Study on Sero-Prevalence and Associated Risk Factors of Lumpy Skin Disease in West Wollega, Ethiopia

MSc Thesis

By

Zelalem Abera

July, 2013

Jimma, Ethiopia

Study on Sero-Prevalence and Associated Risk Factors of Lumpy Skin Disease in West Wollega, Ethiopia

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By

Zelalem Abera

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As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by **Zelalem Abera** entitled **Study on Sero-Prevalence and Associated Risk Factors of Lumpy Skin Disease in West Wollega, Ethiopia**

We recommend it to be submitted as fulfilling thesis requirement.

Hailu Degefu (DVM, M.Sc.) _____ _____

Major Advisor Date Signature

Getachew Gari (DVM, MSc, DRU, PhD) _____

_____

Co-Advisor Date Signature

As member of the *Board of Examiners* of the M.Sc. *Thesis Open Defense Examination,* We certify that we have read, evaluate the thesis prepared by **Zelalem Abera** and examined the candidate. We recommended that the thesis could be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Veterinary Epidemiology.

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DEDICATION

This paper is dedicated to my parents who have grown up me and challenged in my school life since I am the fruit of their long year struggle. It also will be in memory, mainly to my beloved wife Ejigayehu Megersa for her ceaseless support, esteem and care throughout my works and to my son Sichen Zelalem who I never forget him.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my work and that all sources of materials used for this thesis have duly acknowledged. It has been submitted in partial fulfillment of the requirements for MSc degree in Veterinary Epidemiology at Jimma University and is deposited at the university's library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the awards of any academic degree, diploma, or certificate.

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Place: Jimma University,
Jimma

BABILIOGRAPHICAL SKETCH

Zelalem was born in January 15/1983 (G.C) from his father Abera Taressa and his mother Aregash Biyena in Werebabo Siben Peasant Association, Lalo Assabi district, West Wollega Zone of Oromia Regional state, Western Ethiopia. He attended his primary school (1-4) at Hatosi Siben in 1991 and junior secondary school (5-8) at Gerjo Siben in 1996 and he attended his high school in Gimbi Comprehensive School from, 2000 to 2003. After he completed his high school, he joined Jimma University College of Agriculture and Veterinary Medicine in 2004/5, attended Veterinary Medicine and graduated with Doctor of veterinary Medicine (DVM) in 2008. After graduation, he has been working in West Wollega Zone of Gimbi district in Livestock Development, Resource and Animal Health Agency Office as Team leader for three years. He joined to Jimma University College of Agriculture and Veterinary Medicine to study his Master of Science in Veterinary Epidemiology from October 2012 to 2013.

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ABBREVIATIONS

AAU	Addis Ababa University
CI	Confidence Interval
CFSPH	Center for Food Security and Public Health
CO_2	Carbondioxide
Cpx	Capri pox
CSA	Central Statistical Authority
DF	Dilution fluid
DNA	Deoxyribonucleic acid
dsDNA	Double stranded Deoxyribonucleic Acid
DVM	Doctor of Veterinary Medicine
ELISA	Enzyme-linked immunosorbent assay
ETB	Ethiopian Birr
EVA	Ethiopian Veterinary Association
FAO	Food and Agricultural Organization
FCS	Fetal Calf Serum
FITC	Fluorescein Isothiocyanate
FVM	Faculty of Veterinary Medicine
GDP	Gross Domestic Product
GTPV	Goat pox virus
HF	Holstein Friesian
IFAT	Indirect fluorescent antibody test
IgG	Immuno gamma-globulin
ILRAD	International Laboratory for Research on Animal Disease
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
KM^2	Square kilometers
KS-1	Kenyan Sheep 1 virus
LSD	Lumpy skin disease
LSDV	Lumpy skin disease virus
M	Mole
m.a.s.l.	Meter above Sea Level
MEM	Minimum Essential Medium Eagle

ml	Mililiter
MRR	Marginal rate of return
NAHDIC	National Animal Health Diagnostic and Investigation Center
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Center
OIE	Office International des Epizooties, World Animal Health
OR	Odd Ratio
PA	Peasant Association
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PH	Power of Hydrogen
PPR	Peste des petits ruminants disease
RR	Risk Ratio
SGPV	Sheep and goat pox virus
SPPV	Sheep pox virus
SPSS	Statistical Package for Social Science
SSDP	Small Scale Dairy Production
$TCID_{50}$	Tissue Culture Infective Dose 50
USD	United State's Dollar
UV	Ultra Violet
VERO cells	Verda Reno (African green monkey kidney cells)
VIRGO	Variability of solar IRradiance and Gravity Oscillations
WHO	World Health Organization
ZNBC	Zambia National Broadcasting Corporation
µl	Microliter

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ABSTRACT

Lumpy skin disease (LSD) is an economically devastating emerging viral disease of cattle caused by a virus associated with the Neethlig poxvirus in the genus *Capripoxvirus* of the family *Poxviridae.* A cross-sectional study was conducted from October, 2012 to May, 2013 in two districts of Western Wollega of Oromia Regional State, with the objectives to determine animal and herd level seroprevalence of lumpy skin disease and to assess the risk factors that contribute to the occurrence of lumpy skin disease. The study population comprised of indigenous and crossbred cattle. Multi-stage sampling method was applied to select cattle and herd owners for the interviews. Totally, 544 sera samples were collected from 252 herds and the serological test was conducted using indirect fluorescent antibody test (IFAT). An overall individual level sero-prevalence of 6.43% (n=35) and herd level seroprevalence of 5.95% (n= 15) were estimated.There was significant variation ($P<0.05$) between the seroprevalence in Gimbi (4.41%) and Lalo Assabi (8.46%) districts at animal level. The sero- prevalence of LSD exposure among breeds (local and cross) was significantly different in that it was found significantly higher in cross breeds (OR = 2.85, p = 0.016) than in local zebu. A summer season showed statistically significant association (p = 0.000, OR = 4.224. CI = 1.13-7.57) with concomitant high levels of insect activity. There was statistically significant difference ($p<0.05$) among the age groups (adult, young and calf) in the sero-prevalence of LSD. However, the sero-prevalence of LSD was very low in calves. The current finding revealed no significant variation between male and female animals ($p>0.05$). In addition, there was no significant association between seropositivity to LSD and the agro-climatic zones (midland and highland). The risk factors considered in the univariate analysis had no significant association with the herd-level seroprevalence of LSD ($p>0.05$). Finally, the present study revealed a moderate distribution of sero-positive cattle in the study area and similarly the risk factors of the disease observed warrants future detailed study on the transmission of the disease in the area.

Key words: *LSD, Cattle; Risk factors, Seroprevalence, West Wollega, Ethiopia*

1. INTRODUCTION

The livestock sector globally is highly dynamic, contributes 40% of the global value of agricultural output, and support the livelihoods and food security of almost a billion people (Thornton, 2010). Beyond their direct role in generating food and income, livestock are a valuable asset, serving as a store of wealth, collateral for credit and an essential safety net during times of crisis (MoA, 2006; FAO, 2009).

In Ethiopia Livestock, production is an integral part of the agricultural system. The livestock sub sector accounts for 40% of the agricultural gross domestic product (GDP) and 20% of the total GDP without considering other contribution like traction power, fertilizing and mean of transport (Aklilu *et al.*, 2002; Gebreegziabhare, 2010). The livestock sector now has significant contribution to the total foreigner currency of the country.

In the future, livestock production will increasingly be affected by competition for natural resources, particularly land and water (Thornton, 2006). Currently the overall livestock production constraints in Ethiopia are feed shortages, livestock diseases, low genetic potential of indigenous livestock, and lack of marketing infrastructure and water shortages (Markos, 1999; Alemayehu, 2009). Among the many other diseases**,** which are known in causing economic losses and of poor productivity in livestock specifically in cattle is the presence of lumpy skin disease in many parts of the country (Gari *et al.*, 2010; Gari *et al.*, 2012; Birhanu, 2012).

Lumpy skin disease (LSD) is a generalized skin disease which is an infectious, eruptive, occasionally fatal disease of cattle caused by a virus associated with the neethlig poxvirus in the genus *Capripoxvirus* of the family *Poxviridae (*Chihota *et al.*, 2003; Stram, 2006; Ahmed and Zaher, 2008; Gari *et al.*, 2011). The economic losses due to this disease is associated with decreased milk production, traction power loss, weight loss, poor growth, abortion, infertility and skin damage. Pneumonia is a common sequel in animals with lesions in the mouth and respiratory tract (Davies, 1991; Kassa *et al.*, 1998; McDermott *et al.*, 1999; Yacob *et al.*, 2008; Ocaido *et al.*, 2008; OIE, 2010).

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 (Babiuk *et al.*, 2008; Gari *et al.*, 2010). Some epidemiological studies have been carried out since the disease has become established in the country, with the diverse agro-ecological and production systems (Gari *et al.*, 2010).

Study based on seroprevalence in southern Ethiopia reported a prevalence of 6% (Gari *et al.*, 2008). Targeted sampling from outbreak areas around Southern Range land, Wolliso town and north Ethiopia reported prevalence's of 11.6%, 27.9% and 28%, respectively (Asegid, 1991; Beshaewure, 1991; Gari *et al.*, 2008). A recent prevalence study (Birhanu, 2012) showed higher herd prevalence recorded in Afar (51%) and Tigray (37%) regions. Published information on the factors that influence the occurrence of LSD are not many as general, however some studies indicated that LSD is a disease which affects all age group; in Africa imported *Bos taurus* appear to be more susceptible than the indigenous breeds (Davies, 1991). The LDSV was found to be associated with Capri poxvirus outbreaks in Kenya (Kitching *et al.*, 1989).

A clinical case of LSD has been reported in other animals: Asian water buffalo from Egypt (Ali *et al.*, 1990). Antibodies have been demonstrated in black and blue wild beests, Elan, Giraffe, greater Kudu and others (Hedger and Hamblin, 1983; Barnard, 1994). Some researchers have made attempt the transmission of the disease with different flies (Chihota *et al.*, 2001; Chihota *et al.*, 2003; Carn and Kitching, 1995b; Carn, 1996). Recently, Tuppurainen *et al.*, (2010) reported the potential role of ixodic tick in the transmission of LSDV. Weather changes such as cold may adversely affect the insect vector and infected saliva may contribute to the spread of the disease (Hiag, 1957). However, there is a gap in epidemiological disease information (Taransboundry diseases) particularly lumpy skin disease in West Wollega zone except few outbreak reports from the area. The study area interfaces with the pastoralists often crossing the border to other African countries (Sudan and South Sudan) and Benishangul Gumuz Regional State of Ethiopia. Thus, the objectives of this research were to:

- ☞ Determine animal and herd level sero-prevalence of lumpy skin disease
- ☞ To assess the risk factors those contribute to the occurrence of lumpy skin disease in the study area.

2. LITERATURE REVIEW

2.1. The Disease

The range of viral skin disease in animals is very wide and involves many agents who also have significant systemic effects (Babiuk *et al*., 2008; Lloyd, 2009; Edward, 2012). Of these Viral Skin Diseases, Lumpy Skin Disease is the most serious, infectious, eruptive and occasionally fatal viral skin disease and other parts of the body of cattle caused by a virus of the family Poxviridae and economically, a significant cattle disease (production losses). It is therefore defined as a notifiable disease by the World Organization for Animal Health (OIE) (Weiss, 1963; Woods, 1988; Davis, 1991; Babiuk *et al.*, 2009; Gari *et al.*, 2010; OIE, 2010; Magori, *et al*., 2012).

It is an endemic in parts of Africa and has the potential to become established in other parts of the world (CFSPH, 2008). It is characterized by disseminated appearance of skin lesions, 2-5 cm in diameter and lymphadenopathy, accompanied by high fever, which can sometimes exceed 41°C and may last up to 2 weeks (Magori *et al.*, 2012).

2.2. Historical Background of the Disease

Historically the disease was confined to South Africa, it has moved north into Kenya, Sudan and Ethiopia (Weiss, 1963; Muktar; Robert, 1994). According to Mweene *et al.*, (1996), the clinical syndrome of lumpy skin disease (LSD) was first described in Zambia in 1929. Initially, it was considered the result of either poisoning or a hypersensitivity to insect bites. Between 1943 and 1945, cases occurred in Botswana, Zimbabwe and the Republic of South Africa (Davies, 1982; Fayez and Ahmed, 2011; Edward, 2012).

