-slips are being used, a cover-slip should be removed, fixed in acetone and stained using H&E. Eosinophilic intracytoplasmic inclusion bodies, which are variable in size but up to half the size of the nucleus and surrounded by a clear halo, are diagnostic for poxvirus infection. The CPE can be prevented or delayed by inclusion in the medium of specific anti-LSD serum. The herpesvirus of pseudo-LSD produces a Cowdry type A intranuclear inclusion body. Formation of syncytia is not a feature of capripoxvirus infection, unlike the herpesvirus causing pseudo-LSD. Strains of capripoxvirus that cause LSD have been adapted to grow on the chorioallantoic membrane of embryonated chicken eggs and African green monkey kidney (Vero) cells. This is not recommended for primary isolation. (Tuppurainen *et al.*, 2005).

Electron microscopy

Before centrifugation, material from the original biopsy suspension is prepared for examination under the transmission electron microscope by floating a 400-mesh hexagon electron microscope grid, with pileoformcarbon substrate activated by glow discharge in pentylamine vapour, on to a drop of the suspension placed on parafilm or a wax plate. After 1 minute, the grid is transferred to a drop of Tris/EDTA buffer, pH 7.8, for 20 seconds and then to a drop of 1% phosphotungstic acid, pH 7.2, for 10 seconds. The grid is drained using filter paper, air-dried and placed in the electron microscope. The capripox virion is brick shaped, covered in short tubular elements and measures approximately 290 × 270 nm. A host-cell-derived membrane may surround some of the virions, and as many as possible should be examined to confirm their appearance (Kitching and Smale, 1998).

The virions of capripoxvirus are indistinguishable from those of orthopoxvirus, but, apart from vaccinia virus and cowpox virus, which are both uncommon in cattle and do not cause generalised infection, no other orthopoxvirus causes lesions in cattle. However, vaccine virus may cause generalised infection in young immunocompromised calves. In contrast, orthopoxviruses are a common cause of skin disease in domestic buffalo causing buffalo pox, a disease that usually manifests as pock lesions on the teats, but may cause skin lesions at other sites, such as the perineum, the medial aspects of the thighs and the head. Orthopoxviruses that cause buffalo pox cannot be readily distinguished from capripoxvirus by electron microscopy. The virions of parapoxvirus that cause bovine papular stomatitis and pseudocowpox are smaller, oval in shape and each is covered in a single continuous tubular element that appears as striations over the virion. The capripoxvirus is also distinct from the herpesvirus that causes pseudo-LSD (Allerton – herpes mammillitis) (Gershon and Black, 1987).

b) Immunological methods

Fluoresent antibody tests

Capripoxvirus antigen can also be identified on the infected cover-slips or tissue culture slides using fluorescent antibody tests. Cover-slips or slides should be washed and air-dried and fixed in cold acetone for 10 minutes. The indirect test using immune cattle sera is subject to high background color and nonspecific reactions. However, a direct conjugate can be prepared from sera from convalescent cattle (or from sheep or goats convalescing from capripox) or from rabbits hyperimmunised with purified capripoxvirus. Uninfected tissue culture should be included as a negative control as cross-reactions, due to antibodies to cell culture, can cause problems (Davies *et al.*, 1971).

Agar gel immunodiffusion

An agar gel immunodiffusion (AGID) test has been used for detecting the precipitating antigen of capripoxvirus, but has the disadvantage that this antigen is shared by parapoxvirus. Agarose (1%) is prepared in borate buffer, pH 8.6, dissolved by heating, and 2 ml is poured on to a glass

microscope slide (76 × 26 mm). When the agar has solidified, wells are cut to give a six-well rosette around a central well. Each well is 5 mm in diameter, with a distance of 7 mm between the middle of the central well and the middle of each peripheral well. The wells are filled as follows: 18 µl of the 10% lesion suspension is added to three of the peripheral wells, alternately with positive control antigen, and 18 µl of positive capripoxvirus control serum is added to the central well. The slides are placed in a humid chamber at room temperature for 48 hours, and examined for visible precipitation lines using a light box. The test material is positive if a precipitation line develops with the control serum that is confluent with that produced by the positive control antigen (Kitching *et al.*, 1986).

To prepare antigen for the AGID, one of two 125 cm^2 flasks of LT cells is infected with capripoxvirus, and harvested when there is 90% CPE (8–12 days). The flask is freeze–thawed twice, and the cells are shaken free of the flask. The contents are centrifuged at 4000 ***g*** for 15 minutes, most of the supernatant is decanted and stored, and the pellet is resuspended in the remaining supernatant. The cells should be lysed using an ultrasonic probe for approximately 60 seconds. This homogenate is then centrifuged as before, the resulting supernatant being pooled with that already collected. The pooled supernatant is then added to an equal volume of saturated ammonium sulphate at pH 7.4 and left at 4°C for 1 hour. This solution is centrifuged at 4000 ***g*** for 15 minutes, and the precipitate is collected and resuspended in a small volume of 0.8% saline for use in the AGID test. The uninfected flask is processed in an identical manner throughout, to produce a tissue culture control antigen (Kitching *et al.*, 1986).

Enzyme-linked immunosorbent assay

Following the cloning of the highly antigenic capripoxvirus structural protein P32, it is possible to use expressed recombinant antigen for the production of diagnostic reagents, including the raising of P32 monospecific polyclonal antiserum and the production of monoclonal antibodies (MAbs). These reagents have facilitated the development of a highly specific ELISA. Using hyperimmune rabbit antiserum, raised by inoculation of rabbits with purified capripoxvirus, capripox antigen from biopsy suspensions or tissue culture supernatant can be trapped on an ELISA plate. The presence of the antigen can then be indicated using guinea-pig serum, raised

against the group-specific structural protein P32, commercial horseradishperoxidase-conjugated rabbit anti-guinea-pig immunoglobulin and a chromogen/substrate solution (Carn, 1995).

c) Nucleic acid recognition methods

The PCR is a fast and sensitive method for the detection of capripoxviurs genome in EDTA blood, biopsy or tissue culture samples. However, it does not allow differentiation between LSD and sheep and goat pox viruses. Primers for the viral attachment protein gene and the viral fusion protein gene are specific for all the strains within the genus *Capripoxvirus* (Ireland and Binepal, 1998). By the use of sequence and phylogenetic analysis; strains of virus can be identified; this work should be done in a Reference Laboratory. Virus isolates can also be characterised by comparing the genome fragments generated by HindIII digestion of their purified DNA (Black *et al.*, 1986). This technique has identified differences between isolates from the different species, but these are not consistent and there is evidence of the movement of strains between species and recombination between strains in the field. The LSD virus genome contains 156 putative genes (Tulman *et al.*, 2001).

d) Serological tests

All the viruses in the *Capripoxvirus* genus share a common major antigen for neutralising antibodies and it is not possible to distinguish strains of capripoxvirus from cattle, sheep or goats using serological techniques (Tuppurainen *et al.*, 2005).

