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Getachew GARI JIMOLU

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Etude épidémiologique de la dermatose nodulaire contagieuse bovine en Ethiopie et évaluation de son impact économique

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JURY
Philippe DORCHIES, Professeur, Emérite, ENVT, Président
Jean-Pierre GANIERE, Professeur du Ministère de l'Agriculture, ENV Nantes, Membre
Stéphane BERTAGNOLI, Maître de conférences, ENVT, Membre
Philippe JACQUIET, Professeur du Ministère de l'Agriculture, ENVT, Membre
François ROGER, Chercheur, CIRAD Montpellier, Membre

Ecole doctorale :
Sciences Ecologiques, Vétérinaires, Agronomiques et Bioingénieries (SEVAB)

Unité de recherche :
Animal et Gestion Intégrée des Risques (AGIRs), CIRAD, Montpellier

Directeur(s) de Thèse :
Philippe JACQUIET, Professeur du Ministère de l'Agriculture, ENVT
François ROGER, Chercheur, CIRAD Montpellier

Rapporteurs :
Etienne THIRY, Professeur du Ministère de l'Agriculture, Université de Liège
Didier CALAVAS, Ingénieur de Recherche, AFSSA Lyon

Epidemiological Study of Lumpy Skin Disease and Its Economic Impact in Ethiopia

Getachew GARI

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List of publications

1. Gari, G., Tilahun, G., Dorchies, P. 2007. Study on Poultry Coccidiosis in Tiyo District, Arsi Zone, Ethiopia. *Intl. J. Poult. Sci.* 7(3):251-256.

2. Gari, G., Tilahun, G., Dorchies, P. 2007. Preliminary study on Natural Resistance of Two Chicken breeds to Acute *E. tenella* Experimental infection. *Ethiop. Vet. J.* 11(2) 25-39.

3. Gari G, Biteau-Coroller F., LeGoff C., Caufour P., Roger F., 2008: Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method. *Vet. Microbiol.*, 129: 269-280.

4. Gari, G., A. Waret-Szkuta, V. Grosbois, P. Jacquiet, F. Roger, 2010: Risk factors Associated to Observed Clinical Lumpy Skin Disease in Ethiopia. *Epidemiol. Infect*., 138:1657-1666.

5. Gari, G., Waret-Szkuta, A., Roger, F., Bonnet, P. Epidemiological aspects and Financial Impact of Lumpy Skin Disease in Ethiopia. Submitted to Prev. Vet. Med.

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Résumé

La dermatose nodulaire contagieuse (DNC) est une des maladies virales les plus importantes économiquement chez les bovins en Ethiopie. Elle est causée par le virus LSD (Lumpy skin disease virus) appartenant au groupe des Capripoxvirus. L'objectif de cette thèse est de mieux comprendre l'épidémiologie de cette maladie afin de proposer des méthodes de contrôle et de prévention efficaces et applicables sur le terrain. Cette thèse est construite en cinq chapitres. Le premier chapitre fait une description générale du système de production agricole en Ethiopie et présente nos connaissances actuelles sur ce virus et cette maladie. Le second chapitre est consacré à l'évaluation d'un test d'immunofluorescence indirecte (IFI) pour le diagnostic sérologique à l'aide de méthodes sans gold standard. Le test de séroneutralisation virale a été utilisé comme second test de comparaison. L'analyse à l'aide d'un modèle bayesien a montré que l'IFI présentait une bonne sensibilité (92%) et une bonne spécificité (88%) ce qui suggère que ce test peut être utilisé pour le diagnostic et le dépistage de masse de la DNC avec une relativement faible proportion d'erreurs. La possibilité de tester un grand nombre de sérums en IFI est un autre avantage de cette technique pour conduire des études épidémiologiques de grande envergure. La sensibilité et la spécificité de la séroneutralisation virale (SNV) étaient respectivement de 78% et de 97%. Les deux tests IFAT et VNT ont donné des résultats conditionnellement indépendants sur l'état de la maladie de l'animal. En conséquence, le test IFI sera préféré pour un dépistage de masse en raison de sa meilleure sensibilité tandis que le test SNV sera réservé à la confirmation.

Une étude épidémiologique transversale a été menée pour estimer la prévalence de la DNC Bovine à l'échelle du troupeau et de l'individu et pour définir les facteurs de risque associés à cette maladie dans le contexte particulier de l'Ethiopie. C'est l'objet de la troisième partie de

cette thèse. Un total de 330 questionnaires d'enquêtes a été collecté de 44 associations paysannes situées dans 15 districts. La prévalence moyenne de la DNC à l'échelle du troupeau était de 42,8% (IC à 95% : 37,5 – 48,3). Elle était significativement plus élevée dans les zones d'altitude moyenne 55,2% (IC à 95% : 47,5 – 62,6) que dans les zones de basse altitude (22,3%) ou les zones de haute altitude (43,5%). La prévalence de la DNC et la mortalité due à cette maladie, observées à l'échelle de l'animal, étaient de 8,1% et de 2,12% respectivement. A nouveau, elles étaient plus élevées dans les zones d'altitude moyenne (10,4% et 3,2% respectivement) que dans les zones de basse et haute altitude ($P < 0,05$). L'analyse de facteurs de risque a montré que trois variables étaient significativement associées avec la prévalence de la DNC : l'effet de la zone agroclimatique, la conduite de troupeaux différents sur les mêmes pâtures et les mêmes lieux d'abreuvement et l'introduction de nouveaux animaux. L'incidence maximale de la DNC était concomitante de l'augmentation des populations d'insectes hématophages : cette association dans le temps était significative (coefficient de Spearman de 0,88 ; 0,79 et 0,79 respectivement pour les zones de haute, moyenne et basse altitude).

L'évaluation de la faisabilité financière et des bénéfices espérés de la vaccination ont constitué la quatrième partie de la thèse. Le coût financier à l'échelle de la ferme des cas cliniques de DNC et le bénéfice économique de son contrôle par la vaccination ont été analysés dans cinq districts de la région Oromia. 747 questionnaires concernant une période de production d'un an ont été collectés. Des données d'épidémiologie descriptive ont été obtenues. L'incidence cumulée sur un an et les taux de mortalité ont été calculés pour chaque race, sexe et groupes d'âge. Le coût annuel des cas cliniques de DNC a été calculé en additionnant les pertes de production dues à la morbidité et à la mortalité. Les paramètres intervenant dans l'estimation des coûts financiers étaient les pertes de lait et de viande, la perte de capacité de travail (traction essentiellement) et

les coûts de traitement et de vaccination. Le coût financier annuel par tête de bétail a été estimé à 6.43 dollars américains (USD) pour le zébu local et 58 USD pour les croisés Holstein dans les troupeaux infectés.

Le bénéfice financier du contrôle du DNC par une année de vaccination prévue a été calculé en utilisant l'analyse du budget partiel et les changements de la production de l'entreprise dûs à l'intervention de contrôle et ont été mesurés à partir des variables de production de lait, de viande et de la puissance de traction. Le taux de rendement marginal (MRR) a profité de l'intervention de contrôle et a été estimé à 76 (7600%) et le bénéfice net par tête était de 3 USD et 33 USD chez le zébu local et HF / bovins croisés respectivement. La réduction des coûts financiers de la DNC par tête de bétail à l'aide d'un plan de vaccination annuel a été évaluée à 40% pour le zébu local et à 58% pour les bovins croisés Holstein. L'analyse comparative entre vaccination et absence de vaccination a permis de montrer que les producteurs locaux pourraient non seulement récupérer un bénéfice financier substantiel de la vaccination mais qu'ils pourraient également assurer la survie à long terme de leur élevage. Finalement, dans la cinquième partie sont présentées une discussion générale de l'étude épidémiologique et des moyens de contrôle ainsi que les questions non résolues qui nécessitent des efforts de recherche supplémentaires. Les résultats de l'étude des facteurs de risque pourrait également apporter des informations utiles pour la connaissance de l'épidémiologie de la DNC bovine dans d'autres pays africains.

Summary

Lumpy skin disease (LSD) is one of economically important viral diseases of cattle in Ethiopia caused by *Lumpy skin disease virus* in the member of the genus *Capripox viruses*. The objective of this thesis is to better understand the epidemiological features of the disease in order to propose practical and applicable control and prevention options. The thesis is classified in five chapters. The first chapter describes the general agricultural production system in Ethiopia and relates the current knowledge on the virus and the disease as given by the literature.The second chapter deals with the performance of indirect fluorescence antibody test (IFAT) as a serological diagnostic and screening tool that was evaluated using methods without gold standard. Virus neutralization test (VNT) was used as the second test for comparison. The analysis of conditional dependent Bayesian model showed that the IFAT had good accuracy both in sensitivity (92%) and specificity (88%) parameters indicating that it could be used for LSD diagnosis and screening (epidemiological studies, epidemiosurveillance) with less misclassification. Its capacity to run large number of samples per plate just like ELISA could be also taken as an advantage for large epidemiological studies. The sensitivity and specificity of VNT was 78%, 97% respectively. The two tests IFAT and VNT were found conditionally independent on the disease status of the animal. Thus, higher sensitivity and throughput for IFAT would render the test being selected for screening purposes and higher specificity performance of VNT would qualify it to be used as a confirmation test.

A cross sectional study was then conducted to estimate the prevalence of LSD at herd and animal-levels and to analyze the risk factors associated with the disease occurrence in Ethiopia. It is presented in the third chapter. A total of 330 questionnaire surveys were collected from 44 peasant associations (PA) distributed in 15 districts. The average herd level LSD prevalence was

42.8% (95% CI: 37.5–48.3) and it was significantly higher in the midland agro-climate 55.2% (95% CI: 47.5–62.6) than in lowland and highland agro-climate zones (22.3% and 43.5%, respectively). The observed LSD prevalence and mortality at animal level were 8.1% and 2.12% respectively which were still higher in the midland zone (10.4% and 3.2%, respectively) than in lowland and highland zones ($P<0.05$). The risk factor analysis showed that three variables: the effect of agro-climates, communal grazing/watering management and introduction of new animals were significantly associated with LSD occurrence. The temporal association between LSD occurrence and increase in the biting-fly population was also positively correlated by Spearman rank correlation coefficient (0.88, 0.79 and 0.79 for highland, midland and lowland zones, respectively) and statistically significant. The need to evaluate the financial feasibility and benefit possibly expected of vaccination led us to the fourth component of the thesis: The financial cost of clinical LSD at the farm level and the economic benefit of its control by vaccination from the farmers' perspective were analyzed in five selected districts in Oromia Regional state, Ethiopia.