In 1970 LSD spread north into the Sudan, by 1974 it had spread west as far as Nigeria, and in 1977. It was reported from Mauritania, Mali, Ghana and Liberia (Kitching, 1995b). Another epidemic of LSD between 1981 and 1986 affected Tanzania, Kenya, Zimbabwe, Somalia and the Cameroon, with reported (Davies,

1991; Fayez and Ahmed, 2011). Lumpy skin disease (LSD) is confined and enzootic in all Sub-Saharan African countries in which it has occurred and has proved impossible to eradicate and Middle East with recent incursion into Israel (Davies, 1981; Radostits *et al.,* 2007).

From 1929 to 1986, the disease was restricted to countries in sub-Saharan Africa, although its potential to extend beyond this range had been suggested (Davies, 1991). In 1988, LSD was recognized clinically in the Suez Governorate of Egypt, where it was thought to have arrived at the local quarantine station with cattle imported from other countries of Africa (CFSPH, 2008).

In 1989, a focus of LSD was identified in Israel and subsequently eliminated by the slaughter of all infected cattle as well as contacts. Ring vaccination with a sheep pox strain was carried out around the focus area and no further clinical cases have occurred (Davies, 1991; CFSPH, 2008). It reappeared in the summer of 1989 and, in a period of five to six months, spread to 22 of the 26 governorates of Egypt.

A rapid reaction to the problem led to the vaccination of nearly two million cattle with a sheep pox vaccine (Davies, 1982). Lumpy skin disease outbreaks tend to be sporadic, depending upon animal movements, immune status, and wind and rainfall patterns affecting vector populations and as a result of this, the most recent outbreaks outside Africa occurred in the Middle East in 2006 and 2007 and in Mauritius in 2008 (OIE, 2010). In summer of 2006, the most recent outbreak of LSD was recorded in several Egyptian governorates (Awadin *et al.,* 2006).

As mentioned by Mebratu *et al.,* (1984), Lumpy Skin Disease was first observed in the Western part of Ethiopia (southwest of Lake Tana) in 1983. According to Gari *et al.,* (2010), a major epidemic outbreak of LSD occurred in different regions of in different years Ethiopia like Amhara and W/ Oromiya Regions in 2000/2001, Oromiya and SNNP regions in 2003/2004 and Tigray, Amhara and Benishangul regions in 2006/2007.

2.3. Causative Agent of the Disease

Lumpy Skin Disease (LSD) is one of a serious poxvirus disease of cattle caused by Lumpy Skin Disease Virus (LSDV), a DNA virus of the genus Capri poxvirus and of the family Poxviridea. The prototype strain is Neethling virus (Babiuk *et al.*, 2008; Gari *et al*., 2010; OIE, 2010; Magori *et al.*, 2012).

It is closely related to Sheep poxvirus (SPPV) and Goat poxvirus (GTPV) (Babiuk *et al.*, 2009). However, although all three viruses are considered distinct species, they cannot be differentiated serologically (OIE, 2008; Tuppurainen, 2011; Magori *et al.*, 2012). Therefore, the only molecular techniques to distinguish LSD from SPPV and GTPV have been developed.

2.4. Epidemiology

2.4.1. Geographic Distribution

LSD occurs in most African countries (including Madagascar) and sporadically in the Middle East region. Recent outbreaks of LSD in Egypt, Israel (2006 and 2007), Oman and Bahrain (2009) raise the possibility that LSDV might become established in the Middle East, and spread to Asia and Europe (El-Kholy *et al.*, 2008; Brenner *et al.*, 2009).

In addition, the occurrence of the disease in some districts (Adola and Yabello districts) in the years 2003–2005 was reported (Gari *et al.*, 2010). Recently one thousand five hundred herds of cattle have died of suspected lumpy skin disease in Zambia (Mazabuka's Nega Nega area) (ZNBC, 2012).

Figure 1. Global Distribution of lumpy skin disease in the World (2012). ***Note:*** The shaded area shows LSD positive countries

Source: *http://www.epizone-eu.net/publicdocs/animal diseases/website LSD*

2.4.2. Sources of the Virus

LSDV is present in cutaneous lesions and crusts. Virus is also present in blood, nasal and lachrymal secretions, milk, semen and saliva, which may be sources for transmission (Davis, 1991; Irons *et al.*, 2005).

All secretions contain LSD virus when nodules on the mucous membranes of the eyes, nose, mouth, rectum, udder and genitalia ulcerate. Shedding in semen may be prolonged since viral DNA has been found in the semen of some bulls for at least 5 months after infection (OIE, 2009).

Approximately 50% of infected animals are likely to show clinical signs; the majority of experimentally infected animals become viraemic and source of the virus. In experimentally infected cattle, LSD virus was demonstrated in saliva for 11 days,

semen for 22 days and in skin nodules for 33 days, but not in urine or faeces (Tuppurainen, 2005; Bagla, 2005; OIE, 2009; Tuppurainen and Oura, 2012).

2.4.3. Occurrence of the Disease

It has been suggested that, while extending its geographical distribution, the virus has increased in pathogenicity causing extensive epidemics and pandemics on the African continent with sporadic cases occurring during inter-epidemic years (Rweyemamu *et al*. 2000).

There is, also the possibility that LSDV might become established in the Middle East, and spread to Asia and Europe (Tuppurainen and Oura, 2012). It may be present in other Middle Eastern countries, which are lacking active surveillance (OIE, 2010; Tuppurainen, 2011). As mentioned by Rweyemamu *et al*. (2000), the only African countries still considered free of the disease are Libya, Algeria, Morocco and Tunisia.

According to Gari *et al*. (2008), about 90% respondents replied that the disease occurs from July to November, which is the season of high moisture and extends up to December in Ethiopia. However, lack of an understanding of why LSD is not yet established outside of Africa and the Middle East is the point would be seen as a gap.

2.4.4. Species Involved and Role of Wildlife in the Spread of LSDV

Most of the time, Capri poxviruses are highly host specific. LSD is primarily a disease of all cattle, particularly thin-skinned European breeds, are susceptible (CFSPH, 2008). Very little data are available on the susceptibility of wild ruminants to LSD (Tuppurainen & Oura, 2012).

Capri pox disease has been reported in domestic Asian water buffalo (Bubalus bubalis) and Arabian Oryx (Oryx leucoryx) (Greth *et al.* 1992), but could have been caused by closely related poxviruses (Davis, 1981; CFSPH, 2008; Tuppurainen, 2011). However, it was not differentiated if these animals were infected with LSDV or sheep pox or goat poxvirus (House, 1990; Tuppurainen, 2011 & Tuppurainen and Oura, 2012).

Recently, the persistence of LSDV nucleic acid was reported in skin samples collected from springbok (*Antidorcas marsupialis*) in South Africa (Lamien *et al*., 2011). Natural infections were reported in Asian water buffalo (*Bubalus bubalis*) during the LSD outbreak in Egypt in 1988, but the morbidity was significantly lower in buffalo (1.6%) than in cattle (30.8%) (Ali *et al*., 1990). Natural cases have not been seen in an impala (*Aepyceros melampus*) and a giraffe (*Giraffa camelopardalis*) but, have been success fully infected experimentally (Woods, 1988 & CFSPH, 2008) and clinical signs of LSD have been demonstrated in both of them after experimental inoculation with LSDV (Young *et al*., 1970).

LSDV will replicate in sheep and goats following inoculation, but the role of these species as potential reservoirs of the virus is yet to be clarified (House, 2004; Bowden *et al*., 2008; Bowden *et al*., 2009 & Tuppurainen, 2011). No carrier status is recognized in cattle following infection with LSDV although live virus can be detected up to 39 days post infection in the skin of infected animal (Woods, 1988; Troyo *et al*., 2008; Ahmed and Zaher, 2008; Tuppurainen, 2011).

According to Barnard (1997), animals with mild or in apparent infection with LSDV do not always show antibody levels detectable with a neutralization assay. Therefore, the actual number of LSDV-infected wild ruminants may be considerably higher than that revealed by this test. Wild animals showing clinical signs of LSD are likely to be more susceptible to predators, which could explain the lack of reports of clinical disease in wildlife species. In addition, the presence of clinical signs of LSD in wildlife is easily missed, as the monitoring of the skin lesions is difficult or impossible, especially in mild cases.

2.4.5. Risk Factors Associated with Lumpy Skin Disease

Capri poxviruses have a long incubation period; animals intentionally infected can travel a considerable distance before showing disease, and can therefore disperse and spread disease.

Pathogen factors: LSDV is remarkably stable, surviving for long periods at ambient temperature, especially in dried scabs. It is very resistant to inactivation, surviving in necrotic skin nodules for up to 33 days (-80°C for 10 years) or longer, desiccated

crusts for up to 35 days, infected tissue culture fluid stored at 4°C for 6 months, (if protected from sunlight) and at least 18 days in air-dried hides (OIE, 2009).

It can remain viable for long periods in the environment, but virus is susceptible to high temperatures (inactivation is achieved by heating at 55ºC for 2 hours, 65°C/30 minutes) and to highly alkaline or acidic pH. LSDV is susceptible to sunlight, but survives well at cold temperatures (OIE, 2009; Tuppurainen, 2011).

Environment Factors: There is a dynamic relationship between the "agent" that transmits the diseases, the "host" that entertains on the one side and the "environment" on the other. The host and the agent operate within a particular environment and depending on that environment disease spreads and is controlled (Thrusfield, 2005).

The potential risk of agro-climate variations in LSD occurrence showed that midland and lowland agro-climates were more likely to be at risk for LSD occurrence than the highland agro-climate (Gari *et al*., 2010). The warm and humid climate in midland and lowland agro-climates has been considered a more favourable environment for the occurrence of large populations of biting flies than the cool temperature in the highlands (Troyo *et al*., 2008; Tuppurainen, 2011).

Herd contact and mixing is likely to occur in communal grazing and watering points and these were found to be significantly associated with LSD occurrence (Gari *et al*., 2010). 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 the speculated mechanical vectors such as Stomoxys spp. and mosquitoes (Aedes aegypti) (Chihota *et al.*, 2001; Gari *et al*., 2010; Waret, 2010).

The Host Factors: Host susceptibility, dose and route of virus inoculation affect the severity of disease. All breeds, age groups and both sex of cattle are considered to be at risk can be infected with severe and serious complications, while Asian water buffalo are also reported to be susceptible. *Bos taurus* breeds of cattle are more susceptible than *Bos indicus* breeds, and in age wise, young calves often experience more severe disease than adults (CFSPH, 2008; OIE, 2010; Tuppurainen, 2011).

The morbidity rate varies widely depending on the immune status of the hosts (host susceptibility) and the abundance of mechanical arthropod vectors (CFSPH, 2011). As more recently reported by Birhanu (2012), an introduction of new animals to the herd was highly associated with the occurrence of LSD. According to OIE (2011), there is no evidence or report that the virus can affect humans.

2.4.6. Transmission

LSDV is thought to be primarily transmitted by biting and blood feeding arthropods, which are believed to act as mechanical rather than biological vectors (Weiss, 1968; OIE 2010; Chihota *et al*., 2001; Tuppurainen, 2011). Recently, new evidence has been published reporting a possible role for hard ticks in the transmission of LSDV (Tuppurainen *et al.*, 2011).

The study showed molecular evidence of transtadial (occurs when an infection is picked up by one stage in the vector's life cycle and transmitted to succeeding stages in its metamorphosis) and transovarial (the transfer of pathogens to succeeding generations through invasion of the ovary and infection of the eggs, such as occurs in arthropods, primarily ticks and mites) transmission of LSDV by *R.* (*B.) decoloratus* ticks and mechanical or intrastadial transmission by *R. appendiculatus* and *A. hebraeum* ticks (Tuppurainen and Oura, 2012). Female *Aedes aegypti* mosquitoes were shown to transmit LSDV from infected to susceptible cattle for 2-6 days post-feeding on experimentally infected animals (Chihota *et al.*, 2001).

However, attempts to transmit LSDV between experimentally infected and susceptible cattle by *Stomoxys calcitrans* have failed (Chihota *et al*., 2003), as did the transmission of LSDV by two species of mosquito (*Anopheles stephensi* and *Culex quinquefasciatus*) and the biting midge (*Culicoides nubeculosus)* (Woods, 1988; Chihota *et al.*, 2003; Troyo *et al*., 2008).

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 (Gari *et al*., 2010).

Tabanidae, *Glossina* and *Culicoides* spp. have all been found in situations where there has been ongoing LSD transmission and have been suspected to be involved. *Stomoxys* spp. has been shown to transmit SGPV successfully (Davis, 1991).