Virus neutralization

A test serum can either be titrated against a constant titre of capripoxvirus (100 $TCID_{50}$ [50% tissue culture infective dose]) or a standard virus strain can be titrated against a constant dilution of test serum in order to calculate a neutralisation index. Because of the variable sensitivity of tissue culture to capripoxvirus, and the consequent difficulty of ensuring the use of 100 $TCID_{50}$, the neutralisation index is the preferred method. The

test is described using 96-well flat-bottomed tissue-culture grade microtitre plates, but it can be performed equally well in tissue culture tubes with the appropriate changes to the volumes used, although it is more difficult to read an end-point in tubes. The use of Vero cells in the virus neutralisation test has been reported to give more consistent results (Davies *et al.*, 1971).

Agar gel immuno diffusion

The AGID test cannot be recommended as a serological test for the diagnosis of LSD because of the crossreaction with antibody to bovine papular stomatitis and pseudocowpox virus. A consequence of this crossreaction is false-positive results. Lack of sensitivity of the test can also lead to false-negative results (Kitching *et al.*, 1986).

Indirect fluorescent antibody test

Capripoxvirus-infected tissue culture grown on flying cover-slips or tissue culture microscope slides can be used for the indirect fluorescent antibody test. Uninfected tissue culture control, and positive and negative control sera, should be included in the test. The infected and control cultures are fixed in acetone at –20°C for 10 minutes and stored at 4°C. Dilutions of test sera are made in PBS, starting at 1/20 or 1/40, and positives are identified using an anti-bovine gamma-globulin conjugated with fluorescein isothiocyanate. Antibody titres may exceed 1/1000 after infection. Sera may be screened at 1/50 and 1/500. Cross-reactions can occur with orf (contagious pustular dermatitis of sheep virus), bovine papular stomatitis and perhaps other poxviruses (Ireland and Binepal, 1998).

Western blot analysis

Western blotting of test sera against capripoxvirus-infected cell lysate provides a sensitive and specific system for the detection of antibody to capripoxvirus structural proteins, although the test is expensive and difficult to carry out (Kitching *et al.*, 1986).

Capripoxvirus-infected LT cells should be harvested when 90% CPE is seen, freeze–thawed three times, and the cellular debris pelleted by centrifugation. The supernatant should be

decanted, and the proteins should be separated by SDS/PAGE (sodium dodecyl sulphate/polyacrylamide gel electrophoresis). A vertical discontinuous gel system, using a stacking gel made up of acrylamide 5% in Tris (125 mM), pH 6.8, and SDS (0.1%), and a resolving gel made up of acrylamide (10–12.5%) in Tris (560 mM), pH 8.7, and SDS (0.1%), is recommended for use with a glycine running buffer containing Tris (250 mM), glycine (2 M), and SDS (0.1%). Samples of supernatant should be prepared by boiling for 5 minutes with an appropriate lysis buffer prior to loading. Alternatively, purified virus or recombinant antigens may replace tissue-culture derived antigen (Davies *et al.*, 1971).

Molecular weight markers should be run concurrently with the protein samples. The separated proteins in the SDS/PAGE gel should be transferred electrophoretically to a nitrocellulose membrane (NCM). After transfer, the NCM is rinsed thoroughly in PBS and blocked in 3% bovine serum albumin (BSA) in PBS, or 5% skimmed milk powder in PBS, on a rotating shaker at 4°C overnight. The NCM can then be separated into strips by employing a commercial apparatus to allow the concurrent testing of multiple serum samples, or may be cut into strips and each strip incubated separately thereafter. The NCM is washed thoroughly with five changes of PBS for 5 minutes on a rotating shaker, and then incubated at room temperature on the shaker for 1.5 hours, with the appropriate serum at a dilution of 1/50 in blocking buffer (3% BSA and 0.05% Tween 20 in PBS; or 5% milk powder and 0.05% Tween 20 in PBS). The membrane is again thoroughly washed and incubated (in blocking buffer) with anti-species immunoglobulin horseradish-peroxidase conjugated immunoglobulins at a dilution determined by titration. After further incubation at room temperature for 1.5 hours, the membrane is washed and a solution of diaminobenzidin tetrahydrochloride (10 mg in 50 ml of 50 mm Tris/HCl, pH 7.5, and 20 µl of 30% [v/v] hydrogen peroxide) is added. This is then incubated for approximately 3–7 minutes at room temperature on a shaker with constant observation, and the reaction is stopped by washing in PBS before excessive background colour is seen. A positive and negative control serum should be used on each occasion (Ireland and Binepal, 1998).

Positive test samples and the positive control will produce a pattern consistent with reaction to proteins of molecular weights 67, 32, 26, 19 and 17 kDa – the major structural proteins of capripoxvirus – whereas negative serum samples will not react with this pattern. Hyperimmune serum prepared against parapoxvirus (bovine papular stomatitis, pseudocowpox) will react with

some of the capripoxvirus proteins, but not the 32 kDa protein that is specific for capripoxvirus (Kitching *et al.*, 1986).

Enzyme-linked immunosorbent assay

A capripoxvirus antibody ELISA has been developed using the expressed structural protein P32 of capripoxvirus and MAbs raised against the P32 protein (Carn *et al.*, 1994)

2.5.4. Differential diagnosis

Listed below are several diseases that should be considered in the differential diagnosis of LSD: As described by James (1998), as follows.

Bovine herpes mammillitis (also called Allerton virus infection caused by Bovine Herpesvirus-2) — The lesions are superficial (involving only the epidermis) and occur predominantly on the cooler parts of the body such as teats and muzzle. Generalized skin lesions can occur accompanied by a transient fever (1 to 3 days). Resolution of the lesion is rapid and results in focal alopecia but no hide damage.

Streptotrichosis (Dermatophilus congolensis infection) — lesions are superficial (often moist and appear as crusts) scabs or 0.5- to 2-cm diameter accumulations of keratinized material. Lesions are common in the skin of the neck, axillary region, inguinal region, and perineum. The organism can be demonstrated by Giemsa staining.

Ringworm — The lesions of ringworm in cattle are grayish, raised, plaque-like, and often pruritic. The organism can be demonstrated with a silver stain.

Hypoderma bovis infection —The parasitic fly larvae of this parasite have a predilection to migrate to the dorsal skin of the back. They cause a nodule with a small central hole through which the larva exits the body, which results in significant hide damage.

Photosensitization — Dry, flaky, inflamed areas are confined to the nonpigmented parts of the skin.

Bovine papular stomatitis — Pox-like lesions occur in the skin of the muzzle, oral cavity, and esophagus. There is no generalized disease.

Insect bites — The trauma from insect bites causes local inflammation, edema, and pruritus. Insects seldom bite mucous membranes.

Urticaria — Delayed hypersensitivity reactions can be confused with LSD. Such lesions generally resolve within 3 to 5 days. An example of this was described by Shimshony (1989) where allergic reactions occurred after vaccination with a foot-and-mouth disease vaccine.

Besnoitiosis (Globidiosis) — Thick-walled cysts in the skin are caused by sporozoan parasites of the genus *Besnoitia*, which are transmitted mechanically by certain biting flies. Histologic sections will reveal the parasites.