A pre-tested questionnaire survey addressing the period of one year production cycle was considered and 747 questionnaires were collected. Descriptive epidemiological results were obtained from the questionnaire survey data. Annual cumulative incidence, mortality and case fatality rates were calculated for each breed, sex and age groups. Annual financial cost due to clinical LSD infection was calculated as the sum of the average production losses due to morbidity and mortality. The variables that accounted for financial cost estimation were milk loss, beef loss, traction power loss, and treatment and vaccination costs. Annual financial costs per head were estimated of 6.43 USD in local zebu and of 58 USD in Holstein Friesian (HF)/ crossbred cattle in infected herds.

The financial benefit of controlling LSD through a one year planned vaccination was calculated using partial budget analysis and the changes in the enterprise outputs from the control intervention were measured from the variables milk production, beef production and draft work-output. The marginal rate of return (MRR) gained from the control intervention was estimated at 76 (7600%) and the net benefit per head was 3 USD and 33 USD in local zebu and HF/crossbreds cattle respectively. This implied that annual vaccination had enabled to reduce the financial costs due to LSD by 40% and 58% per head in local zebu and HF/crossbreds respectively. The analysis of the planned vaccination as compared to a non vaccination scenario for a one year time horizon have shown that the livestock producers would get substantial benefit not only from financial gain perspective but also to secure and maintain sustainable farm business. Finally in the fifth chapter, general discussion on the epidemiological study and control options were presented along with persistent knowledge gaps that requires further research efforts to fine-tune the proposed control and prevention options. The result from the risk factor analysis could also shed light on the epidemiology of LSD in other African countries suffering from the disease.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AGID	Agar gel immunodiffusion
CaPV	Capripox virus
CI	Confidence interval
CIRAD	Centre International de Recherche Agronomique pour le Développement
CPE	Cytopathic effect
CSA	Central Statistics Authority
CuI	Cumulative incidence
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immune sorbent assay
FAO	World Food Organization
FITC	Fluorescein Isothiocyanate
FMD	Foot and mouth disease
GDP	Gross domestic product
GP	Goat pox disease
GTPV	Goat pox virus
HF	Holstein Friesian
IFAT	Indirect fluorescent antibody test
IgG	Immunoglobulin G
KS-1	Kenyan sheep pox strain 1
LSD	Lumpy skin disease
LSDV	Lumpy skin disease virus
m.a.s.l.	meter above sea level
MLE	Maximum likelihood estimate
mm	millimeter
MoARD	Ministry of Agriculture and Rural Development
mRNA	messenger ribonucleic acid
MRR	Marginal return rate
NB	Net benefit
nm	nanometer
NVI	National Veterinary Institute

OA3.Ts	lamb testis cell line
OIE	Office International des Epizooties, World Animal Health
OR	Odds ratio
PA	Peasant Association
PARC	Pan African Rinderpest Campaign
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
PPR	Peste des Petits Ruminants
RVF	Rift valley fever
Se	Sensitivity
SGPV	Sheep goat pox virus
SNNPR	Southern nation nationalities and peoples region
SP	Sheep pox disease
Sp	Specificity
SPPV	Sheep pox virus
SSDP	Small scale dairy production
$TCID_{50}$	Tissue culture Infective dose 50%
TCV	Total costs that vary
TLU	Tropical Livestock unit
USD	United States of Americas' Dollar
UV	Ultra violet
Vero-cells	African green monkey kidney cells
VNT	Virus neutralization test

CHAPTER I.

Literature Review

Literature Review

1. General Introduction

Ethiopia's topography

Ethiopia is located in Eastern Africa. It borders Sudan on the West, Eritrea on the North, Djibouti and Somalia on the East and Kenya on the South. The total area of the country is 1,127,127 square kilometers. The capital city is Addis Ababa, which is located in the center of the country.

Ethiopia's topography consists of a central high plateau bisected by the Ethiopian segment of the Great Rift Valley into northern and southern highlands and surrounded by lowlands, more extensive on the east and southeast than on the south and west. The plateau varies from 1500 – 3000 meters above sea level (m.a.s.l.). The highest mountain point is Ras Dashen at 4620 m.a.s.l. in the northern highlands. In the eastern part of the rift valley, the Denakil depression is 115 meters below the sea level and is one of the hottest places on earth. The diversity of Ethiopia's terrain determines regional variations in climate, natural vegetation, soil composition and settlement patterns (Anon., 2005; Alemayehu, 2009).

Climate

Altitude-induced climate conditions form the basis for three climatic zones: cool, temperate and hot which have been known to Ethiopians as *Dega*, *Weinadega* and *Kola* respectively. The cool zone (highland) above 2300 m.a.s.l. has a temperature ranging from 16°C to near freezing. A temperate zone with a daytime temperature between 16°C- 30°C occurs in the mid highland zone ranging from 1500 m.a.s.l. to 2300 m.a.s.l. In areas below 1500 m.a.s.l. classified as lowlands,

such as the rift valley, the southeast, the southern and western border lands, daytime temperature ranges from 30°C to over 50°C in Denakil depression (Anon., 2005; Alemayehu, 2009).

Precipitations are determined by differences in elevation and by seasonal shifts in monsoon winds. The highlands receive by far the most rainfall than lower elevations. Rainfall has two major seasons: the *Belg*, a lighter rainy season that usually begins in mid-February and continues up to end of April and the *Kiremt*, the major rainy season starting mid-June and ending mid-September. In general, relative humidity and rainfall decrease from south to north and are always meager in the eastern and south-eastern lowlands (Figure 1) (Alemayehu, 2009).

Population

The Ethiopian administrative structure encompasses 9 regions and 2 city administrations that include about 546 districts. Each district is composed of a different number of *Kebeles* which are the lower administrative level in Ethiopia. In 2008, estimated Ethiopia's population was about 80 million (CSA, 2006). The annual population growth rate is estimated at 2.6%. The population is concentrated in the northern and southern highlands. The lowlands in the southeast, south and west are mostly being sparsely populated.

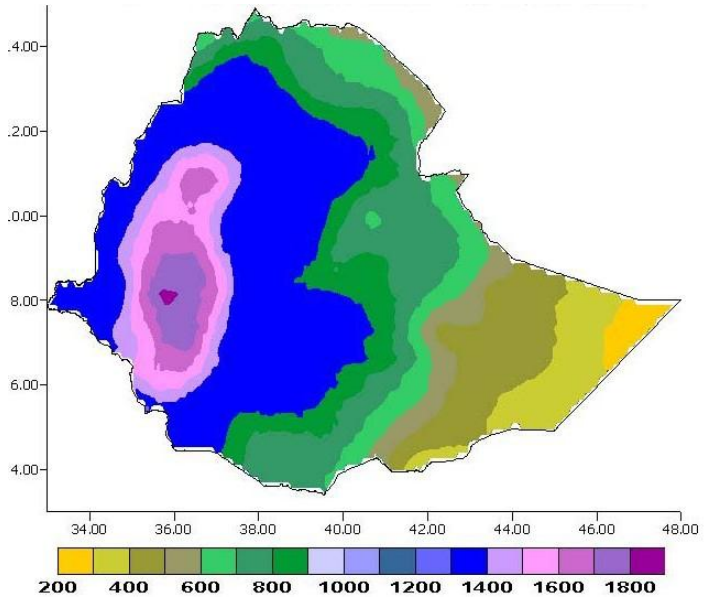


Figure 1: Long term average annual rainfall (mm); Source: Alemayehu, 2009

The agricultural sector

Agriculture is the cornerstone of Ethiopia's economy on which 84% of the rural populations sustain their livelihood. The agricultural production system is mainly a sedentary mixed crop-livestock production system in the midlands and highlands whereas in most of lowlands semi-pastoral and pastoral production systems are dominant (herd owners move their animals seasonally in search of feed and water sometimes over a long distance) (Alemayehu, 2009). The crops grown vary according to the soil types and altitude variations. The main cereal staples are wheat, barley, teff (*Eragrostis abyssinica*), maize and sorghum. Cash crops include coffee, oilseeds and spices.

Livestock production is an integral part of the country's agricultural system. The livestock subsector 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). In 2004 the livestock sector has contributed around 12% of the total foreign currency earning (Anon., 2009). Livestock are significant components of small

scale mixed crop livestock production systems. Draft-oxen are used for ploughing to produce crops. Manure is the cheapest and easily available fertilizer to increase soil fertility. In the lowland parts of Ethiopia, the livelihood of pastoralists and semi-pastoralists relies on livestock production for their food, income source, cultural and social prestige. Common grasslands provide extensive pasture and browse in most parts of the country. Animals are free-ranging in the communal grazing fields and different species are herded together. Natural grass, post-harvest crop residuals and straw are the main source of feed. Concentrate feeds and feed-additives are seldom used (Alemayehu, 2009).

The livestock population is estimated at 48.9 million tropical livestock units (TLU) which includes 41.5 million cattle, 14.6 million sheep, 13.7 million goats, 5.8 million equids, 447 842 camels and 43 million chickens (CSA, 2006). The main cattle breeds classified based on genetical and geographical locations are the Arsi (highland zebu), Boran, Fogera, Horo, Sheko (Gimira), Nuer (Abigar) and Adal (Afar). The Fogera and Horo are well known for their milk production and reared around Lake Tana and in the East Wellega Zone respectively. The Boran, a dual purpose breed, is found in the southern and eastern part of the country. The Sheko and Nuer breeds in the Southwest and Sheko breed is considered to have tolerance to high tse-tse challenge (Lemecha *et al.*, 2006). Exotic breeds such as Holstein Friesian and Jersey have been imported and used for cross breeding with the indigenous cattle (Alemayehu, 2009).