Transmission of LSDV through semen has not been experimentally demonstrated, but LSDV has been isolated in the semen of experimentally infected bulls (Tuppurainen, 2005; Bagla, 2005; Tuppurainen, 2011). Transmission via infected saliva, ocular and nasal discharge, contaminated food and water by infected feces and urine needs to be demonstrated under experimental conditions (Tuppurainen and Venter, 2005).

2.4.7. Morbidity and Mortality

The incidence of disease is highest in wet, warm weather, and decreases during the dry season. New foci of disease can appear at distant sites; in these cases, the virus is thought to be carried by insects (CFSPH, 2008).

According to Woods (1988), recorded morbidity rates have varied greatly from as low as 5% to 100%. Mortality, except in exceptional circumstances, rarely rises above 5%. In outbreaks of the disease, the morbidity rate varies widely depending on the immune status of the hosts (host susceptibility) and the abundance of mechanical arthropod vectors and usually ranges from 3% to 85% (Bennett and IJpelaar, 2005; Kivaria *et al*., 2007;CFSPH, 2008; CFSPH, 2011;Tuppurainen,2011; Tuppurainen and Oura, 2012).

Additionally, according to Davis (1991), morbidity rates of 1 to 2% may be contrasted with those of 80 to 90% in different situations. Mortality rates of 10 to 40% and even higher have been reported on occasion but the much lower range of 1 to 5% is more usual.

However, as more recently reported, more severe disease is seen in *Bos taurus,* particularly Channel Island breeds, than zebu cattle. Calves and lactating cows tend to be most susceptible to disease (Davis, 1991; CFSPH 2008 & Tuppurainen, & Oura, 2012). In general, mortality rate is low (1-3%) but in some occasions up to 75% mortality has been reported (CFSPH, 2011 & Magori *et al.*, 2012).

2.4.8. Mechanism of Pathogenicity

During the acute stage of skin lesions, histopathological changes include vasculitis and lymphangitis with concomitant thrombosis and infarction, which result in to oedema and necrosis.

LSD skin nodules may exude serum initially but develop a characteristic inverted greyish pink conical zone of necrosis. Adjacent tissue exhibits congestion, haemorrhages and oedema. The necrotic cores become separated from the adjacent skin and are referred to as 'sit-fasts'. Enlarged lymph nodes are found and secondary bacterial infections are common within the necrotic cores. Multiple virus-encoded factors are produced during infection, which influence pathogenesis and disease (Prozesky *and* Barnard, 1982; Tuppurainen, 2011).

2.4.9. Incubation Period and Clinical Signs

Incubation period: Lumpy skin disease has an incubation period of 2 to 4 weeks in the field. In experimentally infected animals, fever can develop in 6 to 9 days and lesions first appear at the inoculation site in 4 to 20 days (CFSPH, 2008; OIE, 2010; Tuppurainen, 2011).

Clinical Signs: LSD is an acute infectious disease of cattle of all ages (Davis, 1991; Kumar, 2011) but young calves often have more severe disease than adults (CFSPH, 2008; CFSPH, 2011). The severity of clinical signs of LSD depends on the strain of Capri poxvirus and the host cattle breed. 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 oedema, lymphadenitis and occasionally death (Woods, 1988; Davis, 1991; Tuppurainen, 2005).

Fever is the initial sign. It is usually followed within two days by the development of nodules on the skin and mucous membranes (CFSPH, 2008). These nodules vary from 1 cm to 7 cm and common on the head, neck, udder, genitalia, perineum and legs. Although they penetrate the epidermis and dermis, subcutaneous tissue, and sometimes they extend into the underlying musculature, which are a nidus for secondary bacterial infections and fly infestations (CFSPH, 2008; CFSPH, 2011; Tuppurainen, 2011).

The skin nodules are painful and could involve tissues up to the musculature (OIE, 2008; Magori *et al*., 2012). Where extensive generalization occurs, animals may become lame and reluctant to move. Abortion may occur because of prolonged fever (Woods, 1988; Davis, 1982; Tuppurainen, 2005; Ocaido *et al.,* 2009; Magori *et al*., 2012). In severely affected animals, ulcerative lesions appear in the mucous membranes of eye and oral/nasal cavities causing excessive salivation, lachrymation and nasal discharge. All these secretions may contain LSDV (Babiuk, *et al*., 2008; OIE, 2008). Pox lesions may also be present in the pharynx, larynx, trachea, lungs and throughout the alimentary tract. Raised, circular, firm, coalescing nodules are common and cores of necrotic material called "sit-fasts".

2.5. Diagnosis of LSD

At present, there are no diagnostic test kits for LSDV commercially available (Tuppurainen, 2011). The tentative diagnosis of LSD is usually based on characteristic clinical signs, differential diagnosis and the clinical diagnosis is confirmed by laboratory tests (using conventional PCR) (Tuppurainen, 2005; CFSPH, 2008; OIE, 2009; OIE, 2011).

2.5.1. Clinical Examination

Clinically, LSD should be suspected when the characteristic skin nodules, fever and enlarged superficial lymph nodes are seen and the mortality rate is usually low (CFSPH, 2008). The appearance of the skin lumps that characterize the disease follows within 48 hours. Nodules may appear anywhere on the body from the nose to the tail. Distribution is in a random pattern and not linear. Similar lesions appear in the mucosa of the mouth, nose, vagina and conjunctiva. A purulent nasal and ocular discharge is common (Weiss, 1968; Woods, 1988).

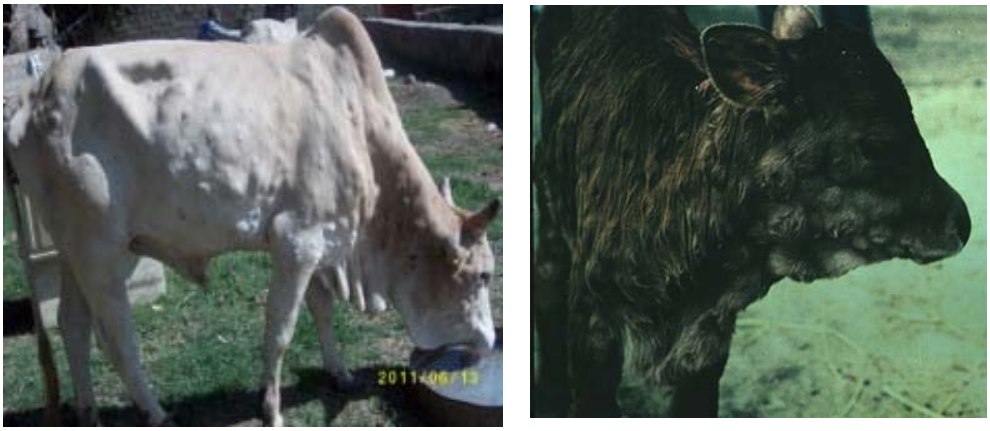

Figure 2. Nodules on the skin of the animals

Source: CFSPH (2011), Iowa State University (B) and Getachew *et al.,* (2012) (A)

2.5.2. Laboratory tests

Laboratory confirmation of LSD is most rapid using a polymerase chain reaction (PCR) method specific for Capri poxviruses or by the demonstration of typical Capri pox virions in biopsy material or desiccated crusts using the transmission electron microscope in combination with a clinical history of a generalised nodular skin disease and enlarged superficial lymph glands in cattle (Davies *et al.,* 1971; OIE, 2010).

As mentioned by CFSPH (2008), OIE (2011); Tuppurainen (2011), routine Diagnostic Techniques are described in the OIE Manual of Diagnostic Tests and Vaccines including: Identification of the agent and Serological tests.

Identification of the agent: Capri poxvirus is distinct from Para poxvirus, which causes bovine popular stomatitis and pseudo cowpox, but cannot be distinguished morphologically from cowpox and vaccinia virus, both orthopoxvirus infections of cattle (OIE, 2010).

Confirmation of lumpy skin disease in a new area requires virus isolation and identification (CFSPH, 2008; OIE, 2011; Tuppurainen, 2011). LSDV will grow in bovine, caprine or ovine cell cultures; the best growth is seen in lamb testis cells (El-Kenawy and El-Tholoth, 2011).

LSDV can be distinguished from the herpes virus that causes pseudo-lumpy skin disease by the cytopathic effect and the intracytoplasmic location of inclusion bodies.

LSDV antigens can be identified with direct immunofluorescence, virus neutralization or enzyme-linked immunosorbent assay (ELISA). LSDV can be detected in cell cultures or directly in tissues by polymerase chain reaction (PCR) assays (CFSPH, 2008; OIE, 2010; Tuppurainen, 2011).

Genome detection using capripoxvirus-specific primers for the fusion protein gene and attachment protein gene has been reported and several conventional and real-time PCR methods have been published for use on blood, tissue and semen samples (OIE, 2010).

Serological tests: Serological tests include an indirect fluorescent antibody test, virus neutralization, ELISA and immunoblotting (Western blotting). Cross-reactions with other poxviruses are seen in some assays. Agar gel immunodiffusion is also available, but cross-reactions occur in this test with bovine papular stomatitis and pseudo cowpox virus (CFSPH, 2008; OIE, 2010). The virus neutralisation test is the most specific serological test, but because immunity to LSD infection is predominantly cell mediated, the test is not sufficiently sensitive to identify animals that have had contact with LSD virus and developed only low levels of neutralizing antibody.

The agar gel immunodiffusion test and indirect immunofluorescent antibody test are less specific due to cross-reactions with antibody to other poxviruses. Western blotting using the reaction between the P32 antigens of LSD virus with test sera is both sensitive and specific, but is difficult and expensive to carry out (OIE, 2010).

Indirect Flouresnt Antibody Test (IFAT): It demonstrated to be suitable for use in retrospective serological surveys in a study carried out in Ethiopia and it was evaluated test for accuracy (Gari *et al*., 2008). The IFAT is a serological test for Capri pox Virus. It was used to detect serum antibody against Capri pox Virus and differentiate serological positive and negative animals.

2.5.3. Differential Diagnosis

Skin lumps appear in cattle due to many conditions other than lumpy skin disease and it is obvious that confusion has been caused by many of them in the past (Woods, 1988). Misdiagnosis and misreporting have probably been common over the years due to veterinarians not having had previous experience of the disease (Woods, 1988; OIE, 2010).

Severe LSD is highly characteristic, but milder forms can be confused with: Pseudo lumpy skin disease (Bovine Herpesvirus$_2$), Bovine papular stomatitis (Para poxvirus), Pseudo cowpox (Para poxvirus), Vaccinia virus and Cowpox virus (Orthopoxviruses) uncommon and not generalised infections, Dermatophilosis, Insect or tick bites, Besnoitiosis, Rinderpest, Demodicosis, Hypoderma bovis infection, Photosensitisation, Urticaria, Cutaneous tuberculosis, Onchocercosis (Siraw, 1987; Davis, 1991; OIE, 2009).

2.5.4. Pathological Lesions

Post mortem lesions can be extensive. Characteristic deep nodules are found in the skin that penetrate into the subcutaneous tissues and muscle with congestion, hemorrhage, and edema. Lesions may also be found in the mucous membranes of the oral and nasal cavities as well as the gastrointestinal tract, lungs, testicles, and urinary bladder. Bronchopneumonia may be present, and enlarged superficial lymph nodes are common (CFSPH, 2008).

Nodules involving all layers of skin, subcutaneous tissue, and often adjacent musculature, with congestion, haemorrhage, oedema, vasculitis and necrosis; Enlargement of lymph nodes draining affected areas with lymphoid proliferation, oedema, congestion and haemorrhage; Pox lesions of mucous membrane of the mouth, the pharynx, epiglottis, tongue and throughout the digestive tract; nasal cavity, trachea and lungs (Fig. 3 and 4).

Oedema and areas of focal lobular atelectasis in lungs; Pleuritis with enlargement of the mediastinal lymph nodes in severe cases; Synovitis and tendosynovitis with fibrin

in the synovial fluid; Pox lesions may be present in the testicles and urinary bladder (OIE, 2009).