2.6. Control and prevention

2.6.1. Treatment

Treatment is directed at preventing or controlling secondary infection. Animals infected with LSDV generally recover (mortality is usually less than 3 percent). Complete recovery may take several months and may be prolonged where secondary bacterial infection occurs. Loss of production results from severe emaciation, lowered milk production, extensive damage to hides, and loss of draft from lameness. It may take up to 6 months for animals severely affected by LSDV to recover fully (Diesel, 1949).

2.6.2. Vaccination

Two live attenuated strains of capripoxvirus have been used as vaccines specifically for the control of LSD (Carn, 1993). A strain of Kenya sheep and goatpox virus passaged 18 times in LT or fetal calf muscle cells, and a strain from South Africa, passaged 60 times in lamb kidney cells and 20 times on the chorioallantoic membrane of embryonated chicken eggs. All strains of capripoxvirus examined so far, whether of bovine, ovine or caprine origin, share a major neutralising site, so that animals recovered from infection with one strain are resistant to infection with any other strain. Consequently, it is possible to protect cattle against LSD using strains of capripoxvirus derived from sheep or goats (Coakley and Capstick 1961). In 1989 and 1990 the Romanian strain of sheep pox vaccine was used to help control the LSD outbreak in Egypt (Michael *et al.*, 1996).

However, it is essential to carry out controlled trials, particularly using the most susceptible breeds in peak lactation, prior to introducing a vaccine strain not usually used in cattle. Protection following vaccination is probably life long, although as immunity wanes, local capripoxvirus replication will occur at the site of inoculation, but the virus will not become generalised. Both strains of capripoxvirus used routinely as vaccines can produce a large local reaction at the site of inoculation in *Bostaurus* breeds (Coetzer, 2004), which some stock owners find unacceptable. This has discouraged the use of vaccine even though the consequences of an outbreak of LSD are invariably more severe (Carn, 1993). A new generation of capripox vaccines is being developed that uses the capripoxvirus genome as a vector for the genes of other ruminant pathogens, for instance genes of rinderpest and peste des petits ruminants viruses. The recombinant vaccine will provide protection against LSD and rinderpest in a single vaccination (Wallace and Viljoen, 2005).

2.6.3. Eradication

The most likely way for LSD to enter a new area is by introduction of infected animals. Biting insects that have fed on infected cattle may travel and be blown for substantial distances. It is likely that LSD spread to Israel via contaminated insects blown across the Sinai Desert. The

movement of contaminated hides represents another potential means for this resistant virus to move (Shimshony, 1989).

If LSD is confirmed in a new area before extensive spread occurs, the area should be quarantined, infected and exposed animals slaughtered, and the premises cleaned and disinfected. Vaccination of susceptible animals within the quarantine should be considered (Davies, 1982).

If the disease has spread over a large area, the most effective means of controlling losses from LSD is vaccination. However, even with vaccination, consideration still should be given to eliminating infected and exposed herds by slaughter, proper disposal of animals and contaminated material, and by cleaning and disinfecting contaminated premises, equipment and facilities. In the Union of South Africa, the control of insects was not effective in preventing the spread of LSD, but current insecticides together with repellents aid in the prevention of the spread of LSD (House, 1990).

3. MATERIALS AND METHODS

3.1. Study area and study population

The study was conducted from October 2009 to April 2010. Two study sites with different farming systems were selected. The first study area was in Amhara Region (North Wello, South Wello and Oromia administrative zone, in northern part of Ethiopia) where the altitude ranges from 1400 to 2230 meters above sea level (Fig 1). Livestock production is extensive system where by animals of different species and age groups share common grazing land and watering point. The breed composition of the sub population is predominantly the local zebu breed. At the time of this study, lumpy skin diseases re-occurred as an outbreak in these study areas. Here animals 6 month to 2 years old are considered as young while, animals >2 years were considered as adults.

The second study sites were in Oromia Region (North Shoawa and East Shoawa zones in centeral part of Ethiopia) where the altitude ranges from 1300 to 2400m.a.s.level. There was concurrent occurrence of LSD and sheep pox outbreak in 2008/2009 in the area and vaccination program was given in place for the last 6 months. Livestock production is extensive and intensive, the intensive system consists of almost all exotic breeds for milk production and animals are indoor. Animals considered in the study were cattle of varying ages. Animals less than one year of age were considered as calves, one to three years old as either bulls or heifers and more than three years old were considered as either oxen or cows in both indigenous and exotic breeds of cattle. Body condition loss and production state losses were also made based on the description provided in the questionnaire format (Annex 1).

Figure 1. Map of Ethiopia and location of the study areas (shaded)

3.2. Study design and methodology

The current study was cross-sectional and retrospective ones. Blood was collected from 263 cattle and administration of questionnaires to 355 herd owners in the LSD outbreak areas. The outbreak occurred in all selected study districts of Ethiopia situated under different production systems. The epidemiological approach carried out generated information about the prevalence, incidence rate, case fatality rate and economic loss due to LSD. Prevalence of LSD occurrence was a proportion of the population at risk that was computed for each respective study areas and were compared. The economic module analysed the impact of LSD incidence from the perspective of herd owners. The losses of the local breed of animals and exotics were compared for the years 2008/2009 during which the LSD outbreaks occurred.

Three districts were selected from the northern study areas based on the occurrence of the LSD. The livestock production system was traditional livestock husbandry. In these study area there was no concurrent occurrence of LSD with sheep pox and hence they were used to determine the prevalence of LSD in the population during its epizootic period through IFAT and VNT.

Three districts from the central study areas which practiced both traditional livestock husbandry and peri-urban small scale dairy production system were selected for generation of the necessary data for retrospective study. These districts were selected based on the pre-known information records of recent LSD outbreak occurrence in the districts. The time for the study assumed a one year period to capture the variable costs and output losses due to LSD occurrence in both production systems. In the centeral study area there was concurrent occurrence of LSD with sheep pox and hence they were not used for serum collection due to fear of contamination and cross-reaction.

3.3. Sample size determination and sampling technique

The purpose of the sampling design was to obtain serological and questionnaire data from the northern and central study areas populations respectively in which LSD occurred and to calculate financial losses resulted from the disease

- Population 1 (P_1): In the northern study area, at the district level, the target Peasant Association (PA) were those with LSD outbreak history. In seven PAs selected using multi-stage sampling strategy from three districts, the herds and animals were randomly selected for sample collection. All animals above 6 months old and both sex groups were subjected to random sampling. In all the places, the sampling was carried out before the deployment of vaccination to control the outbreak. Here is no concurrent occurrence of sheep pox.

- Population 2 (P_2): In the central study area, 355 herd owners were selected from 25 PAs distributed in 3 districts which were selected from North and East Showa of Oromiya region that have known history of LSD outbreak on 2008/2009. The selection of the 25 PAs was performed using a multi-stage sampling strategy with four hierarchical stages. The region followed by districts were purposively selected to include the current LSD outbreak occurrence and different production systems. At the third level, the selection of PAs from each district was based on severe classical LSD occurrence and accessibility in consultation with district experts. The sample frame for PA was obtained from respective district agricultural offices. Finally, individual herd owners and their respective herds were selected for interview based on willingness to participate in the study. The questionnaire was administered by the researcher during face-to-face interviews using local language.