Animal Health

The major cause of economic losses and of poor productivity in livestock is the prevalence of a wide range of diseases such as Contagious Bovine Pleuropneumoniae (CBPP), Foot and Mouth Disease (FMD), Lumpy Skin Disease (LSD), Contagious Caprine Pleuropneumoniae (CCPP), Peste des Petits Ruminants (PPR), African Horse Sickness (AHS), Trypanosomosis and the

presence of internal and external parasites. In general animal diseases are considered to account for 50 to 60% decrease in productivity per year by retarded growth, low fertility, decreased milk production and work output, increased mortality, and by restricting the introduction of more productive exotic breeds. The losses due to mortality are estimated to range from 4-7% for cattle, 7-11% for sheep and 7-11% for goats per annum (Abraham Gopilo, 2005). Other major impacts of livestock diseases are the consequences from sanitary barrier to livestock export trade and direct human losses in case of zoonosis (disease transmissible from animal to human). Public sector expenditures on the control of these livestock diseases, for surveillance and monitoring would also constitute a substantial economic loss for the country as the money used could have been allocated for other developmental purposes (Rich and Perry, 2010).

2. Pox viruses of Vertebrates

Eight genera are found within the *Chordopoxvirinae* subfamily of the *Poxviridae* (Table 1 and 2). The members of this family are among the largest of all viruses, brick shaped or ovoid virions measuring 220-450 nanometer (nm) by 140-266nm. The virions have an external coat containing lipid and an irregular arrangement of tubules on the outer membrane in most genera except the Parapox viruses that have regular spiral arrangement of "tubules" on the outer membrane (Fenner et al., 1987; Sharma and Adlakha, 1995). The virions contain about 30 structural proteins and several enzymes. The nucleic acid is a double stranded Deoxyribo Nucleic Acid (DNA) of molecular weight in the range between 150 and $240*10^6$ daltons. The evolutionary biology of the poxviruses, phylogeny, with particular emphasis on transfer of poxviruses across host species boundaries were reviewed (Xing et al., 2006; Hughes et al., 2010) (Figure 2). The multiplication takes place in the cytoplasm and the cytoplasmic accumulations produce A type

inclusion bodies (Fenner et al., 1987; Coetzer et al., 1994; Sharma and Adlakha, 1995; Bertagnoli and Séverac, 2010).

The members of some genera are ether resistant while other genera are ether sensitive. The pox viruses withstand drying for months and even storage at room temperature. They are destroyed by moist heat at 60^{o} C within 10 minutes. They are also resistant to many common disinfectants (Fenner, *et al.,* 1987). The spread of infection occurs by the respiratory route or through the skin. Some members are also mechanically transmitted by arthropods (Fenner et al., 1987; Coetzer et al., 1994; Sharma and Adlakha, 1995; Bertagnoli and Séverac, 2010).

No	Genera	Prototype virus
1	*Orthopox virus*	*Vaccinia*
2	*Parapox virus*	*Orf virus*
3	*Capripox virus*	*Sheep pox virus*
4	*Suipox virus*	*Swine pox virus*
5	*Leporipox virus*	*Myxoma virus*
6	*Avipox virus*	*Fowl pox virus*
7	*Yatapoxvirus*	*Yaba monkey tumor virus*
8	*Molluscipoxvirus*	*Molluscum contagiosum virus*

Table 1: Classification of Poxviruses of vertebrates: Subfamily *Chordopoxvirinae*

Genus	Virus	Animals naturally affected	Host range	Geographical Distribution
Parapoxvirus	*Pseudocowpox virus*	Cattle, human	Narrow	Worldwide
	Bov. Papular stomatitis virus	Cattle, human	Narrow	Worldwide
	Orf virus	Sheep, goat, human	Narrow	Worldwide
Capripoxvirus	*Sheeppox virus*	Sheep, goat	Narrow	Africa, Asia
	Goatpox virus	Goat, Sheep	Narrow	Africa, Asia
	LSD virus	Cattle, buffalo	Narrow	Africa
Suipoxvirus	*Swine pox virus*	Swine	Narrow	Worldwide
Leporipoxvirus	*Myxoma virus, Hare fibroma virus, Rabbit fibroma virus, Squirrel fibroma virus*	Rabbit Hare Squirrel	Narrow	Americas, Europe, Australia
Avipoxvirus	*Fowlcholera virus, Canary pox virus, Pigeon pox virus, Turkey pox virus,Quailpox virus*	Chickens, turkey, other birds	Narrow	Worldwide
Orthopoxviruses	*Vaccinia virus*	Human, cow, buffalo, pig, rabbit	Broad	Worldwide
	Cowpox virus, Buffalo pox virus	Cow, human, numerous spp.	Broad	Europe Asia
	Ectromelia virus, Rabbit pox virus	Mice Rabbit	Narrow	Europe
	Monkeypox virus	Monkeys, Squirrel, many others	Broad	West and Central Africa
	Uasin Gishu virus	Horse	Broad	East Africa

Table 2: Poxviruses of veterinary importance that affect domestic and laboratory animals;

Source: Fenner *et al*., 1987

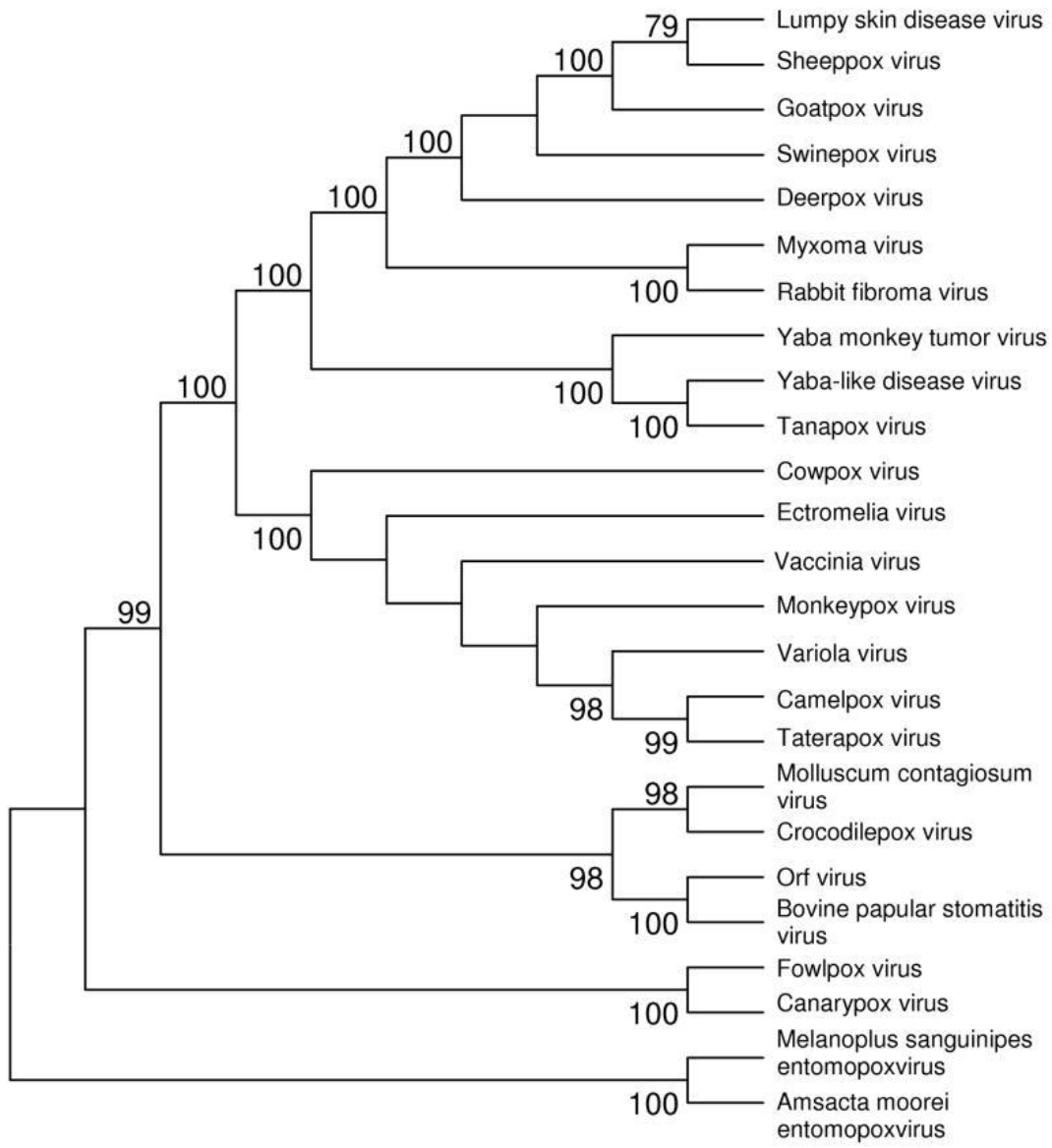


Figure 2: Phylogeny tree (NJ tree) of poxviruses based on concatenated amino acid sequences from 29 conserved orthologous proteins (13, 475 aligned sites); Source: Hughes et al., 2010

The tree was constructed on the basis of the JTT amino acid distance, assuming that rate variation among sites follows a gamma distribution (shape parameter $a = 0.86$). Numbers on the branches represent percentages of 1000 bootstrap samples supporting each branch; only values ≥ 50 % are shown (Hughes et al., 2010).

Viral replication

Replication of poxvirus occurs in the cytoplasm. After fusion of the virion with the plasma membrane or via endocytosis, the viral core is released into the cytoplasm. Transcription is initiated by viral transcriptase and functional capped and polyadenylated messenger Ribonucleic Acid (mRNAs) are produced within minutes after infection. The polypeptides produced by translation of these mRNAs complete the uncoating of the core and about half of the viral genome is transcribed prior to replication, comprising genes encoding proteins involved in host interactions, viral DNA synthesis, and intermediate gene expression. With the onset of DNA replication 1.5 to 6 hours after infection, there is a dramatic shift in the gene expression and almost the entire genome is transcribed, but transcripts from the early genes (i.e. those transcribed before DNA replication begins) are not translated. Two forms of virions are released from the infected cells (virions with one membrane, and virions with two membranes) and both types are infectious (Fenner *et al.*, 1987; Bertagnoli and Séverac, 2010).

3. Diseases caused by Capri-poxviruses (CaPV)

Capripoxviruses (CaPVs) represent one of the eight genera within the *Chordopoxvirinae* subfamily of the *Poxviridae*. The *capripoxvirus* genus is comprised of *Lumpy skin disease virus* (LSDV), *Sheeppox virus* (SPPV), and *Goatpox virus* (GTPV). These viruses are responsible for some of the most economically significant diseases of domestic ruminants in Africa and Asia. CaPV infections have specific geographic distributions (Davies, 1991; Coetzer et al., 1994). Sheeppox and Goatpox viruses are endemic throughout southwest and central Asia, the Indian subcontinent, and northern and central Africa (Figure 3). In contrast, LSDV occurs largely in southern, central, eastern and western Africa with a few sporadic reports in the Middle East

Asian countries (Figure 4) (Bhanuprakash et al., 2006; Babiuk et al., 2008a; ANON., 2010; Fassi-Fehri, 2010; Lefèvre and Gourreau, 2010).