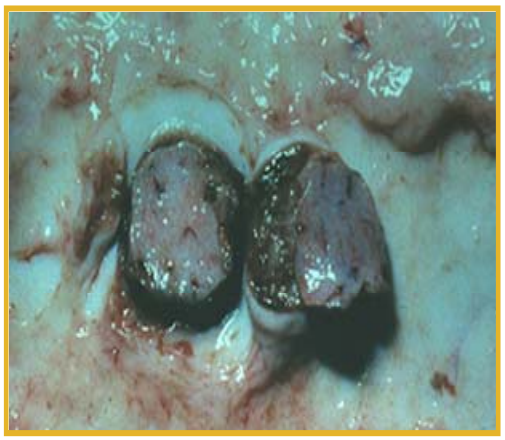

Figure 3. Nodules in lungs

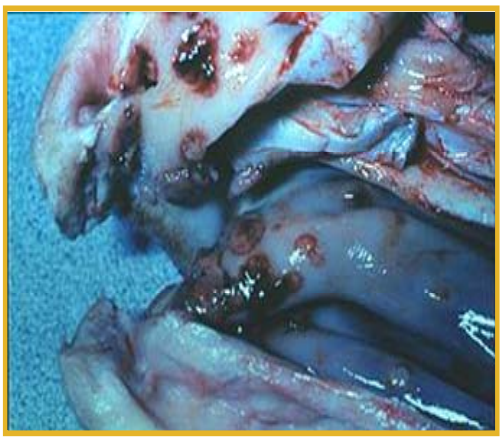

Figure 4. Lesions in the m/m throughout the GIT

Source: CFSPH (2011), Iowa State University

2.6. Treatment, Control and Prevention

Treatment: There is no specific antiviral treatment available for LSD infected cattle. Sick animals may be removed from the herd and given supportive treatment consisting of local wound dressing to discourage fly worry and prevent secondary infections (Davis, 1982; CFSPH, 2008; OIE, 2009; Tuppurainen, 2011).

Control and prevention: LSD should appear in cattle in another country beyond its previous range (Davis, 1991). Therefore, it needs to stop if occurred and to block if new and this can be carried out either by sanitary prophylaxis or medical prophylaxis. In case of sanitary prophylaxis, import restrictions on livestock, carcasses, hides, skins and semen can help to prevent the introduction of LSD in to the disease free countries (Thrusfield, 2005; Dijkhuizen *et al.*, 1995; Rushton *et al.*, 1999; Rushton, 2009).

It is mainly spread to new areas by infected animals, but it could also be transmitted in contaminated hides and other products. However, in infected countries, infected

insects are suspected to have spread LSDV to new areas during some outbreaks. Outbreaks can be eradicated by strict quarantines to avoid introduction of infected animals into safe herds, isolation and prohibition of animal movements, slaughtering of all sick and infected animals (depopulation of infected and exposed animals), proper disposal of carcasses (incineration), cleaning and disinfection of the premises and insect control (Davis, 1982; CFSPH, 2008; OIE, 2009; Tuppurainen, 2011; Edward, 2012).

Medically, with the exception of vaccination, control measures are usually not effective. Vaccination will greatly reduce the morbidity and economic effects of an epizootic but may not completely limit the extension of-LSD. Follow-up vaccination of calves and re-vaccination programmes over a period-of two to three years will greatly reduce the incidence of clinical disease (OIE, 2009).

Two different vaccines have been widely and successfully used for the prevention of LSD in cattle populations in Africa (Davis, 1982). Homologous live attenuated virus vaccine: Neethling strain: immunity conferred lasts up to 3 years and heterologous live attenuated virus vaccine: Sheep or goat pox vaccine, but can sometimes cause severe local reactions. Not advised in countries free from sheep and goat pox (OIE, 2009; Tuppurainen, 2011).

As reported by Davis (1991), two other strains of sheep pox vaccine have recently been used as a prophylaxis against LSD. The Romanian strain, prepared in the skin of lambs for use against sheep pox, was used in several million cattle in Egypt and appeared to be immunogenic. No complications have followed the use of these strains in cattle. No country in sub-Saharan Africa, however, has succeeded in eradicating LSD once it has occurred.

LSDV is susceptible to ether (20%), chloroform, formalin (1%), and some detergents, as well as phenol (2% for 15 minutes). This virus can survive for long periods in the environment: up to 35 days in desiccated scabs and for at least 18 days in air-dried hides (CFSPH, 2008).

2.7. Status of Lumpy Skin Disease in Ethiopia

The Ethiopian economy is highly dependent on agriculture, which in the 2004/05 fiscal year, contributed about 48% of the GDP, followed by 39% from the service sector, and 13% from the industrial sector. Livestock disease is one of the major livestock production constraints in Ethiopia (Gebreegzabheher, 2010).

Lumpy skin disease has been one of the newly emerging diseases of cattle in Ethiopia. Lumpy Skin Disease was first observed in southwest of Lake Tana in 1983 (Mabratu, 1984).

A major epidemic outbreak of LSD occurred in 2000/2001: in Amhara and Western part of Oromiya Region, in 2003/2004: again in Oromiya and Southern Nations and Nationalities People regions and in 2006/2007: in Amhara and Benishangul regions (Table 1).

In terms of the size and magnitude of its occurrence, an epidemic of LSD covering a number of PAs was reported to have occurred in some districts (Adola and Yabello districts) in the years 2003-2005 (Gari *et al.*, 2010) (Table 1). In Somali regional state, the first case of an epidemic of Lumpy Skin Disease in cattle was reported in Somali Region in 2005 *(http://reliefweb.int/sites/reliefweb.int/files/resources)*.

Table 1. Official reported and non-reported LSD outbreaks in the different of districts of some Administrative Regions of Ethiopia from years 2000-2007

Region/district	Years of an outbreak of the disease								Total
	2000	2001	2002	2003	2004	2005	2006	2007	
Amhara									
Laygayint	-	5	-	-	-	-	-	-	5
Farta	5		-	-	-	-	-	2	7
Gozamen	1	5	-	-	-	-	-	1	7
Kobo	-	-	-	-	-	-	-	5	5
Oromiya									
Adola	-	-	-	-	1	14	3	-	18
Yabello	-	1	3	13	8	6	4	1	36
Sebeta-Awas	-	1	2	1	2	1	1	2	8
Bako-Tibe	-	-	-	-	-	-	-	-	2
Chora	-	-	-	10	-	-	-	-	10
Fentale	-	-	-	3	-	-	-	-	3
SNNP									
Kabiena	-	-	-	-	-	-	1	-	1
Afar									
Awash-Fentale	-	-	1	-	-	-	-	-	1
Total	**6**	**12**	**6**	**27**	**11**	**21**	**14**	**6**	**103**

Source: G. Gari (2010)

As reported by Gari *et al.*, (2010), LSD has been extensively circulating across diverse agro-climatic zones of Ethiopia with large variations between districts that could be attributed to their respective agro-ecological zones and farming practices.

Additionally, the same author (2012) explained that, animal and herd sero-prevalence was higher in the midland agro-climate than in highland and lowland agro-climate zones and suggested that the prevalence of LSD infection in Ethiopia is higher than what has been previously reported.

In addition, recent survey which assesses the risk factors and financial impacts of LSD in selected districts of North-eastern Ethiopia (Tigray and Afar Regional States) conducted by Birhanu, (2012) reported a higher herd prevalence of 51% and 37% was recorded in Afar and Tigray Region respectively (Table 2).

Table 2. Reported outbreaks and LSD affected populations in different regions of Ethiopia from 2007-2011

Regions	**Years of reported outbreaks**					
	2007	**2008**	**2009**	**2010**	**2011**	**Total**
Addis Ababa	-	-	3	7	1	11
Afar	-	-	3	2	2	7
Amhara	92	68	35	40	22	257
Ben.Gumuz	3	-	-	-	5	8
Gambela	-	-	-	1	9	10
Oromiya	95	154	219	268	160	896
SNNP	18	18	14	32	17	99
Somali	-	-	3	9	4	16
Tigray	7	8	2	18	13	48
Grand Total	**215**	**248**	**276**	**375**	**233**	**1347**

Source: Birhanu, (2012).

3. MATERIALS AND METHODS

3.1. Description of Study Areas

The present study was conducted in two selected districts (Gimbi and Lalo Asabi) of West Wollega Zone of Oromiya Regional State; Western Ethiopia. West Wollega is one of the 18 Administrative Zones of Oromiya National Regional State. Administratively, the Zone has 21 districts, of which 19 are rural districts and 2 are urban administrations which are again subdivided into 533 kebele administrative units (487 rural and 46 urban Kebeles). Gimbi Town, which is located at a distance of 441 km from Addis Ababa, is the capital of the Zone, it is located between $8^{o}12'$ - $10^{o}03'$ N (latitudes) and $34^{o}08'$ - $36^{o}10'$E (longitudes). The Zone shares borderes with Benishangul-Gumuz Regional State, Qellem Wollega Zone, East Wollega Zone, Illubabor Zone and Gambella Regional State in the Northwest, Northeast and east; West, East, and in the South directions, respectively. The land area of the Zone is estimated to be 14,160.29 km^2. It experiences tropical climate with relatively high mean annual temperature. Generally, mean annual temperature of the Zone varies from 15^{o}C to over 25^{o}C (Socio-economic Abstract of Districts of West Wollega Zone, 2008/09).

The annual rainfall pattern in the Zone decreases from East to West following the physiographic nature of the Zone. The mean annual rainfall of the Eastern high lands ranges from 1800-2000 mm, while in the central plateaus. It ranges between 1600-1800 mm and in the remaining parts of the Zone, it becomes between 1200-1600 mm. In the Southwestern parts of the Zone, it is even less than 1200 mm *(http://www.oromiyaa.com/english/images/West%20WollegaProfile).*

Livestock population of West Wollega Zone is 1,775,404 Bovines, 385,098 Ovine, 353,385 caprines, 137,926 Equines, 2,066,678 poultry and 620,397 Bee colonies (West Wollega Zonal Livestock Development & Health Agency Office, 2011).

The farming system in the zone is mixed (Livestock production integrated with crop Production). Livestock production system is usually extensive, and the most common breeds are the local zebu breeds. Common grasslands provide extensive pasture for all parts of the areas of both study districts.

From the total (21) districts of the Zone, two of the rural districts (9.5%) were selected considered as representative of the rest districts of the Zone.

Gimbi District: is located between $90^{0}10^{0}$- $9^{0}17^{0}$ North latitude and $35^{0}44^{0}$- $36^{0}09^{0}$ East longitudes. The mean minimum and maximum annual temperature ranges between 10^{o}C and 30^{o}C. The mean annual rainfall is 1400-1800ml. It lies at altitudinal range of 1200m-2222m above sea level (a.s.l.). As reported by Ghimbi District Finance and Economic Development office (2001), the district has high livestock potential with 107,334 cattle, 13,476 Ovine, 5124 Caprine, 5211 Equine, and Poultry 44144 and 25600 Bee Colonies (Ghimbi District Finance and Economic Development Office 2001) (*http://www.oromiyaa.com/english/images/Ghimbi).*

Lalo Asabi District: has an area of 418Km^2 and located in the Eastern part of West Wellega Zone. It shares common boundaries with Gimbi, Guliso, Bodji and Yubdo districts, and Benishangul-Gumuz Regional State. Enango town is its capital town that is about 23km far away from the Capital of the Zone (Gimbi). Altitudine ranges between 1500 and 1900 m.a.s.l. The district has an estimated 37, 279 cattle, 13870 Ovine, 565 Caprine, 4383 Equine, 50,109 Poultry. The district is classified into kola (2.2%) and Woinadega (97.8%) agro climatic zones (Socio-Economic Profile of Lalo Assabi District, 2011) *(http://www.oromiyaa.com/index.php*).

The differences between both districts were altitudinal ranges, livestock size which is very low in Lalo Assabi District and agro climatic zones in which all types were found in Gimbi District.