Sample size for blood collection were obtain by taking an average prevalence of 12%, (Gari *et al.*, 2008), absolute desired precision of 5% and confidence level of 95% for estimating prevalence. The sample size was calculated using the formula as follows:

Where, $$n = \frac{1.96^2 P\exp(1 - P\exp),}{d^2}$$

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

The calculated sample size for estimating prevalence was 131.5. In order to adjust the sample size that was required for this multi stage stratified random sampling method for an estimate of the disease prevalence at a 5% level of precision, the sample size was inflated two times. Therefore, a total of 263 animals were sampled proportionally for blood sampling.

3.4. Study methods and data sources

3.4.1. Serological Survey

Blood samples of 5–7 ml were collected in plain vacutainer tubes from the jugular veins. The samples were allowed to clot for 2–3 hrs at room temperature. Then the sera were extracted by spinning at 2500 rpm and preserved at -20^{0}C until tested.

Indirect fluorescent antibody test (IFAT)

The indirect fluorescent antibody test was used to detect serum antibodies against lumpy skin disease. Antibodies of capripox virus can be detected from day 2 after the onset of clinical signs and remain detectable for about 7 months, but a significant rise in titre is usually seen between days 21 and 42 (Lefe`vre *et al.*, 2003; OIE, 2004). The serum samples were processed carefully for the test. The antigen used to detect the serum antibody against lumpy skin disease was KS1 (Kenyan sheep pox virus) strain which is genetically identical with Neethling virus (Gershon and Black, 1988). The KS1 strain was obtained from CIRAD Laboratory and the lamb testis cells were infected using 50 µl of 100TCID50 viral suspension per well cultured in 96-well flat-bottomed tissue-culture grade microtitre plate. The infected monolayer cells were fixed after 48 hrs using 80% acetone. The test serum was diluted in 1/25 in 0.5% lamb serum blocking buffer (blocking buffer is to avoid the nonspecific background reaction) and each serum was tested in duplicate wells. The reference positive and negative control sera were also included in each plate. Fluorescein isothiocyanate conjugated anti-bovine gamma-globuline (IgG) of rabbit was diluted in 1/40 in 0.5% lamb serum blocking buffer. It was added to each well (Standard Operating Protocol of CIRAD). The plates were read using Zeiss Fluorescent microscope under 40x

magnification. The positive test serum appeared bright fluorescence foci where the antibody reacted with the virus and the negative serum appeared as dark field or dim gray foci.

Virus neutralization test (VNT)

Serial dilutions of the test sera were done in 1/5, 1/25, 1/125, 1/625 and 1/3125 and each serum was tested in duplicate wells. The KS1 strain virus in 100 $TCIDS_{50}$ was put in each well and a constant titration was maintained in each well. The Vero cell was used for the test and cultured in 96-well flat-bottomed tissue culture grade microtitre plates (OIE, 2004). The preference of Vero cells was that they are less sensitive to capripox virus and to reduce the problem of "breakthrough" in which the virus dissociate the antibody binding and relapse to infect the cells (OIE, 2004; Bhanuprakash *et al.*, 2006). The plates were incubated at 37^0C, at 5% carbon dioxide (CO_2) for 9 days. The plates were examined under inverted microscope for the presence of cytopathic effect (CPE) starting from day 4. The final reading was taken on day 9 and the result was recorded from the highest dilution which inhibited the CPE in both or either of the duplicate wells. The test result was recorded as the reciprocal of the log titration. The interpretation of the result is that the wells with no CPE in 1/25 and more dilutions were considered as positive serum. This indicates that the antibody against the LSD virus has reacted with the KSI virus and inhibited the growth of the virus not to produce CPE.

3.4.2. Questionnaire survey

The questionnaire was designed based on the previous knowledge of the disease and was pre-tested. It was then modified to include questions on factors either known or thought to influence the spread of LSD. It was carfully administered to collect the necessary data from the farmers

Thirteen questions were structured under six main sections, (Annex 1). The 'herd' was defined as a collective group of cattle from a single owners or a family member. When at least one animal in a herd was reported as having the infection in the past, the entire herd was considered infected. All the data were entered and stored electronically in Microsoft Excel XP 2003.

Epidemiological and economic data relevant to LSD were collected from the farmers, district agricultural experts and documented records.

- District cattle population, herd demographic structure, off-take rates and crude mortality rates,
- The number, sex and age of affected animals or subsequently died,
- Vaccination and treatment interventions that were applied during/after the courses of the disease.
- Estimated time elapse between the subsequent LSD out break occurrences and
- Estimated production losses (level of severity in affected animals) associated with LSD outbreaks were recorded.

Monetary value of all inputs and outputs were assessed based on market prices such as market price of cattle (of different age groups), products (milk and meat) and draft power were assessed. Cost of feed, labor, veterinary treatment were collected from respective district agricultural office and from other relevant institutions. Reduced in work output of draft oxen due to effect of the disease could be an important loss in sedentary crop- livestock production system. But, valuation of the production loss for draft power depended on the time of crop season at which the oxen had got sick and the degree of clinical severity of the disease. At present, vaccination cost for LSD and veterinary service charge did not go to private farmer expenditure and could be accounted as a cost incurred by the government in the analysis. Generally, evaluation of the physical effects and estimation of the production losses were based on the market price and economic values during the year 2009/10.

3.4.3. Economic assessment

The financial losses due to the outbreakof LSD effect (severity) were calculated (i.e. among affected population, the proportion of animals that were showed overt clinical disease and the production loss). Then the magnitude of physical disease effects were determined based on the annual incidence rate (Bennett and IJpelaar, 2005; Kivaria *et al.*, 2007).

1. case specific annual morbidity
2. case specific annual mortality
3. annual cost of treatment and vaccination
4. annual loss of milk production
5. annual loss of beef production
6. reduction in work output

Therefore, by using the following models shown below the financial losses were estimated

$$C = (M + B + W_{op}) + (V + T)$$

C: Direct economic losses

M: Milk loss

B: Beef loss

W_{op}: Work output

V: Vaccination costs

T: Treatment costs

$$(M + B + W_{op}) = P*I_d* I_e*E*U*$$

$$(V+T) = P*I_{tv}*U_{tv}$$

P = size of population at risk

I_d = annual incidence of disease as a proportion of the population at risk

I_e = incidence of disease effects as a proportion of the affected population (among affected animals, the proportion of animals that falls into different disease effect category i.e. severe; mild; low).

E= magnitude of physical disease effects (lit. of milk, kg of beef, working days)

U= unit value of lost output ($/lit. of milk lost, $/working days lost)

I_{tv}= proportion of population at risk treated/ vaccinated

U_{tv}= cost of treatment/ vaccination per animal.

3.5. Data management and analysis

Data obtained from, the test sera and questionnaire for the occurrence of LSD and its positive value in the study districts were used as outcome.

The data encoded and entered into Microsoft excel spread sheets for descriptive, statistical and economic analysis. The prevalence was defined as the proportion of the number of positive animals to the total number of animals that were examined through IFAT and expressed in percent.

Data from the national disease outbreak report database and from district agricultural office documentation were compared to assess whether these two sources agree about the occurrence of LSD in the selected districts. Data from both sources were then explored to investigate the disease distribution pattern and LSD occurrence history in the selected districts.