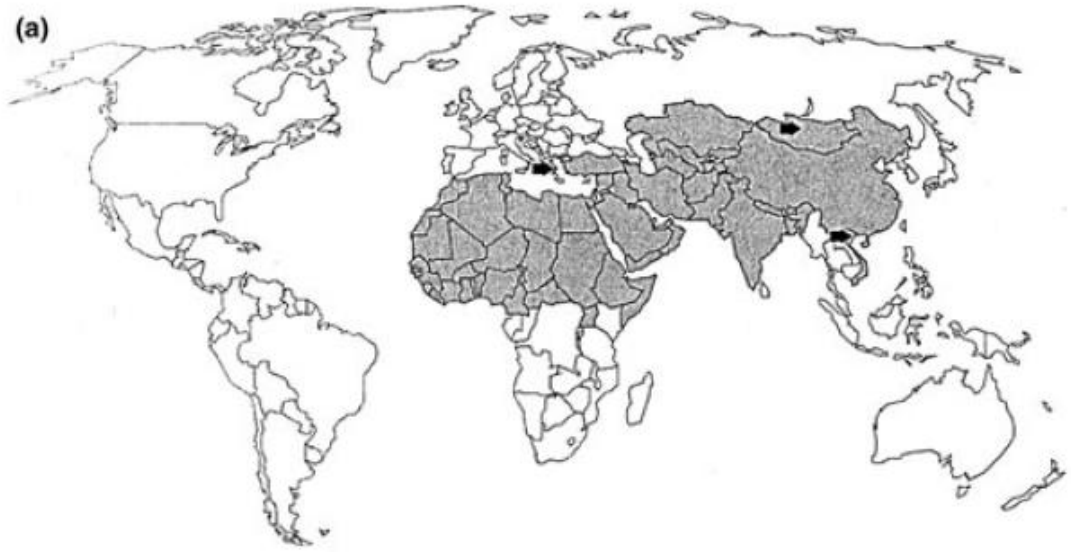


Figure 3: Distribution of Sheeppox and Goatpox diseases in the World. The arrows show the recent outbreak reported parts of the world; Source: Babiuk et al., 2008a

CaPVs are, however, serologically indistinguishable from each other. Restriction enzyme analysis or partial and complete DNA sequence data also support a close relationship between CaPVs (Gershon and Black, 1987; Kitching et al., 1989). CaPVs are generally considered to be host specific (Capstick and Coackley, 1961a). This has been shown specifically for Nigerian, Middle Eastern, and Indian strains of SPPV and GTPV and for LSDV (Stevenson et al., 2000). However, the ability of SPPV and GTPV strains to naturally or experimentally cross-infect and cause disease in both host species has been described previously (Davies, 1982). They are able to induce heterologous cross-protection (Carn, 1993; Barnard et al., 1994).This similarity between Sheeppox and Goatpox has led to the suggestion that they are part of a disease complex caused by a single viral species and that observable host range specificities result of regional virus

adaptations to sheep or goat hosts. However, restriction endonuclease analysis and cross-hybridization studies of SPPV and GTPV indicate that these viruses, although closely related (estimated 96 to 97% nucleotide identity), can be distinguished from one another and may undergo recombination in nature (Tulman *et al*., 2002). SPPV and GTPV DNA sequence analysis also indicate a high degree of similarity to LSDV, which genome sequence contains a conserved ChPV-like complement of replicative genes and a unique complement of virulence and host range genes (Gershon and Black, 1987; Kitching *et al*., 1989; Tulman *et al*., 2002). Moreover, restriction enzyme analyses of the genome of the Kenya SGPV and LSDV strains have shown that they appear to be identical (Kitching et al., 1987; Davies, 1991; Tulman et al., 2001; Le Goff et al., 2009).

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

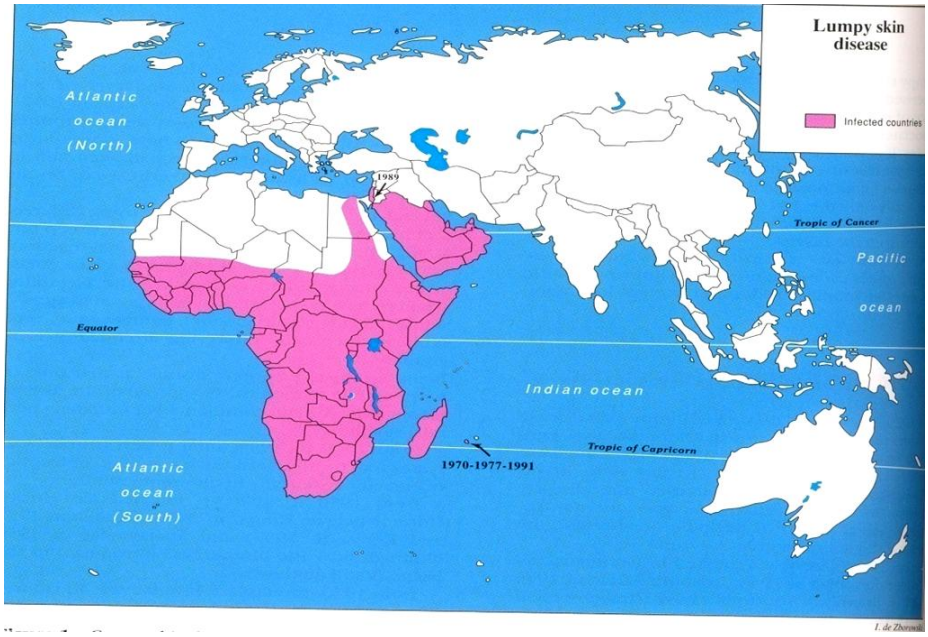


Figure 4: Geographical distribution of LSD; Source: Lefèvre, P C, Gourreau, J M, 2010

4. Lumpy Skin Disease (LSD)

LSD is an acute to sub acute viral disease of cattle that can cause mild to severe symptoms including fever, nodules in the skin, in the mucous membranes and in the internal organs, skin oedema, lymphadenitis and sometimes death. The disease can result in economic losses due to 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; OIE, 2010).

History of LSD

The clinical syndrome of LSD was first described in Zambia (formerly Northern Rhodesia) in 1929. Between 1943 and 1945, cases occurred in Botswana (Bechuanaland), Zimbabwe (Southern Rhodesia) and the Republic of South Africa. The infectious nature of the disease was recognized at this time. A panzootic in South Africa, which lasted until 1949, affected some

eight million cattle and consequently incurred enormous economic losses (Diesel, 1949; Davies, 1991).

LSD was first identified in East Africa in Kenya in 1957 and Sudan in 1972, then in West Africa in Nigeria in 1974, and it was reported in 1977 in Mauritania, Mali, Ghana, and Liberia (OIE, 2010). Another epizootic of LSD between 1981 and 1986 affected Tanzania, Kenya, Zimbabwe, Cameroon, Somalia and Ethiopia. In May 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 Africa. The disease spread locally in the summer of 1988 and apparently overwintered with little or no manifestation of clinical disease. 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 (Ali et al., 1990).

In 1989, a focus of LSD was identified in Israel and subsequently eliminated by the slaughter of all infected cattle as well as contacts. But another outbreak reappeared recently in 2006 (Yeruham et al., 1995; Brenner et al., 2006). LSD has continued to be reported from the Middle East countries, Palestinian Autonomous Territory and Oman since 2006 (OIE, 2010). Cases have also been reported in Yemen (OIE, 1990). Sporadic reports were recorded in Kuwait in 1986-1988 (OIE, disease report, 1988), Bahrain in 1993 (not confirmed by virus isolation), La Réunion Island in 1993, Mauritius in 2000 (Barnard et al., 1994; Lefèvre and Gourreau, 2010).

Etiology

LSD is caused by *Lumpy Skin Disease virus* (LSDV) within the genus *Capripoxvirus* and the prototype strain is Neethling Virus. It is an enveloped DNA virus, ovoid shape with a molecular size of 350*300nm and a molecular weight that ranges from 73 to 91 (Kilodalton) KDa. LSDV

genome sequences were assembled into a contiguous sequence of 150.8 kilobase pair (kbp) which is in accordance with previous size estimates of 145 to 152 kbp (Tulman *et al.*, 2002; Kara *et al.*, 2003). These genes encode several poxviral proteins known to be structural or involved in virion morphogenesis and assembly. The terminal genomic sequences contain a unique complement of at least 34 genes which are responsible in virulence, host range and/or immune evasion (Tulman *et al.*, 2002; Johnston and McFadden, 2003; Kara *et al.*, 2003). LSDV is genetically and antigenically closely related to a strain of sheep and goat pox virus (Alexander et al., 1957). Comparison of LSDV genome with published restriction fragment analysis of the SPPV and GTPV genome indicates that there may be additional terminal sequences of less than 200 bp present (Gershon and Black, 1987; Kitching et al., 1989; Tulman et al., 2002).

LSDV is susceptible to sun light and detergents containing lipid solvents. The virus could be inactivated after heating for 1 hour at 55°C (Davies and Otema, 1981; Coetzer et al., 1994; Lefèvre and Gourreau, 2010). However, it withstands drying, pH changes if not an extreme pH and can remain viable for months in dark room such as infected animal shade off its host. LSDV can persist in skin plugs for about 42 days (Babiuk et al., 2008b; Lefèvre and Gourreau, 2010). It is likely that the viral A type inclusion body protein in infected cells may protect the virion after the scab has disintegrated, although this has not yet been proven (Babiuk et al., 2008a).

Geographical distribution

LSD distribution has extended from sub-Saharan countries to Egypt and Western Africa. Outside the African continent Israel has reported LSD outbreaks and sporadically some Middle East countries which showed that there is a real potential risk of the disease to establish endemically there (Brenner et al., 2006). Epidemiological trend of LSD suggests that there could

also be a considerable potential risk of the disease spreading further into North Africa, into the Middle East countries and to Mediterranean regions because of global climatic changes and trade movement in animals and animal products (Davies, 1991; Babiuk et al., 2008a).