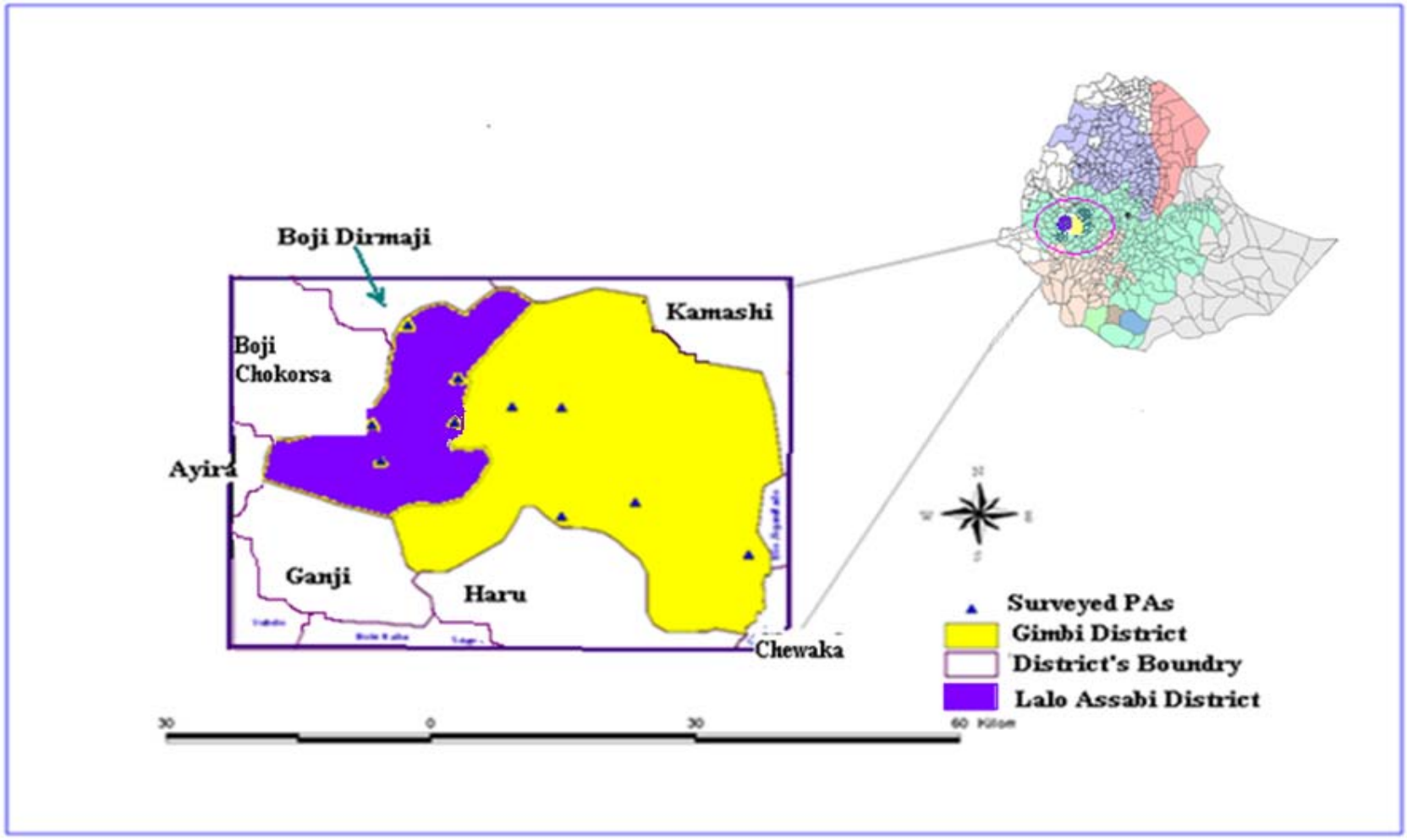

Figure 5. Map of the study Area

3.2. Study Population

Study animals involved in this study were all indigenous zebu and crossbred cattle population of all age groups kept in extensive management system in Gimbi (107,334) and Lolo Asabi (37,279) districts of West Wollega Zone of Oromia Regional State.

The districts were purposively selected based on the accessibility, presence of livestock markets activity, production and management system, history of contact with wild life and transboundary animal's movement from other pastoralist area of neighbouring Regional States of Ethiopia. These districts share similar farming system but different in agrological locations.

3.3. Study Design

A cross-sectional study was carried out from October, 2012 – May, 2013 to determine the sero-prevalence and risk factors for LSD occurrence in the study area. Multi-stage sampling method was followed to select the sampling units and districts, PAs, herds and animals were selected to be included in the study.

An animals included in the study were distributed over the purposively selected districts. Five PAs were randomly selected from each district in consultation with the respective district Agricultural Office; especially Livestock Resource, Development and Health Agency expert's based on location and accessibility.

From selected PA's, a herd was selected as a primary epidemiological unit, and by assuming an average number of 10 animals per herd; from total of 5,028 herd population, 252 of them were randomly selected. In each PA, the number of selected herds range from 22 (Were Seyo of Gimbi District) to 28 (Bikiltu Tokuma of Gimbi and Dongoro Dissi of Lalo Assabi Districts) in both Districts. The term "herd" mean that cluster or aggregate of animals' those have similar resource of feeding, drinking and etc. Additionally, the extensive management system implies that animals from the same

kebele (PAs) share communal grazing and watering resources and experience the same environmental and climatic conditions.

Animals were sampled from both districts and those selected animals were used as a secondary epidemiological unit to be included in the study based on the representativeness of the Kebeles (PAs) and Weredas. So, range of one to three (1-3) cattle from the selected herds were randomly selected as secondary epidemiological units and fourty four to sixty three (44-63) animals from each PA and a total of 544 cattle were sampled in order to have representative sample size to estimate sero-prevalence in both districts.

3.4. Sampling Technique and Sample Size Determination

The simple random sampling technique was followed, to select individual animals to be used for the study in the study area. Minimum sample size for this cross-sectional study was calculated using the formula by Thrusfield (2007) with 95% confidence level and 5% absolute precision.

The sample size was achieved by assuming the expected national prevalence of Lumpy Skin Disease (LSD) at animal level 23% (Gari *et al.*, 2010). Accordingly, 272 desired sample sizes for the study were calculated.

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

Where: n = required sample size;
exp = expected prevalence;
d = desired absolute precision.

Therefore, $n = \frac{(1.96)^2 * 0.23 * (1-0.23)}{(0.05)^2} =$ **272**

To increase precision, the obtained minimum sample size (272) was inflated by two folds, to account for the effect of randomness and representative population in multistage sampling (Thrusfiled, 1995). Therefore, a sample size of 544 was considered for this study. The limits of the associated interval indicate the specified bounds within which the estimate will lie with the defined level of confidence.

Generally, based on the consultation with the respective experts of Livestock Resource, Development and Health Agency Offices of both districts and herd owners whether they introduced new animal (which might vaccinated or not) into the herd, samples were collected from non-vaccinated indigenous zebu cattle breeds and crossbred cattle population in 252 herds distributed in 10 PAs of 2 (Two) districts of the Zone.

During sample collection, the estimated age of each sampled animal was determined by consulting the owners of the cattle. Accordingly, the sampled animals were categorized as calves (>0.5-2years), young (>2- 4years) and adults (>4years) (Berecha *et al*., 2011).

3.5. Sample collection, submission and preparation

3.5.1. Serum Sample Collection and Handling

By using disposable 10 ml sterile vacutainer tubes, full of whole blood samples were collected from the jugular vein of each animal. The tubes were then kept protected from direct sun light at room temperature in slant position until the blood clotted and sera were separated within 12 hours. The separated sera were transferred to sterile cryovials; bearing the names of PAs, animal number, age and sex and kept in icebox at the field.

Finally, the samples were transported to the National Animal Health Diagnostic and Investigation Centre (NAHDIC), Sebeta, for serological examination using Indirect Fluorescent Antibody Test (IFAT). Gari *et al.* (2008) reported that, IFAT has a reasonable high accuracy to be used for the diagnosis and sero-surveillance analysis of LSD in the target population.

In the laboratory; the sera were preserved at -20^{o}C until laboratory investigation (Hemagen Diagnostics, 2001). Additionally, test principles and test procedures for Indirect Flouresnt Antibody Test (IFAT) set by Hemagen Diagnostics Inc, were used (**Annex- II**).

3.6. Questionnaire survey

About fifteen (15) questions were designed to capture information of the disease and associated risk factors those associated with the occurrence of the disease in the study area.

The contents of the questionnaire survey include general information of the respondents (age, sex, religion, educational status, bases of their livelihood). It also includes history of disease occurrence (common constraints of livestock, major livestock diseases, season of occurrence and duration of outbreak).

Additionally, herd management (Herd size, farming system, introduction of new animal, watering or grazing points, contact of animals with different areas and presence of disease transmission, Biting flies & existence of livestock markets), breed and sex were collected. The risk factors for the outbreak of the disease would be observed in the study area (**Annex 1**).

The survey was carried out in two purposively selected districts after serum collection had been finalized. Ten (10) PAs that were selected for serological survey were included in the questionnaire survey to assess factors for LSD occurrence in the study area.

A questionnaire which equivalent to the herd size was administered to the herd owners (household) and interview face to face by using local language, which was 'Oromiffaa' (Afan Oromo) that taken 10-15 minutes was carried out.

Accordingly, 127 and 125 households or farmers were included in questionnaire survey from Gimbi and Lalo Assabi districts respectively. So, a total of 252 households or farmers were interviewed using prepared semi-structured questionnaire.

The selected farmers then were asked questions related to the composition of the herd, the management system used, and if vaccination or any other treatment had been applied during or after the course of the disease.

Additionally, they were asked to explain the symptoms of the disease and clinical observation of sick animals related to LSD would be observed during sample collection in order to crosscheck whether the disease is surely lumpy skin disease or not.

Finally, valuable information was collected through questionnaire from randomly selected herd owners found in each PAs of the districts.

3.7. Procedures of the Test

Procedurally four main points were listed. These were cell seeding, cell infection, cell fixation and testing of sera. Under each point, there were line-by-line procedures. Initially, VERO cell was the cell line used to grow the virus.

The antigen used to detect the serum antibody against lumpy skin disease was SGPV (Sheep and Goat Pox Virus) and Phosphate-buffered saline (PBS) Tween buffer was diluted at 1:40 concentration using distilled water and the PH of the prepared PBS was measured before use.

Required amount of 1% skimmed milk was prepared and serum was diluted at 1:20 concentration using prepared 1% skimmed milk (blocking buffer). About 50µl of substrate solution were dispensed into each well of the microtiter plates and incubated for 30 min at room temperature. Duplicates of 50µl of diluted controls and test sera were dispensed into wells. The plates were then covered with adhesive plate cover and incubated for 30 min at 37^0C.

The incubated microplates were rinsed three times by filling each well of the plates with about 200µl PBS. After washing the plates, Fluorescence isothiocyanate (FITC)

conjugated anti-sheep immunoglobulin G(IgG) of rabbit was diluted at 1/40 in 0.5% lamb serum blocking buffer and 50µl was added to each well & kept in 37°c for 30min.

Finally, 50 µl PBS was added to each well after the plates were washed 3 times by adding 200 µl PBS and ready to observe under UV light microscope **(Appendix I: A&B).**

3.8. Data management and Analysis

Data entry and management was made using Microsoft Excel sheets. Data analysis was made using Statistical Package for Social Science (SPSS 2007, version 16) software.

An explanatory variables or independent predictors includes all the risk factors those contributed the disease occurrence and dependent or response variables includes the test result of the study.

Univariate or multivariate logistic regression was used to analyze the risk factors and its association with exposure to the disease. In all the analyses, confidence levels at 95% were calculated, and a $P<0.05$ was used for statistical significance level.

The risk of association such as Odd Ratio (OR) was analyzed for the risk factors and sero-positivity of the disease to determine the degree of association risk factors and the disease.

Descriptive statistics like prevalence was used to calculate sero-positivity by dividing the number of LSD positive animals by the total number of animals tested and the herd prevalence was determined by dividing positive herds to total number of herds and the herd would be considered positive if one or more animal in the herd would be positive to lumpy skin disease.

4. RESULTS

4.1. Animal level seroprevalence

The overall sero prevalence of lumpy skin disease in the study areas was 6.43 % (35/544). Between the two districts included in the study, the sero prevalence was significantly higher (P< 0.05) in Lalo Assabi animals as compared to animals from Gimbi District (Table 3).

Table 3. Sero-prevalence of Lumpy Skin Disease at animal level in Gimbi and Lalo Assabi Districts of West Wollega Zone

District	**Animal tested**	**N^{o} of Sero Positive (%)**	**P-value**	**OR (95%CI)**	**95% CI**
Gimbi	272	12 (4.41)			2.30-7.52
Lalo Assabi	272	23 (8.46)	0.05	2(0.90-8.03)	5.43-12.41
Total	**544**	**35 (6.43)**			**4.52-8.83**

4.2. Herd level sero prevalence

Among the 252 herds investigated in this study, 15 (5.95%, 95% CI = 3.38-9.66) of the herds had at least one positive using IFAT for LSD.