Descriptive statistics of the studied variables were obtained. Sero prevalence and animal level prevalence as well as the frequencies associated with the study variables were estimated.

Analysis for the associations of the risk factors with LSD was first done using Chi-square test for categorical variables.
Statistical analysis was conducted using SPSS version 11.5 and STATA version 8.0 (Stata Corp. USA, 1984-2003)

4. RESULTS

4.1. Seroprevalence

The result of IFAT and VNT in extensive farms of the northern study area showed that 41.8% (110/263) and 32.3% (85/263), respectively (Table 1). This blood result was taken in agreement from Getachew Gari at NAHDC Sebeta.

Table 1: Test agreement between IFAT and VNT from serum samples.

		VNT		Total
		+	-	
IFAT	+	80	30	110
	-	5	148	153
Total		85	178	263

Since, the sensitivity and specificity of the tests being used were known and hence according to the Rogan-Gladen estimate the true prevalence was calculated from the apparent prevalence for both IFAT and VNT as follows:

$$P = \frac{P^* + Sp - 1}{Se + Sp - 1}$$

where, P = The true prevalence

P* = The apparent prevalence

Se = Sensitivity

Sp= Specificity

According to Gari *et al.* (2008), the sensitivity and specificity of the tests were:

$Se_{IFAT} = 0.92$ $Se_{VNT} = 0.78$

$Sp_{IFAT} = 0.88$ $Sp_{VNT} = 0.97$

Therefore, from the apparent prevalence of IFAT (0.418) the true prevalence calculated was (0.37), and the apparent prevalence of VNT (0.323) then the true prevalence calculated was (0.39).

As in Table 1, the test agreement between the two tests using Kappa statistics was Kappa = 0.86 showing that the two tests have almost perfect agreement according to the interpretation of Kappa result (Dohoo *et al.*, 2003).

The seroprevalence of the disease in two age-groups (6 month up to 2 years as young and > 2 years as adult) showed that 31.3%(21/67) and 45% (89/196) for young and adults by IFAT, respectively (Table 2). Accordingly, VNT revealed 25.4% (17/67) and 34.7(68/196) for young and adults, respectively (Table 2).

As shown in Table 2, the chi-square test for association of risk factors with the prevalence obtained by Indirect Fluorescent Antibody Test (IFAT) and Virus Neutralization Test (VNT) for different age and sex groups were not significant (*P=0.05).*

Table2: Summary results of the Chi-square test for association of the risk factors with LSD of cattle using IFAT and VNT tests

Risk factor	No. tested	No.Posetive (prevalence)	*P*-value
Sex-IFAT			
Male	151	51 (33.8%)	0.337
Female	112	43 (38.4%)	
Age- IFAT			
Young	67	21 (31.3%)	0.237
Adult	196	89 (45.4%)	
Sex-VNT			
Male	151	51 (33.8%)	0.558
Female	112	34 (30.4%)	
Age-VNT			
Young	67	17 (25.4%)	0.159
Adult	196	68 (34.7%)	

4.1.1. Questionnaire survey results

Of the 355 sampled herds in the central study areas (North and East Shoawa), which were examined using questionnaires administered in 25 PAs, 102 herds were from intensive small scale dairy farms and the rest 253 herds were from extensive system. Different vernacular names of LSD were recorded from the three different sampling districts, such as 'Guribrib', 'Ekek', 'Fentata'.

Farmers in all the study districts described farming systems as sedentary. Communal grazing and watering points were dominant in extensive farming system while zero grazing was dominant in intensive farms.

A proportion of farmers (2.1%) reported introduction of new animals in to herds following purchase (for replacement, herd expansion, fattening), cattle exchange without any screening for the health status of them. The frequency of introduction of new animals was almost equal in extensive farms (1.3%) and intensives farms (1.1%).

Out of the total 2954 animals sampled in both farming systems, 537 (18.2%) animals were infected (Table 3). The proportion was higher in intensive farms (31.75%) than extensive farms (13.8%) and a 2.7% mortality rate was recorded. The farming system specific proportion was higher in intensive farms (7.24%) than in extensive farms (1.25%). Observed prevalence and observed mortalities were significantly higher in intensive farms (31.75%) than extensive farms (7.2%) ($P<0.05$) (Table 3). No association of the variables because all the sampled animals were taken from population already under risk (at LSD occurrence).

Table 3: Summary of LSD occurrence assessed by the questionnaire survey

Events recorded	Intensive	Extensive	Overall
Lumpy skin disease (LSD) status			
Total herds investigated	102	253	355
Total head of cattle sampled	718	2236	2954
Total head of cattle declared as affected	228	309	537
Apparent LSD prevalence at animal level (%)	31.8 (28.3-35.2)	13.8 (12.4-15.3)	18.2 (16.8-19.6)
Apparent mortality due to LSD (%)	7.24 (5.3-9.1)	1.25 (0.8-1.7)	2.7 (1.3-4.1)

Values in parentheses are 95% confidence intervals

Table 4: Summary of LSD occurrence assessed by the questionnaire among different age groups.

Events recorded	Calf	Yearling	Adult	Overall
Lussmpy skin disease (LSD) status				
Total head of cattle sampled	423	707	1824	355
'Total head of cattle declared as affected	51	123	363	2954
Apparent LSD prevalence at animal level (%)	12 (8.3-15.7)	17.4 (13.7-21.4)	19.9 (16.2-23.6)	18.2 (16.8-19.6)
Apparent mortality due to LSD (%)	4.5 (3-8.9)	2.4 (1.2-3.6)	2.4 (1.7-3.1)	2.7 (1.3-4.1)

Values in parentheses are 95% confidence intervals

The observed prevalence due to LSD for cattle in different age-groups such as calves, yearlings and adults in both farming systems were 12% (51/423), 17.4% (123/707) and 19.9% (363/1824) respectively (Table 4). The observed mortality among the age-groups were 4.5% (19/423), 2.4% (17/707) and 2.4 %(44/1824) respectively. Observed prevalence was higher in adult animals (19.9%) than yearlings (17.4%) and calves (12%). Whereas, the observed mortality of 4.5%in calves was higher than those of yearlings (2.4%) and adults (2.4%) (P <0.05) (Table 4).

4.2. Monetary analysis

The financial losses due to the occurrence of LSD as a proportion of the affected population were calculated (i.e among affected population, the proportion of animals that were shown overt clinical disease and subject to production loss). The magnitudes of physical effects were determined based on the annual incidence rates as follows: for the small scale dairy farms in

intensive system and for the local cattle in extensive system. The analytical estimates of the production losses of herds were the only investigated in the study period. The prices used in the model were from the average of the annual aggregate of prices for each item. They were gathered from market price monitoring documents kept at the respective district agricultural offices.