In Ethiopia, LSD was first observed in 1983 in the western part of the country (southwest of Lake Tana) (Mebratu et al., 1984). After its first appearance, an explosive sudden epidemic spread from the north through the central to the southern part of the country. In the subsequent three to five years, it had covered the vast area of the highland and midland parts of the country. LSD is one of reported diseases in Ethiopia which deserves outbreak notification to the National veterinary services. However, a variable degree of under-reporting of the outbreak cases could exist from different parts of the country. Data investigations from the national disease outbreak report database during the period 2000-2009 showed that major epidemic outbreaks of LSD occurred in 2000/2001 in the northern parts of the country in Amhara and West Oromia regions. Then it extended to the central and the southern parts of the country in 2003/04 covering large parts of Oromia and Southern Nation, Nationalities and Peoples (SNNP) regions. In 2006/07 another extensive outbreak reappeared in Tigray, Amhara and Benishangul regions in the northern and north-western parts of the country. From 2007 up to 2009 the outbreak number progressively increased in Oromia Region situated in the central part of the country while it seemed to be gradually decreasing in the northern part of the country including Tigray, Amhara and Benishangul regions. This showed that an epidemic reoccurs after an interval of 5-6 years cycle in unvaccinated cattle population. The national disease outbreak report during these 10 years showed that LSD has spread virtually to all the regions in the country and in different agro-climatic zones (Figure 5) (MoARD, Epidemiology Section Personal communication).

Studies based on clinical disease observation done around Nekemt town, Wolliso town and in Southern rangeland in Ethiopia have reported different animal level prevalence of LSD ranging from 7 to 28% (Asegid, 1991; Beshahwured, 1991; Regassa, 2003). A mortality of 1-3% was observed in the same study and was similar to a previous report by Davies (1991). However, epidemiological studies carried out in Ethiopia to date were of limited scopes and did not elucidate the full image of its distribution in the country.

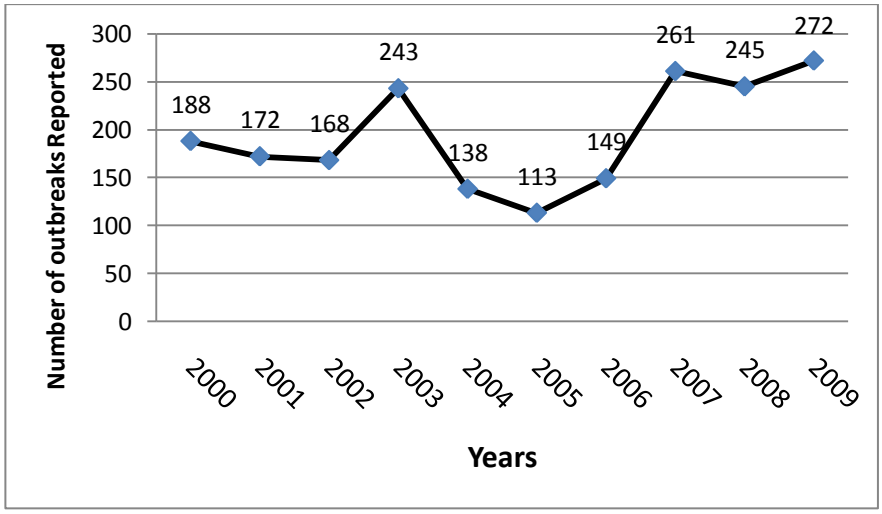


Figure 5: Number of LSD outbreaks reported based on outbreak notification reports from year 2000 to 2009

Epidemiology and pattern of the disease

The incubation period of LSD is 6 to 10 days in experimentally infected animals (Babiuk et al., 2008b) but is thought to be 2 to 4 weeks in naturally-infected animals (Barnard et al., 1994). The World Organization for animal health (OIE) Code gives the maximum incubation period of 28 days for regulatory purposes.

The morbidity of LSD varies enormously and is usually estimated at 10% in endemic areas which may be contrasted with those of 80 to 90% in different situations like in South Africa (Barnard et al., 1994; Babiuk et al., 2008a). In southern, West and East Africa, higher morbidities have been encountered in epizootics, yet much lower morbidity may also occur during other epizootics (Davies, 1991). Mortality of 10 to 40% and even higher have been reported on occasion but the lower range of 1 to 5% is more usual (Davies, 1991; Barnard et al., 1994; Babiuk et al., 2008a). The reason why morbidity and mortality enormously vary during the epizootic of LSD infection is not yet clearly known, but different factors attributed to this variation are cattle breed, health status of the animal, viral isolates and insect vectors involved in the transmission. Thus in general, 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., 2008a). Diseases and factors which compromise the immune status of the animal such as trypanosomosis in the western part of Ethiopia might add to the severity of LSD infection. The distribution and relative abundance of insect vectors are also thought to reflect the differences in morbidity rates in the various habitats. Finally, mechanical insect vectors which are capable to pierce deep in to the tissue feeding from intravenous blood are assumed to cause severe clinical LSD (Kitching and Mellor, 1986; Carn and Kitching, 1995; Chihota et al., 2001).

LSD occurs in many different biotopes from the temperate high altitude through to the various wet and dry savannah ecotypes and the dry semi-arid and thorn scrub. It can also spread extensively in irrigated lands like in Sudan and Egypt (Davies, 1991). The disease usually occurs during wet seasons and shortly after the major rainy season. It has a feature that an epidemic reoccurs after an interval of 5-6 years in susceptible cattle population (Woods, 1988; Barnard et

al., 1994). However, further study of the risk factors associated with the disease occurrence is needed.

Mode of Transmission and Host Range

The virus of LSD does not spread readily among animals held in insect-proof pens. While infection by contact can occur, it is not considered a major component of transmission during epizootics (Carn and Kitching, 1995). Most infection is thought to be the result of blood sucking arthropods mechanically (Thomas and Mare, 1945; Von Backstrom, 1945; Diesel, 1949; MacOwan, 1959; Kitching and Mellor, 1986; Chihota et al., 2001). The multiplication of *LSDV* in the vector insects has not been demonstrated. In the infected animal virus is present in blood, nasal and lachrymal secretions, semen and saliva, which may be sources for transmission (Irons et al., 2005; Babiuk et al., 2008b). LSD is transmissible to suckling calves through infected milk. Direct transmission can occur when the animals share the same drinking trough due to contamination by nasal and salivary discharges from infected animals (Barnard et al., 1994; Lefèvre and Gourreau, 2010). The virus enters the host either through the skin or the digestive tract mucosa.

Particular types of insects incriminated in the transmission of LSDV are not all elucidated. Virus has been isolated from *Stomoxys* species and *Biomyia fasciata* species commonly associated with cattle and found in large numbers during LSD epizootics (Weiss, 1968). *S. calcitrans* has been thought as the most likely insect to have a role in the epidemiology of LSD based on the detection and isolation of virus from flies that had fed on infected cattle during an outbreak (Diesel, 1949; MacOwan, 1959; Anon., 2008). *Stomoxys* spp have been shown to transmit SGPV successfully (Kitching and Mellor, 1986). In 1989 the LSD outbreak in Israel was

attributed to infected *S. calcitrans* carried over by wind from Ismailiya in Egypt (Yeruham et al., 1995). The introduction of LSD to La Réunion in 1991 was also exclusively attributed to Stomoxys despite all the official quarantine and prohibition of cattle movement measures were implemented (Lefèvre and Gourreau, 2010). However, there are still doubtful issues on this assumption which could raise some questions on the very nature of mechanical transmission that requires short time period to transmit the pathogens, and the distance that these flies could be blown by wind, if any because of the large size of *Stomoxys* flies which might unlikely be able to blow by wind like mosquitoes to far distances. In an experimental transmission attempt, *Aedes aegyti* (Diptera: *Culicidae*) was reported to transmit LSDV in cattle (Chihota et al., 2001) whereas the transmission by *Stomoxys* spp. was not successful (Chihota *et al*., 2003). Other biting flies like Tabanids, *Glossina* spp, *Culicoides* spp have been suspected to be involved. The potential of Ixodid ticks to transmit LSDV was also reported (Tuppurainen *et al.*, 2010). An embarrassing gap in our knowledge requires defining the transmission mechanisms of LSD and research efforts are required to understand the prevalence of the different biting flies potentially associated with LSDV transmission in the various biotypes of countries.

Some wild species like Giraffe (*Giraffa camelopardalis*), Impala (*Aepyceros melampus*), and Thomson's gazelle have been infected experimentally by parenteral inoculation with LSDV and have developed characteristic lesions. However, under natural conditions, lesions of LSD have not been seen on these animals when they have been present during epizootics of the disease (Young et al., 1970). Sheep and goats do not become infected during outbreaks of LSD even when held in close contact with infected cattle. African buffaloes (*Syncerus caffer*) and Asian water buffaloes (*Bubalus bubalis)* do not show lesions in the field during epizootics of LSD but both buffalo types may suffer an unapparent infection and seroconvert (Davies, 1991). In an

enzootic area of LSD in Kenya, many African buffaloes had high titers of antibodies to Capripox virus whereas in another area, no antibody was found (Davies, 1991). Infection has been reported in Arabian Oryx in Saudi Arabia (Greth et al., 1992). In general the role of wildlife in the transmission and maintenance of LSDV was found almost negligible (Hedger and Hamblin, 1983). The absence of reservoir host for LSD virus might lead us to the assumption that infection might persist in the endemic areas at a low level as unapparent or mild form in the cattle population (Woods, 1988; Lefèvre and Gourreau, 2010).

Clinical Signs and Pathogenesis

The characteristic clinical signs of LSD are a fever of 40–41.5^{o}C that may last 6–72 hours and occasionally up to 10 days which is accompanied by watering eyes, increased nasal and pharyngeal secretions, loss of appetite, reduction in milk production, some depression and reluctance to move.

Within 1–2 days onset of the clinical signs there is a cutaneous eruption of nodules or lumps, which may cover the whole of the body. The most common sites are the head and neck, perineum, genitalia and udder, and the limbs. The nodules are 0.5–5 cm in diameter, appearing as round circumscribed areas of erect hair, firm and slightly raised from the surrounding skin (Figure 6). The lesions are full skin thickness involving the epidermis, dermis and subcutis, which may be oedematous. Regional lymph nodes are enlarged and oedematous.