In this study, herd-level risk factors were considered and examined by logistic regression for presence of any association with herd-level sero positivity to Lumpy skin disease virus. Except breed of animals, age and summer season in which the biting flies reach at the peak, none of the risk factors considered in the analysis had significant effect on herd-level sero prevalence to LSD (p>0.05).

Table 4. Sero-prevalence of LSD at the herd level in Gimbi and Lalo Assabi Districts

District	N^{o} of examined Herds	N^{o} of positive Herds (%)	P-value	OR (95%CI)	95% CI
Gimbi	127	4 (4.15)	-	-	-
Lalo Assabi	125	11(8.8)	0.069	2.97	0.92-9.66
Total	**252**	**15 (5.95)**	**0.035**		

On the other hand, there was variation in the sero-prevalence of LSD occurrence among the cattle of different Kebeles selected for the study. Relatively high seroprevalence records were observed in Dongoro Dissi (15%) and Lelisa Yesus (13.11%) villages. On the contrary, all sera samples taken from Jogir and Were Seyo showed zero positivity for IFAT test we used in this study (Table 5).

Table 5. Descriptive and Analytic Results of Sero prevalence of LSD for Cattle of defferent Kebeles

PAs in both districts	N^{o} of sampled	N^{o} of Sero Positive (%)	P-value	OR (95%CI)	95%CI
Were Seyo	63	0 (0)	0.363	-	-
BikiltuTokuma*	58	1 (1.72)	0.031	-	0.04-9.23
Jogir	46	0 (0)	-	-	-
Chutta Kaki	44	3 (6.81)	0.004**	0.2	1.42-28.65
LelisaYesus	61	8 (13.11)	0.023**	8.6	5.83-27.22
Horda Daleti	48	1 (2.12)	0.208	1.2	0.05-11.07
Nebo Daleti	57	5 (8.77)	0.766	5.4	2.91-19.29
Werebabo Siben	52	3 (5.77)	0.049**	4.4	1.29-15.94
Haroji Serdo	55	5 (9.1)	0.305	5.1	3.01-19.93
Dongoro Dissi	60	9 (15)	0.003**	10	7.10-26.57
Ground Total	**544**	**35 (6.43)**	**0.175**	**3.49**	**4.52-8.83**

*Reference variable for OR, ** Statistical significance

4.3. Sero prevalence of LSD based on Sex, Age, Breed and Altitude differences

Comparison was made on the sero-prevalence between female and male animals. Out of animals sampled, the majority or 64.0% were females while about 36.0% of them were males. The sero-prevalences were 7.65% and 5.74% in female and male, respectively (Table 6). However, there was no statistical difference between the two sexes.

Analysis of age wise prevalence of Lumpy Skin Disease indicated that the difference in prevalence among the three age groups were relatively high in adult group (Table 6) than the young and calf age groups with statistically significant variation ($P < 0.05$).

The breed of the sampled animals showed a significant variation, where the seroprevalence of LSD recorded in cross breed cattle that was about 3 times more frequent than local zebu animals [OR (95%) = 1.2- 6.9, P = 0.016] (Table 6).

Based on altitude differences the target area was broadly classified in to midland or 'Weynadega' (1200-1900 masl) and highland or 'Dega' (> 1900 masl). Thus, comparison was made on the sero-prevalences of the Highland ('Dega') having 9.48% and Midland ('Weynadega') with 5.55% (Table 6). There was no significant variation in sero-prevalence between the two-agro climates at individual level ($p > 0.05$).

Table 6. Sero-prevalence of Lumpy Skin Disease According to Sex, Age, Breed and Altitude differences in the area

Risk factors	Animal tested	Number of positive (%)	P-value	OR (95%)	95% CI
Age					
Adult	251	22 (8.78)	0.097	3.41 (0.8-30.3)	5.56 - 12.96
Young	220	11 (5)	0.899	1.050 (0.49-2.24)	2.52 - 8.77
Calves*	73	2 (2.74)	0.005**	0.12 (0.03- 0.52)	0.3 - 9.54
Sex					
Male	348	20 (5.74)	-		3.54 - 8.73
Female	196	15 (7.65)	0.384		4.34 - 12.31
Breed					
Local*	496	28 (5.61)	-	-	2.3-19.8
Cross	48	7 (14.58)	0.016**	2.85 (1.2-6.9)	3.7-8.77
Altitude					
Highland	274	20 (7.23)			4.51-11.11
Midland	270	15 (5.55)	0.41	1.3 (0.63-2.91)	3.31-8.90
Ground Total	**544**	**35 (6.43)**			

**Reference variable for OR, ** Statistical significance*

4.4. Result of Questionnaire Survey

4.4.1. Description of the interview respondents

During the study period, 252 (n=252) respondents (individuals of herd owners) in selected 10 PAs of two (2) districts of the Zone were interviewed.

Based on their religion and educational status, the respondents were classified as Muslim; 4.0%, Christian; 93.3% and others (Wakefeta); 2.8% and as literate; 80.2% while illiterate; 19.8%. According to their sex and age, 81.3% of them were males while 18.7% of them were females and 83.3% of them were adult while 16.7% of them were young.

Additionally, based on their livelihood; 73.8% practicing mixed agriculture; 12.3% practicing mixed agriculture and trading; 7.9% practicing mixed agriculture, trading and employed and 6.0% of them were practicing others. Agro climate of the surveyed PAs of the two districts were mostly high lands and midlands.

Generally, most Christian, literate and male respondents those running their livelihood with mixed agriculture were included in this study (Table 7).

Table 7. Summary of related information from respondents in both districts

Parameters	**Categories**	**Frequency**	**Percentage (%)**
Sex of owners	Females	47	18.7
	Males	205	81.3
Age of owners	Adults	210	83.3
	Young	42	16.7
Religion	Christian	235	93.3
	Muslim	10	4.0
	Others	7	2.8
Education	Literate	202	80.2
	Illiterate	50	19.8
Livelihood	Mixed agriculture only	186	73.8
(Occupation)	Mixed agriculture & trading	31	12.3
	Mixed agriculture, trading & employed	20	7.9
	Others*	15	6.0

*(Mixed agriculture & employees, mixed agriculture & trading)

4.4.2. Associated Risk Factors of Lumpy Skin Disease Occurrence in the Study Area

Sero prevalence study and questionnaire surveys were carried out simultaneously and it has been tried to identify risk factors for LSD occurrence. The seasons were compared and about 74.2% of respondents informed that, summer was the season at which the disease occurred in the area. At the spring season, disease occurrence was the lowest (3.85%) in the area.

Also about 95.6% of respondents informed that they introduced new cattle to their herds without knowing whether the animal was vaccinated or not and the result confirmed that, only 5.8% of herds were sero positives. As 50.8% of respondents replied that, their cattle had not been vaccinated and according to a proportion of 68.3% herd owners, there was no seasonal movement of animals from place to place for search of feed and water; that means most of the farming system in the area was sedentary.

In both districts, cattle grazed communally and higher frequency of communal grazing type 63.5% and 100.0% communal watering point was reported by herd owners during this study. As the result indicated that, 98.8%, 65.5%, 91.7% and 61.5% of herd owners was reported as the presence of animals contact with different PAs, district, Zone or regions and countries respectively. A 91.7% of them reported that, dry season (Bona) was a season at which contact of animals is high in the area (Table 8).

However, it has no statistically significant association with the occurrence of lumpy skin disease. As 67.1% of herd owners informed, a summer season (Ganna) was the leading season of the year. A summer season showed statistically significant association ($p = 0.000$, OR = 4.224. CI = 1.13-7.57) with concomitant high levels of insect activity (Table 8).

In addition, factors with p-values less than or equal to 0.20 (vaccination history, seasonal movement and contact with other districts livestock's) were fitted into the multivariate logistic regression model. Nevertheless, except summer season with a high of activity of flies, none of the farm-level risk factors were found statistically significant ($p>0.05$).

Table 8. Summary of associated risk factors and its effect on herd-level sero-positivity to LSD in the study area

Major Risk Factors for LSD Occurrence	Categories	Percentage (%)	N^{o} of herd examined	N^{o} of LSD Positive (%)	P-Value	OR (95%CI)	95%CI
Season of outbreak	Autumn (Birra#)	26 (10.3)	26	3 (11.5)	0.30	3.39	0.33-34,9
	Winter (Bona#)	12 (4.8)	12	0 (0)	-	-	-
	Spring (Arfasa#)*	27 (10.7)	27	1(3.85)	-	-	-
	Summer (Ganna#)	187 (74.2)	187	11(5.9)	0.64	1.63	0.20-13.1
An introduction of new cattle	No	11 (4.4)	11	1 (9.1)	0.65	0.61	0.07-5.16
	Yes	241 (95.6)	241	14 (5.8)			
Seasonal movement of animals	No	172 (68.3)	172	10 (5.8)			
	Yes	80 (31.7)	80	5 (6.3)	0.14	1.08	0.35-3.24
Grazing Type	Communal	160 (63.5)	160	12 (7.5)			
	Separate	48 (19.0)	48	2 (4.2)	0.54	1.45	0.31-5.45
	Both*	40 (15.9)	40	1 (2.5)	0.34	0.25	0.31-5.45
Watering point	Communal	30 (100.0)	30	100.0	-	-	-
Contact of animals with different Pas	No	3 (1.2)	3	0 (0)	-	-	-
	Yes	249 (98.8)	249	15 (6.02)			
Contact of animals with different district	No	87 (34.5)	87	3 (3.4)	0.23	2.2	0.60-8.00
	Yes	165 (65.5)	165	84 (7.3)			
Contact with different Zone or regions	No	21 (8.3)	21	1 (4.7)	0.81	1.3	0.16-10.32
	Yes	231 (91.7)	231	14 (6.1)			
Contact with other country	No	97 (38.5)	97	4 (4.1)	0.33	1.7	0.55-5.74
	Yes	155 (61.5)	155	11 (7.4)			
Season of contact	Dry season	231 (91.7)	231	15 (6.5)	-	-	-
	Wet season	21 (8.3)	21	0 (0)	-	-	-
Season at activity of biting flies high	Summer (Ganna#)	169 (67.1)	162	12 (7.1)	0.000**	4.224	1.13-7.57
	Autumn (Arfasa#)*	40 (15.9)	47	3 (6.4)	-	3.982	3.98-8.98
	Spring (Birra#)	43 (17.1)	43	0 (0)	-	-	-
Existence of livestock marketing	No	175 (69.4)	175	12 (6.9)	0.36	0.54	0.18-1.99
	Yes	77 (30.6)	77	3 (4.0)			

Note: Number of examined herds is equal to the number of respondents (Herd owners) included into the study during the questionnaire survey and # Local language for seasons, *Reference variable for OR, **Statistical significance

4.4.3. Major livestock constraints in the area

There are different major constraints limiting livestock in the area. During the study period, it was tried to identify the major constraints those commonly encountered in daily activity of livestock production system in the study area. Generally, the respondents were reported that diseases, shortage of feed, shortage of water, predators and flies are main problems/constraints. Although a single interviewer reported one or more than one constraints and as indicated below (Table 9), diseases were firstly reported by a proportion of 36.9% respondents, which indicated as the first most important constraint or the leading problems for livestock.

Table 9. Summary of the major constraints by their rank in the study area

Major constraints	**Frequency**	**Percentage**
Diseases	93	36.9
Diseases, water shortage & flies	33	13.1
Diseases, feed shortage & water shortage	30	11.9
Diseases, feed shortage, water shortage, predators & flies	18	7.1
Diseases & water shortage	16	6.3
Feed shortage & water shortage	13	5.2
Water shortage & flies	12	4.8
Diseases, water shortage & predators	12	4.8
Diseases, feed shortage, water shortage & predators	8	3.2
Flies*	6	2.4
Diseases & predators	4	1.6
Diseases, feed shortage & predators	3	1.2
Diseases, feed shortage, water shortage & flies	3	1.2
Diseases and feed shortage	1	0.4
Total	**252**	**100.0**

NB: * constraints those were play a major role for the disease transmission in the area.

4.4.4. Major diseases of animals in the area

Most of the time, Trypanosomosis and external and internal parasites were repeatedly reported as the main diseases from the areas. However, including the above diseases of livestock, the respondents in the study areas commonly reported major diseases. As usually reported from the area, a proportion of 19.8% (50) respondents reported Trypanosomosis and ticks infestation as the leading diseases in their area. These diseases were listed below in (Table 10) according to their local and scientific names.