4.2.1. Losses investigated in intensive farms

Of the total cattle investigated in intensive herds 133, 206 and 379 were calves, yearlings and adults, respectively. Overall, these herds had high genetic pool of exotic breeds of cattle. The magnitudes of physical disease effects were determined based on the annual incidence rates. The parameter values used are given as follows:

1. Case specific annual morbidity = 31.75%
2. Case specific annual mortality = 7.2%
3. Annual cost of treatment = 217 birr/animal
4. Annual cost of vaccination = 5 birr/animal
5. Annual loss of milk production = 396 lit/cow
6. Annual loss of beef production = 75kg loss/infected animal
7. Reduction in work out put = 0 birr (no draft work in intensive farms)

Therefore, the total direct economic losses being experienced by these herds due to LSD were estimated as follows:

$$C = (M + B + Wop) + (V+T)$$

$$M + B + W_{op} = P \times I_d \times I_e \times E \times U$$ = output losses

$$V + T = P \times I_{tv} \times U_{tv}$$ = Expenditure losses

The total output losses experienced by the intensive farms were estimated after analyzing the exact value of each variable of the model from the data as given above, therefore the values obtained are given as follows:

P = 718, population under the study at risk

I_d = 0.32, annual incidence of LSD in the population.

I_e = 0.25, 0.55 and 0.2 were proportion of cattle experienced severe, mild and low disease effects, respectively.

E = 396 lit/lactating cow, 75kg body wt. loss in infected cattle and 52 cattle due to death were losses due to LSD annually.

U = 5.75birr/liter of milk, 8000 birr/ animal and 56birr/kg of beef were the price values experienced during 2008/2009 in the study areas.

I_{tv} = 0.65 and 0.32 were proportion of the population vaccinated and or treated for LSD, respectively.

U_{tv} = 5birr/animal and 217birr/animal were the annual average vaccination and treatment costs, respectively for LSD during 2008/2009.

Therefore, substituting the above values in the model generated the following:

M + B + Wop	= 27,913,680 birr,	where as
V + T	= 167024 birr	
Total loss	= 28,080,704 birr	
Mortality loss	= 416,000 birr	
Grand total loss	= 28,496,704 birr	

These were the amount of total monetary losses experienced by 102 dairy herds in Debre zeit town in 2008/2009 which were totally intensive. The losses estimated above were losses which have their values interms of price but, there are other losses experienced by the disease effects the (LSD), since these losses were difficult to estimate, such as increase in calving interval were estimated as 1.73 months and abortion rate of 1.9%. These losses are complex and difficult to

estimate in price values, therefore the farms/herds had experienced much losses than the above estimated grand value.

4.2.2. Losses investigated in extensive farms

The total number of cattle sampled in the investigated 253 herds were 2236 out of which 290, 501 and 1445 were calves, yearlings and adults respectively. The population consisted of local breeds of cattle and few cross-breeds which were generally considered as local breeds in this study. The total monetary losses experienced were estimated from the data generated by questionnaire survey in two selected districts of East and North Shoawa namely; Debre zeit and Yaya Gullele woredas. The districts were recorded by observed epidemic of LSD on 2008/2009. The total annual monetary losses due to LSD in the districts were estimated for that year using the values of the following parameters:

1. Case specific annual morbidity = 13.8%
2. Case specific annual mortality = 1.2%
3. annual cost of treatment = 38birr/animal
4. annual cost of vaccination = 0.5birr/animal
5. annual loss of milk production = 116 lit/lactating cow
6. annual loss of beef production = 45 kg loss/infected animal
7. reduction in work output = 15.6 hectar /ox annually

The method of computing the total monetary losses was therefore, the total output losses experienced by the extensive herds. The following values

P = 2236, were population under the study at risk

I_d = 0.14, were annual incidence of LSD in the population

I_e = 0.57, 0.38 and 0.03 were proportion of cattle experienced severe, mild and low disease effects respectively.

E = 116lit/ lactating cow, 15.6 hectar/ox, 45kg body wt. loss in infected cattle and 28 cattle due to death were losses due to LSD annually.

U = 5.7birr/liter of milk 100 birr/hectar/ox, 2453 birr/animal and 47 birr/kg of beef were the price values experienced during 2008/2009 in the study areas.

I_{tv} = 0.12 and 0.14 were proportion of the population vaccinated and or treated for LSD respectively

U_{tv} = 0.5birr/animal and 38birr/animal were the annual average vaccination and treatment costs respectively for LSD during 2008/2009.

Therefore, by substituted in the models of output losses and expenditure, i.e.

$$M + B + Wop = P \times I_d \times I_e \times E \times U$$ = output loss

$$V + T = P \times I_{tv} \times U_{tv}$$ = expenditure losses

M + B + Wop = 4,973,909,012 birr

V + T = 714 birr

Total loss = 4,973,909,726 birr

Mortality loss = 68,684 birr

Grand total loss= 4,973,978,410 birr

These were the amount of total monetary losses experienced by the herds that experienced LSD outbreaks under the study in extensive farming system. The other losses which were complex and difficult to evaluate in terms of price such as increase in calving interval, poor growth and abortion rates were not included. Therefore the total monetary losses due to the effect of LSD were more than the above estimated costs.

5. DISCUSSION

The extensive livestock production system allows maximum chances for mixing of different herds during communal grazing and watering points. Under this system, it is likely to speculate that the introduction, transmission and spread of LSD infection to susceptible herds are favorable. Uncontrolled cattle movements due to trade, pastoralism, presence of vector populations and dynamics, wet climates which favor increases of densities of insects as well as multiplications could favor potential risk factors for the transmission of the disease from herd to herd and from place to place (Toma *et al.*, 1999). Seasonal characteristics of LSD occurrence implies that the transmission of the disease might be linked with the optimum season for the development of vector insects population (Kitching and Mellor, 1986; Chihota *et al.*, 2001, 2003). However, there is still a paucity of hard evidence to incriminate specific insect vectors in the transmission of LSDV and this may deserve further study to elaborate the principal vectors.

The immune response against LSD involves predominantly cell mediated immune and the humoral immune responses. The latter lasts for short period of mostly 7 months (Lefe'vre *et al.*, 2003; OIE, 2004). Hence studies based on serological detection of the disease should take into consideration the short lifespan of detectable antibodies in the blood. The blood samples were collected, in the natural infected cattle population (P1) during active disease outbreak. The questionnaire was administered to the population (P2) of cattle owners with the same disease status. These approaches enhanced to know the extent of the distribution and associated monetary losses that resulted from the disease impact during its occurrence.

An optimum consideration was taken during the laboratory techniques to limit the possible cross-reaction of parapox and orthopox virus with LSD virus and the information obtained through epidemiological disease investigation records was also used to understand the clinical disease situation in the study population. The members of capripox virus genus are antigenically very close related, which makes not possible to distinguish them by serological tests (Davies and Otema, 1981). However, capripox virus is highly host-specific under natural environment (Capstick and Coackley, 1961). Crossreaction of cowpox virus was observed to occur with LSD virus at lower dilution of 1/8 (Davies and Otema, 1981). But in this study, the test sera was

diluted at 1/25 for IFAT. This probably reduced the possibility of cross-reacting globulins and nonspecific background reactions. However, the cross-reaction of cowpox with LSD virus observed in IFAT has not been demonstrated in VNT (Davies and Otema, 1981).

The current serological study helps us to relate the sero-prevalence and risk factors obtained from the questionnaire data. The questionnaire survey results have allowed us to evaluate the annual retrospective disease information and related production losses due to LSD in the study areas. The scope of the retrospective study was limited to one year period to minimize the possibility of memory loss regarding numerical estimates of reported facts by the farmers. And thereby reducing potential recall bias.