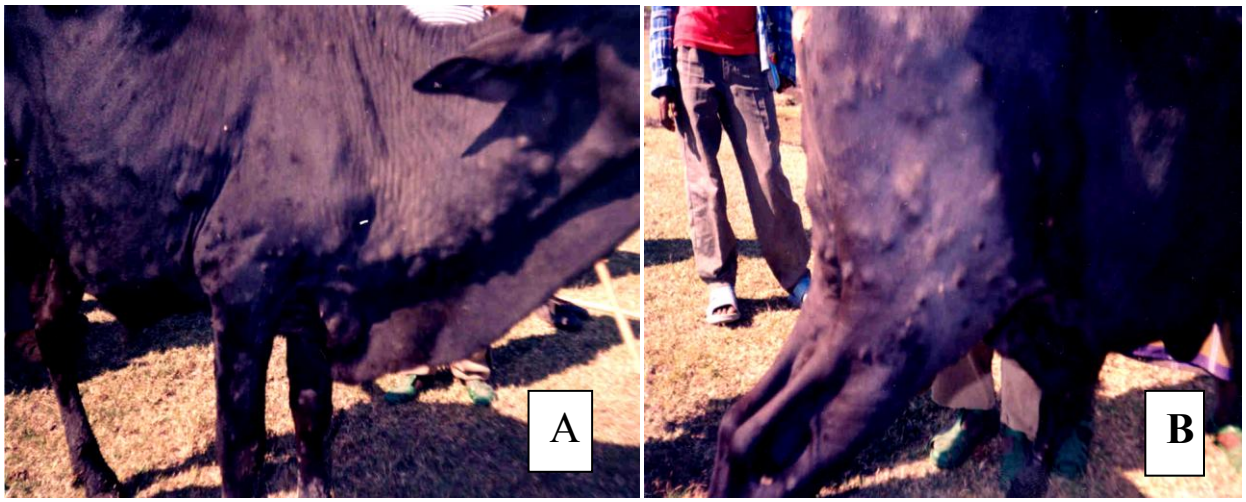


Figure 6: Clinical case of LSD in Dawa-Chefa District in 2008 (Ethiopia). Circumscribed nodules on the skin all over the body and swollen superficial lymphnodes (A,B).

Lesions develop on the muzzle, in the nostrils, and in the mouth and pharynx. They show a ring-like margin where there has been separation from the surrounding healthy epithelium. Lesions in the larynx and trachea, and throughout the alimentary tract, especially the abomasum, become ulcerated and necrotic. Mucopurulent nasal discharges, persistent dribbling of infected saliva, coughing and stertorous (snoring) and often distressed breathing are manifested. Inflammation and hyperemia of the conjunctiva and cornea of the eyes is common (Davies, 1991; Bowden et al., 2008).

Inflammatory and oedematous swellings of the limbs, brisket and genitalia may develop. Skin lesions become necrotic. Some remain *in situ* and others slough leaving a full skin thickness hole, known as a ‚sitfast', which becomes infected by pus-forming bacteria and can also be infested by fly strike. Large areas of skin may slough causing substantial down grade of the hide quality (Green, 1959). Lesions in the skin, subcutaneous tissue, and muscles of the limbs, together with the severe skin inflammation caused by secondary infection of lesions, greatly

reduce mobility. Rapid deterioration in body condition results and animals that recover may remain in poor condition for 1-3 months and in extreme cases for up to 6 months.

Pneumonia is a common and often fatal complication. Absence of oestrus cycles during the severe debility and abortion is frequent in the early stages due to prolonged fever (Ahmad and Zaher, 2008). Painful genitalia in bulls can prevent from serving for long periods. Foetus born to infected cows may show skin lesions at birth presumably acquired through intra-uterine infection (Davies, 1991).

Pathological lesions

On autopsy, nodules may be found in the subcutaneous tissue, muscle fascia and in muscles, which are grey-pink with caseous necrotic cores. The subcutis is infiltrated by red watery fluid. Similar nodules may be scattered through the nasopharynx, trachea, bronchi, lungs, rumen, abomasum, renal cortex, testicles and uterus (Prozesky and Barnard, 1982).

Histopathological examination shows that the epidermis is extensively necrotic. While in the intact areas, some ballooning degeneration of squamous epithelial cells with occasional intra-cytoplasmic inclusions is seen. Prominent lesions of vasculitic necrosis with cell debris and severe diffuse infiltration with inflammatory cells mainly neutrophils, have been seen in the superficial and deep dermis (Prozesky and Barnard, 1982). There is a vasculitis and perivascular infiltration with white cells which causes a thrombosis of the vessels in the dermis and subcutis (Figure 7). The cells infiltrating the lesion are of a predominantly epithelioid type, which was described in sheep pox (Burdin, 1959; Davies, 1991; Brenner et al., 2006). There are also eosinophilic intracytoplasmic inclusions in the epidermal elements of the lesion and the inflammatory cells. The lesions gradually become necrotic as a result of the thrombosis (Burdin, 1959).

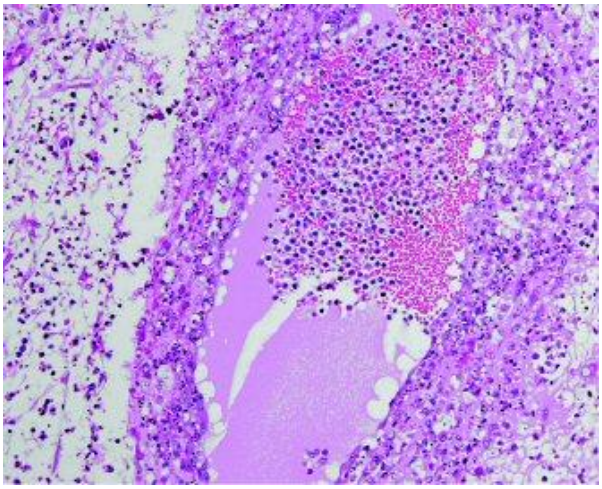


Figure 7: Vasculitic necrosis with cell debris and severe diffuse infiltration with inflammatory cells mainly neutrophils, are seen in the superficial and deep dermis; Source: Brenner et al., 2006.

Diagnosis

LSD can be clinically diagnosed by its pathognomic nodular lesions on the skin, mucous membranes, swelling of the superficial lymph nodes and systemic involved symptoms by experienced practitioners. Confirmation of the diagnosis through laboratory techniques can be done using various methods.

Virus isolation and identification

Rapid confirmation can be made by demonstration of the typical capripox virion in biopsy material or desiccated crusts using the transmission electro-microscope in combination with the clinical history of a generalized nodular skin disease and enlarged superficial lymph nodes in cattle (Figure 8). Capripox is morphologically distinct from Parapox virus which causes bovine pustular stomatitis and pseudocow pox, but cannot be differentiated from Cowpox and Vaccinia viruses in Orthopox virus. But neither of these causes a generalized infection and both are uncommon in cattle (Fenner et al., 1987; Babiuk et al., 2008a; OIE, 2010). LSDV causes a

characteristic cytopathic effect and intracytoplasmic inclusion bodies, and is distinct from the virus of pseudo-LSD (Allotron- Herpes mammilitis), which is a herpesvirus producing syncytia and intranuclear inclusion bodies (Babiuk et al., 2008a).

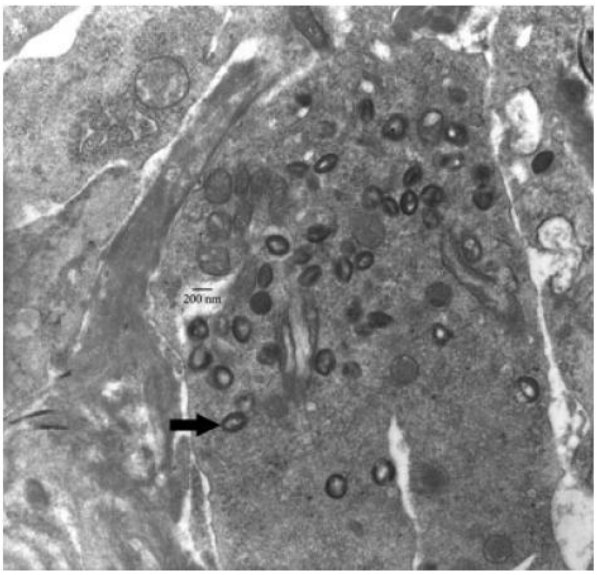


Figure 8: A Capripox virion from the skin of Capripox infected goat; the virus particle is indicated by arrow; Source: Babiuk et al., 2008a

Virus isolation could be attempted and is best carried out in primary lamb kidney cell or lamb testis cell cultures. Secondary lamb testis cell line (OA3.Ts) has been proved to replace the primary cell cultures for better efficiency and easily managed to grow Capripoxvirus (Babiuk et al., 2007). LSDV can be grown on a variety of sheep, goat and cattle cells (Binepal et al., 2001). The LSDV isolation can be confirmed by Immunostaining technique using anti- Capripoxvirus serum which allows the visualization of the LSDV plaques in the cell culture (Babiuk et al., 2007). Antigen detection can be demonstrated in tissue culture using immunoperoxidase or immunofluorescent staining (OIE, 2010). A Polymerase Chain Reaction (PCR) technique to detect capripoxvirus antigen from cell culture and biopsy specimens has been developed and the reagents are available commercially (Irland and Binepal, 1998; Heine et al., 1999; Tuppurainen

et al., 2005; Bowden et al., 2008). An immunocapture Enzyme-linked immunosorbent assay (ELISA) for the detection of Capripoxvirus antigen is also reported (Rao et al., 1997).

Serodiagnosis

Neutralizing antibody appears 3-4 days after the onset of the clinical signs and reaches the peak titre level in 2-3 weeks. Both complement fixing and precipitating antibodies are present in the serum of infected and recovered animals. Immunological defense against capripoxvirus relies mainly on cell-mediated immune response and humoral immunity would remain in the circulation for a short period within the time range of mostly seven to eight months (Capstick and Coackley, 1962; Lefèvre and Gourreau, 2010; OIE, 2010).