Table 10. Summary of the Local and scientific name of major diseases in the study sites

Diseases local name	**Scientific name of the diseases**	**Frequency**	**Percentage**
Gandi	Trypanosomosis	44	17.5
Abba Sanga or chita	Anthrax	12	4.8
Masa or Okolcha	Foot and mouth disease	7	2.8
Gororsa	Pasteurollosis	30	11.9
Somba (Kufa)	Bovine TB	12	4.8
Dibe guru	Mastitis	11	4.4
Dibe goga	Skin Diseases	16	6.3
Dulandula	Leach	8	3.2
Gandi and Bokoksa	Trypanosomosis and Bloat	17	6.7
Bokoksa & Gororsa	Bloat and Pasteurollosis	17	6.7
Gandi and Sinchi	Trypanosomosis & Black leg	2	0.8
Gandi and silmi	Trypanosomosis &Tick infest^{n}	50	19.8
Others*	_	26	10.3
Total		**252**	**100.0**

NB: *[Dhukuba sare (Rabies), Dhukuba tiruu (Fasciolosis), Hidda arrabaa (Toxocity), Ciniinnaa (Colic)

Figure 6. A lumpy skin diseased cows (Local and crossbreds) showing skin nodules covering the entire body at Dongoro Dissi village of Lalo Assabi district.

5. DISCUSSION

In the present study, Lumpy Skin Disease Virus (LSDv) exposure was investigated in the two administrative districts of West-Wollega Zone (Gimbi and Lalo-Assabi) by applying field study, serological analysis and questionnaire surveys.

5.1. Animal level Sero-prevalence and Associated Risk Factors

The 6.43% seroprevalence of Lumpy Skin Disease recorded in cattle of the study was close to the animal level (6%) and overall (8.1%) sero prevalences recorded by Gari *et al.,* (2008) in southern Ethiopia and Gari *et al.,* (2010) for the different agro-ecological zones in Ethiopia. It is also worth to mention that, other studies based clinical observation on the disease were made around Nekemet which is close to this study area and, 7% prevalence was reported (Regassa, 2003). Again, targeted study on outbreak areas of Southern Range land, around Wolliso town and in three districts of eastern Amhara region reported prevalence of 11.6%, 27.9% and 28%; respectively (Asegid, 1991; Beshawarad, 1991; Gari *et al.,* 2008).

In the present investigation, the overall animal sero-prevalences of LSD in the two administrative districts of West-Wollega namely Gimbi (4.41%) and Lalo-Assabi (8.46%) showed a significant variation with logistic regression analysis. Similarly the overall prevalence observed in Lelisa Yesus (13.11%) and Dongoro Dissi (15%) was significantly high as compared to the rest of the Kebeles which was due to factors like sharing common boundary with Beni-Shangul Gumuz Regional state, focal grazing point and high livestock trade activity.

This finding agrees well with the finding of Gari *et al.* (2008), who stated a difference in the frequency of occurrence of LSD across 15 districts they selected for their study. In addition, many factors such as season, insect vector activity, the health status and breed of the animals can affect the magnitude and the occurrence of LSD (Kitching and Mellor, 1986, 1991; Barnard *et al.,* 1994; Chihota *et al.,* 2001; Babiuk *et al.*, 2008).

In the present study, an attempt has been made to compare the susceptibility of the indigenous (Zebu) and crossbred (Zebu x Frisian) breeds of cattle raised in the same management system. The result revealed a significantly higher sero-postivity result in the cross breed (OR = 2.85, P = 0.02). This result some how goes with the previously suggested idea that, the breeds of *Bos taurus*, imported into Africa from Europe, or Australia are far more susceptible than the indigenous *Bos indicus* cattle (Davies, 1991; Barnard *et al.*, 1994; Babiuk *et al.*, 2008).

Analysis of the association between age and sero-positivity for LSD revealed no statistically significant variation among the three age categories; however, the sero prevalence in calves is very low as compared to adult and young age groups. This may be indicative of prevailing passive maternal immunity and low frequency of exposure.

Similar to this finding, Rweyemamu *et al.* (2000) reported that, suckling calves showed the lowest attack rate, though in the dynamic model younger cattle did not show higher susceptibility to infection in their study of mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus during a certain outbreak. There were no previous reports of age related susceptibility to LSD.

A possible alternative explanation for the lower sero prevalence recorded in calves in this study may be associated with lower susceptibility of calves to biting by flies as previously described (Troyo *et al.*, 2007). Another potential explanation can be associated with location, as the lowest prevalence was documented in the calves, which were kept at homestead where there is less insect vector activity. The study revealed high sero prevalence (8.78%) in adults, in which the maternal immunity level drops and exposed to diseases, as the age increases.

The absence of significant association ($p>0.05$) between sex and sero positivity to LSD was observed in current investigation using univariate analysis, but Tuppurainen, (2011) reported that lactating cows seem to be the most susceptible. On the contrary, Blood *et al.* (1983) indicated that, male zebu cattle had higher cumulative incidence

than females and this might be attributable to the stress factor of exhaustion and fatigue rather than to a biological reason.

Another reason given by Gari *et al.* (2010) also mentioned that, the majority of male animals were draft oxen used for heavy labour, which might contribute to an increase in susceptibility. The same authors also reported as draft oxen cannot protect themselves well from biting flies when harnessed in the yolk, and the beat scratches on their skin induced while ploughing may attract biting flies potentially capable of transmitting LSD infection.

Relatively higher sero-prevalences were found in the highland (7.23%) than midland (5.55%) with no statistical variation in this study. On the other hand, Gari *et al.* (2008) found out that LSD occurrence to be high in midland and lowland agro-climates than the highland agro-climate in some other parts of Ethiopia. In addition, a recent study done by the same authors in 2012 based on serology estimated by using a Bayesian model and herd level seroprevalence was higher in the midland (64%) as compared to the lowland (50%) and the highland (26%) agro-climatic Zones of Ethiopia.

5.2. Herd Level Sero prevalence and Associated Risk Factors

The overall herd prevalence recorded in this study (5.97%) was very low when compared to the previous herd level reports of 64%, 26% and 50% for midland, highland and low land agro climate zones of Ethiopia (Gari *et al.*, 2012). However, the presence of a single sero positive herd could also be in support of herd level endemicity of LSD in the area.

Even if the sample size was sufficient in the present study for LSD, the strength of associations between most of risk factors considered and analysed for their associated with sero-positivity status were not significant. However, none of the factors considered for herd-level prevalence in the study were significant, the influences of management related risk factors and characteristics of the population for occurrence

of infection in a herd are reported to have an important role (Gari *et al.*, 2010; Gari *et al.*, 2012).

Associated Risk Factors: Herd those selected for serological survey were also included in the questionnaire survey to assess factors for LSD occurrence in the study area. Beside the blood collection, questionnaires were commonly applied in epidemiological investigation to collect information on disease occurrence and associated risk factors and they have been used successfully.

The study was under taken to identify risk factors that contribute to the occurrence of lumpy skin disease in the study area. This has been reported (Ali *et al.*, 1990 and Tuppurainen and Oura, 2012) that the outbreak of the disease was mostly associated with the prevalence of insect vectors, host susceptibility, livestock density at the grazing and watering points, husbandry systems, wet seasons and agro ecologic conditions, presence of moist, humidity, market conditions and an introduction of new animals without any examination.

As the questionnaire survey result indicated Lumpy Skin Disease (LSD) was dominates the area due to one or more factors those attributes the occurrence of the disease. From the result, 65.1% of respondents informed presence of the disease in their area and it agrees well with the finding of Gari *et al.* (2010), in which about 42.8% of the interviewees reported occurrence of LSD in their herd.

This finding revealed that, 95.6% of herd owners informed that they introduced new cattle to their safe herds without identifying whether the animal was vaccinated or not and the result of analysis confirmed that, 5.8% herds were sero positives. This indicated that, most herd owners from both districts acquired cattle through purchasing from auction markets and very few of them claimed that they acquired cattle from inheritance or dowry. It also showed, cattle keepers were tilted towards commercial farming than traditional cattle keeping in which there is a chance to introduce a LSD positive animal into the LSD free herds. However, it was not statistically significant ($p>0.05$) for the occurrence of the disease in the area.

Similar finding was reported by Gari *et al.* (2010), as the frequency of introduction of new animals was higher in the midland agro-climate zone (40.6%) than in the highland and the lowland zones (25.2% and 21%, respectively). The same authors also reported that the introduction of new animals to a herd had a strong association with an increased risk of disease in the herd and a noticeable proportion of farmers (32.1%) reported introducing new animals to their herd following purchase (for replacement, herd expansion, fattening), receiving cultural gifts or cattle exchange without any screening for the health status of the new animal.

Another attempt has been made in the present study to compare the season at which an outbreak of the disease can be occurred and about 74.2% of respondents reported; it was high in summer season and the lowest Spring seasons was (3.85%) in the area. This could be due to rainy nature of the season and livestock production system in which the extensive system was dominant in the area, that exposes animals to the biting flies those are active for interrupting feeding.

Similar to this finding, Tuppurainen and Oura, (2012) and Gari *et al. (*2010) mentioned, LSD outbreaks were associated with wet and warm weather conditions due to an abundance of blood-feeding arthropod populations in the summer season.

According to a proportion of 68.3% (172) herd owners, there was no seasonal movement of animal from place to place for search of feed and water; that means most of the farming system in the area was sedentary. As a result of analysis revealed, there is a slight difference of 6.3% sero-positivity for LSD in herds those move as compared to the herds not move from place to place with no statistically significant variation among (p=0.14, OR=1.08, CI=0.35-3.24).

Higher frequency of communal grazing type 63.5% and 100.0% communal watering point was reported by herd owners during this study. In both districts, cattle grazed communally (Table 8) although those with a few cattle had them tethered. Even though there was, an increment report found with communal grazing and watering points, multivariate logistic regression analysis revealed statistically insignificant effect among these risk factors and occurrence of the disease in the area.

However, Gari *et al.* (2010) mentioned that communal grazing and watering points were found to be associated with the occurrence of LSD. Additionally, different authors (Chihota *et al.*, 2001; Kitching, 1985; Waret-Szkuta *et al*., 2009) were reported as sharing watering points, grazing plots and post-harvest fields would allow contact and intermingling of different herds that would probably increase the risk of exposure.

A questionnaire result of the present study showed that, 67.1% of respondents reported that, a summer (wet season) was a season at which the activity of biting flies is high and showed statistically significant association (p = 0.000, OR = 4.224. CI = 1.13-1.57). Flies activity was four times (4x) more likely to be high in the summer (wet) as compared to other seasons for the occurrence of the disease in the area (Table 9).

Similar to this finding, Davies (1981) and Mac Owen (1959) reported that biting insects play the major role in the transmission of LSDV. Troyo *et al.*, (2008) also mentioned that, epidemics of LSD are associated with rainy seasons. Currently, it is widely agreed that LSDV is transmitted mechanically via arthropod vectors.

This result also agrees well with the finding of Gari *et al*. (2010), who described the warm and humid climate in midland and lowland agro-climates has been considered a more favourable environment for the occurrence of large populations of biting flies than the cool temperature in the highlands. The same authors also stated that both biting-fly activity and disease outbreak frequencies begin to increase from April reaching a maximum in September. In conclusion, the result of this work strongly supported that LSDV is transmitted mechanically via arthropod vectors.

Out of 252 interviewees; 98.8 %, 65.5%, 91.7% and 61.5% of them reported that their cattle could travel and be in contact with different animals of different PAs, district, Zone or regions and countries, respectively. This could be due to animal's movement from place to place for the purpose of vaccination, trade activity, searching for feed and water during dry season and other activities, which is a risk factor in contracting cattle diseases such LSD. A 69.4% of respondents were declared, as there was no existence of livestock marketing in the area.

During this study period, it has been tried to identify the major constraints commonly encountered in daily activity of livestock production system in the study area. As herd owners informed diseases, shortage of feed and water, predators and flies were the main problems or constraints of livestock in the area. Although a single interviewer and diseases reported, one or more than one constraints were firstly reported by a proportion of 36.9% respondents, which indicated as the first most important constraint or the leading problems for livestock production and followed by water shortage and flies by 13.1% in the area.

Similarly, Nibret and Basaznew and Belay *et al.* (2012) stated that diseases were the main constraints of livestock. Some other constraints observed in both districts were lack of veterinary extension services and poor breeds. So, the findings of the major constraints limiting livestock production during the present study in the area was tended to agree with findings found Nibret and Basaznew (2012).