The sero-prevalence of 32.3% and of 41.8% of LSD recorded in this cross-sectional study in VNT and IFAT respectively in LSD outbreak occurred northern study areas are comparable with a previous result, which was recorded in an outbreak around Wolliso town of a prevalence of 27.9% (Gari, 2008). Morbidity for LSD varies from 3% to 85% and this wide range probably likely depends on the presence and densities of the mechanical insect vectors and the susceptibility of the cattle (Mac Owen, 1959). This highlights the observation of the epizootiology of lumpy skin disease in the study areas in Ethiopia.

Agreement between tests is evidence of validity and this study showed that the accuracy of IFAT was fairly good in both sensitivity and specificity parameters indicating that it can be used for LSD diagnosis and screening with low misclassification. Its capacity to run large number of sample per plate (45 samples per plate) could be also taken as an advantage to use for large epidemiological studies of LSD. The drawback in using IFAT is that it requires longer time and may be more costly as compared to ELISA technique.

The retrospective study was carried out in the small scale intensive dairy farms and free ranging extensively managed farms. It was based on the symptomatic disease identification experience of the herd-owners complemented by veterinary official epidemiological records. Endemically, occurring skin diseases of cattle such as bovine herps mammilitis, detmatophilosis, demodecosis and ringworm were taken into consideration in the differential diagnosis. However, confusion

with pseudo- LSD cases might have occurred although its presence has not yet been confirmed in Ethiopia.

The magnitudes and frequencies of LSD occurrences varied across districts and farming systems during the study period. The observed overall LSD prevalence at animal level in local cattle in this study was 13.8% which was very close to the 10% reported by Davies (Davies, 1991) and Babiuk (Babiuk *et al.*, 2008) in endemic areas of Africa. However, sero-prevalence of LSD in Southern Ethiopia was 6%, which is less than the estimate reported here. This difference might be attributable to the facts that the determination of sero-prevalence could underestimate the sero-prevalence of LSD (Gari *et al.*, 2008). The observed LSD apparent prevalence at animal level in intensive small scale dairy farms in this study was 31.8% which was significantly high. This might have been due to the susceptibility of exotic breeds. According to Davies certain breeds of cattle are more susceptible than others, especially those that are thin-skinned such as Jersey and Guernsey breeds and African Sanga cattle such as the fogera in Ethiopia (Davies, 1991).

The overall apparent mortality in the present study was 2.7% which is in agreement with a range of 1% to 3%, reported by Davies and Babiuk (Davies, 1991; Babiuk *et al.*, 2008). Accordingly, the apparent mortalities among different age-groups were 4.5%, 2.4%, and 2.4% in calves, yearlings and adults, respectively. The apparent mortality was significantly high in calves. This might have been due to the influence of age factor susceptibility. All ages of cattle can be affected, but young calves are usually more severely affected (Davies, 1991). The overall observed prevalence among age-groups in the present retrospective study was 12%, 17.4% and 19.9% in calves, yearlings and adults respectively therefore, the increases in observed prevalence in adults and yearlings than calves might be related to the frequent exposure of these age-groups to vector insects relatively because of the fact that communal grazing and watering points were found to be significantly associated with LSD occurrence which would allow contact and intermingling of different herds that would probably increase the risk of exposure and enhance the viral transmission through the speculated mechanical vectors (Gari *et al.*, 2008). Since calves are usually kept indoors until they wean in extensive farms of Ethiopia and hence calves have less probabilities of acquiring LSD.

This study showed that LSD has been extensively circulating across divers agro-climatic zones with large variation among districts. This variation could be attributed to the different agro-ecological zones and farming practices. Moreover, factors such as viral isolates, the breeds of cattle, the immunological levels of populations and the vectors involved in the transmission should also be considered for the variations observed in these findings (Davies, 1991; Babiuk *et al.*, 2008; Anon, 2009).

According to the monetary losses resulted from the disease (LSD) were estimated using two models. The result from this models provided useful insights into the economic impacts of this disease. These analyses have identified the main monetary impacts of the disease and its relative economic importance in both intensive and extensive production systems. The total monetary losses from 102 herds of sampled 718 dairy cattle was estimated at 28,496,704 birr per year due to LSD and the losses from 253 herds of sampled 2236 local cattle herds was estimated at 4,973,978,410 birr per year. The overall losses in both systems were 5,002,475,114 birr. Other losses that were difficult to estimate monetarily, such as abortion, infertility, increase in calving intervals were not incorporated in the model and hence the estimate results are lower than the actual ones. Nevertheless, the findings are indicators of how much LSD hampers the improvements of the productions especially in the small scale dairy farms and the gross national economy of the country.

The, LSD is one of cattle disease which causes high economic loss due to its chronicity of infected cattle, poor growth, infertility, abortion and sometimes deaths of animals. Milk production ceases and pneumonia is a common sequele in animals with lesions in the mouth and respiratory tract (Davies, 1991; OIE, 2008). Severe and permanent damage to hides from the skin lesions could also bring considerable losses to the tannery industries and loss of foreign currency as hide is one of the export commodities of the country (Bayou *et al.*, 1998; Anonumous, 2008). High susceptibility of exotic breeds to LSD would pose a considerable concern to the development of small scale dairy industry in Ethiopia. Moreover, it contributes to the livestock diseases which hamper the international livestock and its products trade due to phyto-sanitary regulations..

6. CONCLUSIONS AND RECOMMENDATIONS

In the present study a high prevalence of LSD were recorded using serological test results and questionnaire data but the overall apparent prevalence at animal level in intensive farms was more than twice the extensive farms and the mortality rate recorded in intensive farms was more than five folds than the extensive farms which indicates the higher susceptibility of exotic breeds to LSD which would pose a considerable concern to the development of small scale dairy industry in many parts of the country. The annual monetary losses due to LSD were estimated at 28.5 million birr in intensive farms and 5 billion birr in extensive farms. This study shows that LSD has been extensively circulating across diverse populations of cattle with large variations between districts that could be attributed to their respective farming systems. Moreover, factors such as viral isolates, the breed of cattle, the immunological status of the population and the vector insects involve in the transmission should also be considered for the creation observed in this finding.

Therefore, based on the above conclusions the following recommendations are forwarded.

- Improving awareness of farmers and veterinary services providers on the potential of the transmission of the
- disease

- Cost effective control and prevention tactics of the disease in herds should be carefully designed with constant evaluations at national and regional levels to avoid the existing economic losses and propagation of the disease.

- Further study should be done to elucidate vector insects incriminated in the transmission of LSDV and their dynamics in different agro ecologies.