Virus Neutralization Test (VNT)*:* VNT is the most common widely used serological test for capripox antibody detection (Davies and Otema, 1981; Babiuk et al., 2008a; OIE, 2010). It has high specificity to rule-out false positives due to cross- reaction with cowpox and Parapoxvirus antibodies but its sensitivity is lower to trace small antibody titration (Davies and Otema, 1981).

Indirect Fluorescence Antibody Test (IFAT): An indirect test using the capripoxvirus antigen fixed in the tissue culture plate can be used to detect antibodies against LSD in the serum. The test was reported to have good sensitivity but cross reacting Parapox and Orthopox viruses might affect its specificity at lower serum dilution rates (Davies and Otema, 1981).

Western blotting assay is a specific and sensitive test, however, it is difficult to perform and interpret (Chand et al., 1994). An antibody ELISA based on P32 recombinant antigen was developed and the preliminary test evaluation done but it is not yet validated to replace the conventional tests (Heine et al., 1999). Indirect ELISA based on inactivated whole antigen from sheeppoxvirus was reported to detect capripox antibody in experimentally infected animals (Babiuk et al., 2009). Recombinant CPV Antigen ELISA was also reported to detect serum

antibody from experimental infected sheep and goats which is still under development (Bowden *et al.*, 2009). Agar gel Immunodiffusion test (AGID) has been used for detecting the precipitating antigen of capripoxvirus, but has the disadvantage that this antigen is shared with Parapoxvirus and has also less sensitivity (OIE, 2010). So far a diagnostic assay that can be easily run for an epidemiological study of LSD is not yet validated and commercially not available. Moreover, the accuracy of the conventional diagnostic techniques which are currently being used for diagnosis purposes have not been evaluated in particular in the context of the target population in Ethiopia.

Differential diagnosis

Skin diseases of cattle that could be considered as differential diagnosis are:

Bovine Herpes Mammilitis (Pseudo-lumpy skin disease): The presence of Bovine Herpes Mammilitis case has not yet been confirmed by laboratory in Ethiopia.

Dermatophilosis: *Dermatophilus congolensis* infection is one of wide spread skin disease of cattle in Ethiopia and lesions could be differentiated from LSD in that the lesions of dermatophilosis are superficial (often moist and appear as crusts of keratinized material) scabs of 0.5- to 2 cm diameter. The organism can be demonstrated by Giemsa staining.

Demodicosis, Besnoitiosis, Photosensitization, insect bites; and Ringworm could also be considered as the differential diagnosis. But epidemiological features could help to distinguish LSD vs. other skin lesions.

5. Control and Prevention

Control and prevention of LSD in endemic countries like Ethiopia relies mainly on vaccination. The experience in the major parts of the country showed that the vaccination approach is commonly chosen and is often that of ring vaccination around a local foci outbreak when it occurs. Animals that recover from virulent LSD infection generate lifelong immunity consisting both of a humoral and cell mediated protective immunity (Kitching et al., 1987). Maternal immunity provides protection from LSD in calves at least for 6 months (Davies 1991). In South Africa, the control of insects was not effective in preventing the spread of LSD, but current insecticides together with repellents might help to reduce the spread of LSD (Davies, 1991). There is no specific treatment for LSD, but early stage antibiotic treatment could reduce secondary bacterial complications to improve recovery process.

Vaccines for LSD control

Attenuated vaccines of different capripoxvirus strain origins are available to protect cattle, sheep and goats. LSD (Neethling strain), Kenya SGPV, Romanian sheep pox and Gorgon goat pox (from Iraq) have all been shown to be serologically identical by fluorescent antibody and serum VNT (Davies and Otema, 1981). Therefore, it is likely that many of these vaccine strains available in different parts of the world would be suitable for the prophylaxis of LSD (Kitching et al., 1987; Davies, 1991; Kitching, 2003). These live attenuated vaccines are mainly stimulating the cell mediated immune response.

Two different vaccines have been widely used for the control of LSD in cattle populations in Africa. In southern Africa, the Neethling strain was passaged 50 times in tissue cultures of lamb kidney cells and then 20 times in embryonated eggs (OIE, 2010). The strain proved to be

innocuous and immunogenic for cattle, although local reactions do occur in a high proportion of animals at the vaccination site. No generalization of infection has ever followed its use. It is produced in tissue culture and issued as a freeze-dried product (Capstick and Coackley, 1961a; Weiss, 1968).

In Kenya, an effective vaccine has been produced from a local strain of sheep and goat pox virus (SGPV). The SGPV was passaged 18 times in pre-pubertal lamb testes or foetal muscle cell cultures and used for vaccination at this level (OIE, 2010). This was shown to immunize cattle against LSD (Capstick and Coackley, 1961a; Carn, 1993). Local reactions have not been seen, but some *Bos taurus* breeds have shown lymphadenitis with signs of mild, generalized LSD-like lesions following vaccination (approximately 0.02%) (Yeruham et al., 1994). These reactions were not reproduced in the laboratory, and no such reactions have ever been observed in *Bos indicus* cattle. In Ethiopia both Kenyan SGPV and Neethling strain vaccines are produced at the National Veterinary Institute (NVI) and the Kenyan SGPV strain is widely used for all cattle, sheep and goats.

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 (Michael et al., 1996). Another sheep pox strain, the RM 65 prepared in tissue culture, was used in Israel. No complications have followed the use of these strains in cattle. However, re-infection of the beef cattle has been reported in Israel during 2006/07 epidemics after vaccination with the RM65 sheeppox vaccine (Brenner et al., 2009). In general problems related to vaccine failure and re-infection of vaccinated animals have been getting higher magnitude which should draw the attention of

researchers and vaccine production institutes to envisage for better immunogenic CPV vaccines in the future.

Studies with both the Neethling and the Kenya SGPV strains showed that an immunizing dose of $10^{3.5}$ $TCID_{50}$ is desirable for field vaccination campaigns. It is suggested that 10 to 50 times the sheep immunizing dose should be used for cattle to protect from LSD (Davies, 1991). After a single inoculation, solid immunity lasts for at least 3 years and probably longer (Capstick and Coackley, 1961a).

Serological studies with vaccinated cattle have shown that many animals resist challenge with virulent LSDV when they have no detectable fluorescent or neutralizing antibody to the virus. Most animals do show a serological response after field infections with wild LSDV, however, the vaccinal strain does not elicit detectable humoral immunity (Babiuk et al., 2008b). There is an important cellular component of the immune response to LSD in cattle, as there is to other pox viruses and based on this principle Capstick and Coackley (1962) developed a hypersensitivity test to determine the susceptibility of cattle to LSD for use in vaccination studies (Capstick and Coackley, 1962). This test can be used to determine the responses to vaccination.

New Recombinant Vaccines

The large size genome and resistance to heat characteristics of LSDV has render this virus a very useful and efficient vector of expression for the construction of recombinant vaccines (Lefèvre and Gourreau, 2010). A new generation of capripox vaccines are being developed that use capripoxvirus genome as a vector for the genes of other ruminant pathogens, for instance genes of Rinderpest (Romero *et al.*, 1993), PPR (Diallo *et al.*, 2002; Berhe *et al.*, 2003), Rabies

(Aspden *et al*., 2002) and RVF (Wallace and Viljoen, 2005; Wallace *et al*., 2006). These prospective recombinant vaccines under development could provide protection against LSD and the counterpart diseases inserted as recombinant antigen in a single dose vaccination (Babiuk, 2002).

Control and Eradication in Disease free countries

If LSD is confirmed in a new area before extensive spread occurs, the area should be quarantined, the infected and in contact animals slaughtered, and the premises cleaned and disinfected as an attempt to eradicate the disease from the country (Davies, 1991; Babiuk et al., 2008a; OIE, 2010). Ring vaccination of cattle within the quarantine in the radius of 25-50km and strict animal movement controls should be considered (Yeruham *et al*., 1995).

If the disease has spread over a large area, the most effective means of controlling losses from LSD is mass vaccination. However, even with vaccination, consideration should still be given to eliminating infected and exposed herds by slaughter, proper disposal of animals and contaminated material, and by cleaning and disinfecting contaminated premises, equipment, and facilities (Anon., 2008, 2010).

6. Economic Importance

Economic losses due to LSD depend on the magnitude of production losses due to morbidity and mortality. Milk yield fall more than 50% in affected herds has been reported and concurrent purulent mastitis which can cause loss of quarters could accentuate the fall in milk production (Woods, 1988; Lefèvre and Gourreau, 2010). The full skin thickness lesions of LSD punch holes right through the hide, thereby causing permanent damage (Green, 1959; Prozesky and Barnard,

1982). Secondary infections of the skin and lung lesions results in further debility often causes culling. Abortion may occur due to prolonged fever lasting up to 72 hours and it is not uncommon in the early stages. Temporarily sterility in bulls and extended delay in coming to estrous in cows due to debility had been recorded. Economic losses to the producers in terms of physical loss impact could be comparable to that caused by FMD (Babiuk et al., 2008a).

High susceptibility of high producing breeds imported from Europe or Australia could also pose a considerable hindrance for the development of small scale and intensive dairy production in Africa and in particular in Ethiopia. Being one of the transboundary diseases, Capripox viruses could have impediments to livestock and livestock product trades. This could affect particularly the economic well-being of the farmers and that of pastoral communities but also more globally the country's economy (Rich and Perry, 2010).

Capripoxviruses in general have a single serotype, have no carrier state, have a limited host range and vaccines are available that provide long lasting immunity. These attributes increase the prospect of successful implementation of regional control programs, leading to the elimination of the virus and conceivably eradication (Babiuk et al., 2008a).

7. The Objective and goals of the PhD research

General objective

The general objective of this thesis is to gain understanding in the epidemiology and economic impact of LSD in Ethiopia in order to propose practical control and prevention options.

Specific objectives

Three specific objectives were envisaged and each would be treated in a separate chapter (Chapter 2, 3 and 4) in the subsequent compilation of the thesis.