The same authors also mentioned that, fasciolosis (32.45%), gastrointestinal parasitism (14.66%), anthrax (10.54%) and blackleg (9.56%), pasteurellosis (7.91%), lumpy skin disease (5.60%) and trypanosomosis (2.31%) were the most frequently observed diseases in cattle.

A 19.8% of respondents reported that Trypanosomosis and ticks infestation as the leading diseases and about 6.3% of skin diseases (Dibe goga in local language) including lumpy skin disease were reported by herd owners in the area in their area in which the result of the finding agrees well with the previous study.

6. CONCLUSION AND RECOMMENDATIONS

The present cross-sectional study indicated that lumpy skin disease is an important disease in the western Wollega zone of Oromia regional state of Ethiopia. Even if the recorded sero-prevalence is moderate, the disease is found to be spreading in to new areas that have been considered previously as free areas (kebeles or districts) of the zone and will be a major livestock health problem.

The result showed that, LSD has been associated with periods (Seasons) of high rainfall and concomitant high levels of insect activity. Some of the risk factors such as sex, age, breed and altitude considered in this study were found to be associated with the sero-positivity of the disease at animal level. This revealed that some of the current cattle management practices executed by livestock owners of the study area, namely: Introduction of new animal to the herd, mixing of cattle in watering and grazing areas, free movement of animals to different areas are very common, can be a risk factors, and could aggravate the spread of lumpy skin disease.

This study provides the first evidence (preliminary information) of the presence of LSDV infection in the West Wollega. This finding also gives attention on the distribution of LSDV in the study area and can assist planners, decision-makers, practitioners and researchers in their efforts. In addition, it could help them in disease surveillance and control activities for risk mitigation and to improve the health of animals.

Therefore, based on these findings the following recommendations are forwarded that might help in preventing losses associated with the occurrence of LSD improving the productivity of cattle: Mass vaccination should be applied for all breeds of cattle in both districts using an effective specific vaccine against LSD, such as the attenuated Neethling strain vaccine. The use of insecticides to control biting flies before raining season should be practiced in the area. Livestock owner need to be introduced with the basic knowledge of risk factors those contribute to disease in the study area. Due to the biggest challenge, that was poor infrastructure that facing during this study, further research is needed to assess the status of the disease and to suggest implementation of appropriate control and prevention methods in the areas.

7. REFERENCES

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8. ANNEXES

Annex I. Questionnaire Design for Study on Sero-Prevalence and Associated Risk Factors of Lumpy Skin Disease in West Wollega, Ethiopia

Dear respondents, I am conducting a Research on the causes of low Animal productivity in two Districts/Woredas of West Wollega Zone (Gimbi and Lalo Assabi). So, this questionnaire is designed to collect reliable and relevant information from you on the issue under study. Hence, you are kindly requested to complete (fill) this questionnaire sincerely and honestly. Please, make 'tick' (√) and write information as necessary, in the space provided. I never forget to acknowledge you for your polite behavior, generous support and spent time to this information.

With best regards!

Name of the Area: _____ Questionnaire n^{o}: ___Date of Interview: _____

Section One: General Information

1. Owner'sName_____Region_____Zone_____Woreda_____Kebele_____Age_ Sex__Religion___EducationalStatus:literate___Illiterate___OthersSpecify_____
2. What are the bases of your livelihood? 1. Livestock's only ☐ 2.MixedAgri. 3. Trading ☐ 4. Employed ☐ 5. Others (Specify)_____☐
3. Agro climate of the area: High lands ☐ Midland ☐ Low land ☐
4. Geo. Reference: N _____ E _____ Alt _____

Section Two: History of Diseases Occurrence

5. Total cattle herd size of the farmer_____: Herd structure Ox____ Bull____ Beef____ Lactating cow_____ Dry cow_____ Heifer_____ Calf_____;
6. If exotic breed cattle specify the breed, number, sex, age_____
7. Other farm animals holdings:

Description	Number
Cattle	
Sheep	
Goats	
Equines	
Others	

8. What are Common constraints of livestock production?
   1._____
   2._____
   3._____
   4._____

9. What are the Major livestock diseases occurring in last three years ranking in its order of importance with their symptoms?

Age of Animals	Name of Disease	Clinical Signs
Calf		
1		
2		
3		
4		
Young		
1		
2		
3		
4		
Adult		
1		
2		
3		
4		

10. Do you know Lumpy Ski Disease (LSD) or 'dibe goga' in your Area? Yes /No?

10.1. If yes, how you can identify the disease (LSD)?

_____

10.2. When did the disease commence in the area (PA)? Season __Mon__ year____

10.3. How long since the outbreak has been seen in the area, < 1yr__ 1-2 Yrs___2-3Yrs___ >3Years___

10.4. In which months/seasons of the year that LSD often seen in your area? _____

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

Section Three: Herd Management

11. Farming System: Sedentary__ transhumant___

12. Herd level Risk Factors Identification

12.1. Did you buy new cattle or introduced new cattle since 6 months before the onset of the outbreak? Yes/No, if yes, origin of the cattle, number, sex and age? _____

12.2. History of vaccination against LSD: Vaccinated, Yes /No?

If yes, at which season (when)? _____

If no, why? Explain_____

12.3. Grazing type: a) Separate b) communal c) both

12.4. Do you have common watering/grazing points with other herds: Yes /No?

12.5. Contact with animals of different area

Did you move your cattle to other grazing place seasonally? Yes /No?

12.6.If yes, when_____, where _____, how long did you keep them there_____.

12.7. Contact with animals of different Kebele: Yes /No?

12.8. Have contact with livestock of different district: Yes /No

12.9. Have contact with livestock of different Zone/regions: Yes /No?

12.10. Have contact with livestock of other country: Yes /No?

12.11. When the animals contact _____

12.12. An introduction of new animals: Yes /No?

13. At which months/season the activity of Biting flies of cattle are highest?

14. Do you know any disease that can be transmitted from wild life to livestock?

Yes No

14.1. If yes, what are they? 1._____ 2._____ 3._____

4._____5._____6._____

15. Does livestock market exists in the villages: Yes /No?

15.1. Name and distance (in km) of livestock market frequently used and the known cattle trade route around their area_____.

Annex II. Sample Collection Format

National Animal Health Diagnostic and Investigation Center (NAHDIC)

Quality Management System

Laboratory Specimen submission Form

Part II Additional information to be filled for Surveillance and certification purpose

Animal ID	Kebele	village/ Abattoir	Species	Sex	Age	Sample	Geo. Reference		Altitude	Lab. code	Date tested	Test Type	Test Result
							N	E					

Annex III. IFAT Plate layout

IFAT Plate prepared on _____

IFAT for _____ Plate No. ____ Date_____ Reader _____

Serum dilution 1/20 FITC dilution1/40 Infection time 48hrs TCID50/well 100

A												
B												
C												
D												
E												
F												
G												
H												

Annex IV. Indirect Fluorescent Antibody Test Laboratory Protocol

A. Preparation of Ag coated Microplates (96 wells Tissue grade Microplate)

1. Prepare cell culture media (MEM +10% Fetal Calf Serum (FCS) + Antibiotics optional)
2. Add 100 µl of lamp testis cells suspension (2*104 cells/ml) to each 96 wells
3. Incubate the plates for 24hrs in 37°c and 5% CO2
4. Prepare the KS1 virus dilution of 2*103 TCID50 /ml in MEM cell media (without FCS)
5. Remove the previous media in the plate slowly using multichannel pipette keeping the plate in gentle slope to remove all
6. Add 50 µl viral suspensions to each well and incubate at 37°c for 2hrs
7. Add 150 µl media with 10% Fetal Calf Serum to all wells and keep in 37°c for 48hrs
8. Remove the media by multi channel pipette in the same way as n° 5 to infect the cell
9. Add 100 µl acetone 80% cooled at 4°c to all wells for fixation and discard by pippete
10. Add 50 µl acetone 80% to each well & keep the plate in -20°c for 30min
11. Remove the acetone, wrap the plate with Aluminum paper and keep in -20°c until the plate is required for examination

B. Procedure to test the sera

1. Add 50 µl PBS- 0.01M to each well and keep for 30min at room temperature.
2. Add 50µl blocking solution (1% skimmed milk or 0.5% lamb serum in 0.01M PBS) to each well and incubate in 37°c for 20min
3. Dilute the test sera in blocking solution at 1/25 dilution
4. Take your record sheet and write the layout of the Microplate to add the test sera, strong +ve, weak +ve and negative control sera.
5. Add 50 µl of diluted serum to the microplate in duplicate wells according to the layout and incubate at 37°c for 30min
6. Rinse each well with 200 µl PBS 0.01M and discard by turning up-side down, repeat 3 times
7. Dilute the Fluorescein isothiocyanate conjugated anti-bovine gamma-globuline (IgG) of Rabbit serum (FITC Rockland) in 1/40 dilution in blocking buffer solution (Note: Antisheep and Anti-goat FITC from Dako also work perfectly like anti bovine FITC)
8. Add 50 µl diluted FITC to each well & keep in 37°c for 30min
9. (6) Wash the plate by 200 µl PBS 3 times
10. Add 50 µl PBS to each well and observe under UV light microscope.

Annex V. Reagents and materials required for the test

Reagents: were Vero cells, viral suspension (Cpx vaccine, etc) properly diluted, cell medium MEM with EARLE salts; 10% fetal calf serum (Fcs) that contain 1% de-glutamine in ml, cell medium MEM with EARLE salts that contain 1% de-glutamine in ml, L-glutamine, Fetal calf serum (Fcs), Trypsin-versene, Trypan blue, PBS1x, Ice, 70% Alcohol, 80% acetone, Anti-PPR monoclonal antibody, Anti-goat antibody conjugated with FITC, Dilution fluid (DF) (monoclonal antibody and conjugated) 1 % skimmed milk in PBS

Materials: were paper roll, Timer, Rotary microtiter plate shaker, Incubator (equipped with rotary microtiter plate shaker), Multichannel pipettor, Single channel pipettor, Glassic Pipettes (1ml, 5ml &13ml), Yellow tips, Troughs and Adhesive plate cover.

Annex VI. Principles of the test

The VIRGO fluorescent antibody assays utilize the indirect method of fluorescent antibody staining; first described by Weller and Coons in 1954. The procedure is carried out in two basic reaction steps. In step one, the serum to be tested is brought into contact with the antigenic substrate. Antibody, if present in the test serum, will attach to the antigen, forming an antigen-antibody complex. If the serum being tested does not contain antibody for this particular antigen, no complex is formed and all the serum components are washed away in the rinse step.

In the second step, the infected cells were fixed and allowed to react with Capri pox antibodies. In addition, it involves the adding of a fluorescein labelled antisheep and goat antibody to the test wells. If the specific antigen-antibody complex is formed in step one, the fluorescein labelled antibody will attach to the antibody moiety of the complex in step two.

The plates were read using Inverted fluorescence microscope under 40× magnifications. The positive tested serum appears with bright fluorescence foci where the antibody has reacted with the virus (apple green when stimulated by UV or blue light) and the negative serum appears as a dark field or dim gray foci.

Annex VII. Figure of cell seeding

Annex VIII. Figure of cell media ready for infection by the virus

Annex IX. Summary of kebeles, herd owners and livestock population in both districts

Name of districts	Nº of total PAs in districts			Name of selected PAs	Total herd owners	Nº of selected herd owners	Cattle Popⁿ in PA	Sample size in PAs	Altitude
	Rural	Urban	Total						
Gimbi	31	1	32	Were Seyo	150	23	1,725	63	1903
				Bikiltu Tokuma	460	28	2,734	58	1821
				Jogir	579	25	2,422	46	1298
				Chutta Kaki	421	23	2,474	44	2016
				Lelisa Yesus	510	28	2,248	61	1851
Subtotal	**31**	**1**	**32**	**5**	**2,120**	**127**	**11,603**	**272**	**-**
Lalo Assabi	27	4	31	Horda Daleti	707	25	994	48	1766
				Nebo Daleti	513	26	1,040	57	1618
				Werebabo Siben	620	23	1,212	52	1933
				Haroji Serdo	652	28	1,344	60	1936
				Dongoro Dissi	416	23	1,724	55	1937
Subtotal	**27**	**4**	**31**	**5**	**2,908**	**125**	**6,314**	**272**	**-**
G. total	**58**	**5**	**63**	**10**	**5,028**	**252**	**17,917**	**544**	

Source: Agency of Livestock Resource, Development and Animal Health Office of the both Districts.