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8. ANNEXES

Annexes 1

Questionnaire format for LUMPY SKIN DIESEASE Investigation

I. Study Location and Administrative levels

Region _____
Zone _____
Wereda _____
PA_____
Village/Got_____

Date _____
Owner's Name_____
Location of sampled PA
Long_____
Latit _____
Altit_____

Total No of LS in PA
Ox_____ Bull_____
L. cow_____D. cow_____
Calf _____ Heifer_____
Exotic cattle_____

Herd structure and size
No of cattle in **Got___**
Ox _____ Bull_____
L. cow _____ D. cow _____
Yearling _____ Heifer _____
Calf _____
Exotic cattle _____

Occupation mobility
Sedentary_____
Nomadic_____
Transhumant

Grazing
Communal_____
Private_____
Zero grazing_____

II. The History of LSAD Occurrence

Epidemiological Information

1. Have you had skin diseases of cattle in your herd? _____
2. Have you had LSD in your cattle? Yes _____
3. When did the disease commence in the area (PA)? Season ____ Mon_____ Year__
   How long since the outbreak has seen in the area, <1ys ____1-2 yrs ___ 2-3 yrs___ >3 yrs ____
4. How frequent LSD reoccurs in the area? Don't know _____ every 1 yr___ Every 2 yrs ___ >3 yrs_____
5. Total cattle herd size of the farmer _____: Herd structure Ox ____ Bulll ___ Beef ___ Lactating cow___ Dry cow ___ Heifer ___ Calf ___

6. How many animals have got sick and died due to LSD among the herd _____

Infected	Sex	< 1year calf	Yearlings	Adult
1.				
2.				
3.				
4.				
5.				
Died/slaughtered	**Sex**	**< 1 year calf**	**Yearlings**	**Adult**
1.				
2.				

III. Herd Management

7. Did you buy new cattle or introduced new cattle since 6 months? Yes/No

   If yes, origin of the cattle, number, sex and age?

   _____

8. Name and distance (in km) of livestock market used and the main cattle trade route km own around their area. _____

9. Did you move your cattle to other grazing place in this year? Yes/No

   If yes, when _____, how long did you keep them there _____?

10. Do you vaccinate your cattle for LSD? Yes _____ No _____

    If yes when? <1 year _____ 1-2 years _____ 2-3 years_____

    >3 years__

IV. Financial loss data

11. Do you consider LSD as an important disease and how do you score it? V. severe _____

Severe _____ Moderate _____ Low not important _____

If you consider as an important disease, what are the major losses you encountered?

a) Animal dies, Number and estimated current price of each?

Died/slaughtered	Sex	< 1 year calf	Yearlings	Adult	Price (Birr)
1.					
2.					
3.					

b) Total number of crude mortality due to any disease/case in that particular year

Died/slaughtered	Sex	< 1 year calf	Yearlings	Adult	Price (Birr)
1.					
2.					
3.					

c) Breed of affected (0) _____ lactating caw (s): _____; dry cow(s)_____and heifer(s)_____

NO	Production stage of	Current Bwt	Estimated Bwt loss (H/M/L)	Parity	Lactation stage during LSD onest (mon.s a/r calving)	Lactation Continued up to present	Lactation Stopped (date)	Milk Prod. loss		
								B/re LSD	A/r LSD	Total Loss

d) Abortion, in number _____

e) Reproductive disorders: ex. Prolonged calving interval, _____(mons); other _____

f) Affected (0) B.wt loss due to LSD (in kg) and off-work days

No	Current Bwt (kg)	Estimated Bwt loss H/M/L	Off-Work days

g) Estimated cultivated land area per ox/day _____

h) Estimated crop production losses due to off-working days: max _____

mod_____

min _____

i) Time and labor cost expended for medical care of sick animal (approximately equivalent to the cost of vet. Service charge/ animal): max _____ min _____

j) Cost of medication in birr/animal max _____ mod _____min _____

k) Herd management: Indoor feeding _____ ; Free ranging _____

l) Feed: Price of straw/donkey pack _____ Hay/hip _____; Silage/kg _____ Concentrates/kg _____

m) Other costs:

Estimated Labor: Herdman labor _____; Milker/cleaner _____

Housing: Fenced stable _____; House barn _____

Total animal sold; _____ ; Slaughtered _____; culled _____ ;others_____

Service charge for breeding _____

V. Reproductive Parameters

12. Reproductive Parameters

a) Annual reproductive rate: local _____; Exotic _____.

b) Calving interval: local _____ ; Exotic _____

No	1st Calving	2nd Calving	Calving Interval	Annual Reprod. Rate	Breed	Remark

c) The average production lifespan of local cows? _____ Exotic _____

d) The average production lifespan of draft oxen? _____

e) The first calving age of heifers? Local _____ Exotic _____

f) The age at which the bull starts to work as draft? _____

g) Off-take rates per year for different sex and age groups _____

h) Crude mortality rates for any disease per year by sex and age groups _____

VI. Market price monitor

1) Market Price monitor

a) Average price of Cattle market in that month, **OX** = AV _____ max _____min_____,

Bull = AV_____ max _____min_____, **L.Cow**=AV_____ max _____min_____,

D.Cow=AV_____ max _____min_____, **Heifer**=AV_____ max _____min_____,

Calf <1year=AV_____ max _____min_____,

b) Average price of **Milk** (lt) in that month: max _____, min _____,

Beef meat (kg) max _____, min _____, Hide= max _____, min _____,

Dung for fuel/sack _____and/or compost/sack_____

Vaccination cost/animal _____

j. Cost of medication in birr/animal max _____ mod _____min _____

k. Herd management: Indoor feeding _____ ; Free ranging _____

l. Feed: Price of straw/donkey pack _____ Hay/hip _____; Silage/kg _____

Concentrates/kg _____

m. Other costs:

Estimated Labor: Herdman labor _____; Milker/cleaner _____

Housing: Fenced stable _____; House barn _____

Total animal sold; _____ ; Slaughtered _____; culled _____ ;others_____

Service charge for breeding _____

12. Reproductive Parameters

a) Annual reproductive rate: local _____; Exotic _____.

b) Calving interval: local _____ ; Exotic _____

No	1st Calving	2nd Calving	Calving Interval	Annual Reprod. Rate	Breed	Remark

c. The average production lifespan of local cows? _____ Exotic _____

d. The average production lifespan of draft oxen? _____

e. The first calving age of heifers? Local _____ Exotic _____

f. The age at which the bull starts to work as draft? _____

g. Off-take rates per year for different sex and age groups _____

h. Crude mortality rates for any disease per year by sex and age groups _____

1. Market Price monitor

a) Average price of Cattle market in that month, **OX** = AV _____ max _____min_____,

Bull = AV_____ max _____min_____, **L.Cow**=AV_____ max _____min_____,

D.Cow=AV_____ max _____min_____, **Heifer**=AV_____ max _____min_____,

Calf<1year=AV_____ max _____min_____,

b) Average price of **Milk** (lt) in that month: max _____, min _____,

Beef meat (kg) max _____, min _____, Hide= max _____, min _____,

Dung for fuel/sack _____and/or compost/sack_____

Vaccination cost/animal _____

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10. SIGNED DECLARATION SHEET

This thesis is my original work, has not been presented for a degree in any university and that all sources of material used for the thesis have been duly acknowledge

Name _____

Signature _____

Date of submission _____

This thesis has been submitted for examination with our approval as university advisors

Dr. Moses Kyule (BVM. MSC, MPVM, PhD, Associate Professor) _____

Dr. Yasmin Jibril (DVM, MSc, Assistant Professor) _____