1. To determine the diagnostic and screening tests that could be used for Epidemiological study of LSD: Lack of information on the performance and characteristic of the diagnostic tests could substantially affect the result and parameter estimates of epidemiological studies and it should be considered as a precursor step. The accuracy of diagnostic tests should also be evaluated for specific target population in Ethiopia. There is no perfect (gold standard) test for the diagnosis of LSD and thus the novel Bayesian approaches to evaluate the performance of diagnostic tests without gold standard was chosen to estimate the accuracy of Indirect Fluorescent Antibody test (IFAT) and Virus neutralization test (VNT) under field study in Ethiopia (Gari *et al*., 2008).
2. To estimate the prevalence at herd-level and animal-level and to identify and quantify the risk factors associated with the disease occurrence in Ethiopia:

   An epidemiological study which encompassed the different agro-ecological zones of Ethiopia was undertaken. Serological sampling was concurrently conducted with the risk factor study to estimate the sero-prevalence and to analyze the risk factors associated with LSD occurrences. The risk factor analysis in this study has enlightened the important risk factors associated with LSD occurrence for the future targeted measures to control and prevent the disease (Gari et al., 2010). Because of time limitation the serological test results could not be included in the thesis. The control opinions proposed should be complemented with economic feasibility investigations for the cost-effective way of controlling the disease at different scales and scenario to provide guidance for

eventual knowledge-based decision process for the control of the disease or to mitigate the risks to an acceptable level. The next study emphasizes on the financial cost-benefit of LSD control.

3. Evaluation of financial cost of clinical LSD at the farm level and the economic benefit of its control by vaccination:

   The farm level financial impact and the economic benefit of LSD control by annual vaccination were evaluated in selected districts in Ethiopia. The result of this study would provide an insight for the producers and the government to endeavour the control of the disease. Manuscript is prepared from this work and it is submitted to Prev. Vet. Med. Journal.

CHAPTER II.

ARTICLE 1 : Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method

Evaluation du test d'immunofluorescence indirect (IFI) pour le diagnostic et le dépistage de la Dermatose Nodulaire Contagieuse Bovine à l'aide de méthodes bayésiennes.

G. Gari, F. Biteau-Coroller, C. LeGoff, P. Caufour, F. Roger

Résumé :

Deux tests de diagnostic sérologique de la Dermatose Nodulaire Contagieuse (DNC) Bovine ont été développés, l'immunofluorescence indirecte (IFI) et la séroneutralisation virale (SNV) mais aucun des deux n'a encore été évalué dans le contexte éthiopien. C'est pourquoi, ce travail a eu pour objectif d'évaluer les performances de l'IFI pour le diagnostic et le dépistage de masse de la DNC en Ethiopie en utilisant le test SNV en comparaison. Une méthode bayésienne a été utilisée dans la mesure où il n'existe pas de gold standard. Des prélèvements de sang ont été effectués dans deux sous populations de bovins, la première dans le nord où des épizooties récentes de DNC ont été observées récemment et la seconde dans le sud du pays. Cette étude a permis de montrer que la spécificité (88%) et la sensibilité (92%) de l'IFI étaient bonnes et que le test pouvait être utilisé dans des dépistages sérologiques avec un relativement faible nombre de faux positifs ou négatifs. Le test SNV, très spécifique mais moins sensible, devrait être réservé à la confirmation de cas douteux.

Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method

G. Gari, F. Biteau-Coroller, C. LeGoff, P. Caufour, F. Roger

Summary

Diagnostic tests are important tools for epidemiological studies in that they enable to detect and quantify non-clinical disease events in the population. Interpretation of sero-diagnostic data is conditioned by the sensitivity and specificity of the tests used and the prevalence of the disease in the study area. One needs to know the accuracy of the diagnostic test to adjust for the misclassification of the disease status of the animal. The accuracy of the conventional sero-diagnostic techniques used for diagnosis of LSD such as Virus Neutralisation Test (VNT), and Indirect Fluorescence Antibody Test (IFAT) have not been evaluated in particular in the context of the target population of Ethiopia (Greiner and Gardner, 2000b). Thus, the objective of this study was to fulfill this gap and evaluate the performance of IFAT for diagnosis and screening of LSD in Ethiopia using VNT as second test for comparison. Bayesian method appeared as particularly well suited for the analysis of diagnostic tests evaluation without gold standard (Enoe et al., 2000; Gardner et al., 2000; Johnson et al., 2001; Georgiadis et al., 2003; Branscum et al., 2005). It has the advantages to provide more stable point and interval estimates than Maximum Likelihood Eestimate (MLE) without the necessity of large sample sizes (Enoe et al., 2000) and to improve parameter estimation by the fact that uncertainty in observational study data is modeled into probability distribution (Figure 9).

Two different study sub-populations were selected in Ethiopia to get two different disease prevalences to comply with the assumption of sampling design for Bayesian analysis. The sub-population from the northern Ethiopia was where recent LSD outbreak had occurred and 263

sera were collected from three districts. The second sub-population was from the southern Ethiopia where 200 sera samples were collected by cross sectional random sampling method in two districts. The result from the analysis showed that the accuracy of IFAT was good in both sensitivity and specificity parameters indicating that it can be used for LSD diagnosis and screening with less misclassification (Figure 10). Its capacity to run large number of samples per plate just like ELISA could be also taken as an advantage for large epidemiological studies. Moreover, the two tests IFAT and VNT were found conditionally independent on the disease status of the animal. This implies that the two tests could be used either in series or in parallel combinations with maximum test efficiency (Gardner *et al.*, 2000; Greiner and Gardner, 2000b; Dohoo *et al.*, 2003). Higher sensitivity and throughput for IFAT would render the test being selected for screening purposes and higher specificity performance of VNT would qualify it to be used as a confirmation test.

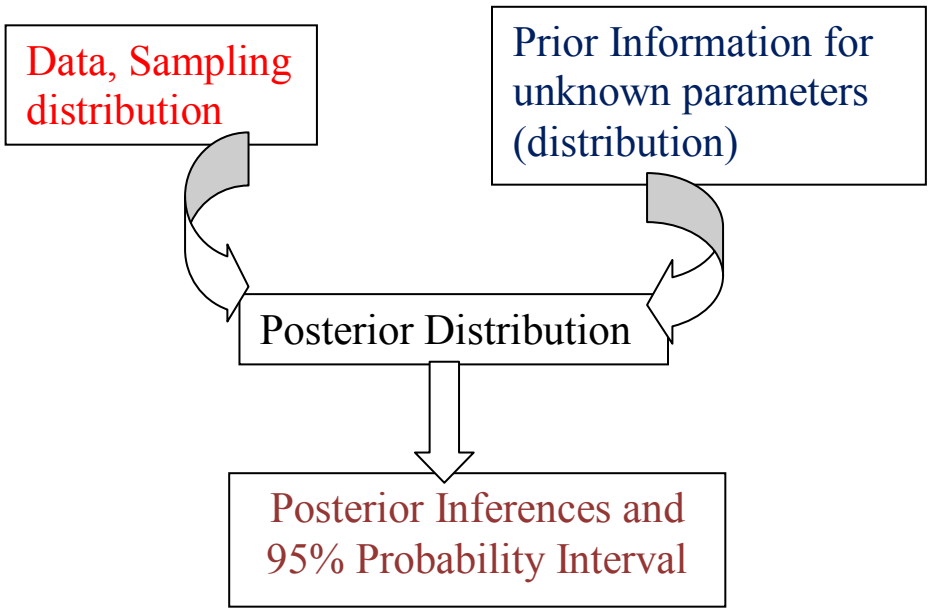
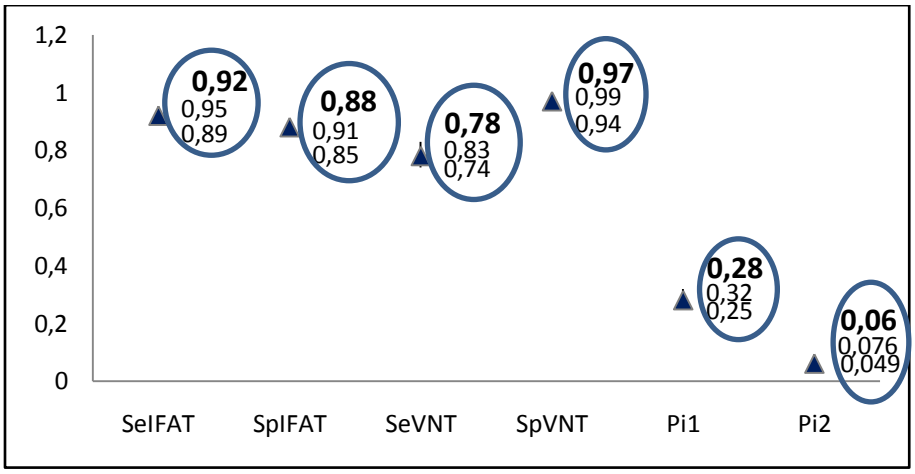


Figure 9: Simple illustration of Bayesian model to generate the inference mean or median values

The specificity of IFAT obtained in this analysis was relatively fine 0.88 (95% CI: 0.85–0.91) and the dilution rate of the test sera applied in our protocol (inverse log 5= 2) might have contributed to reduce the possibility of cross-reacting globulins and nonspecific background reactions. However, the specificity of VNT was very high which allowed to rule-out the possible crossreaction of Parapox and some Orthopox antibodies with LSD and was in congruent with the previous report (Davies and Otema, 1981).

The result of our study demonstrated that IFAT could be used for sero-surveillance study of LSD in the target population. Although, accuracy measure would not be the only basis for test selection, IFAT would come as a first choice where there is no an appropriate ELISA kit for epidemiological study of LSD. Then adjusted true prevalence of LSD in Ethiopia could be calculated based on the sensitivity and specificity parameters obtained from the sero-prevalence study as described by (Dohoo et al., 2003).



95% confidence interval is given below the mean estimates

Pi1 and Pi2 are prevalence of sub-population 1 (Northern Ethiopia) and 2 (Southern Ethiopia) respectively.

Figure 10: The sensitivity, specificity and prevalence estimations by Bayesian model

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Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method

G. Gari [a,b,*], F. Biteau-Coroller [b], C. LeGoff [c], P. Caufour [c], F. Roger [b]

[a] *Sebeta National Animal Health Research Center, PO Box 04, Sebeta, Ethiopia*
[b] *CIRAD, Epidemiology and Ecology of Animal Diseases Unit, TA A-16/E, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France*
[c] *CIRAD, Emerging and Exotic Animal Disease Control Unit, TA A-15/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France*

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Abstract

The performance of indirect fluorescence antibody test (IFAT) for serological diagnosis and screening of lumpy skin disease (LSD) was evaluated using methods without gold standard. Virus neutralization test (VNT) was used as the second test and the study sites were selected from two different geographical places in Ethiopia to get different disease prevalence. The analysis of conditional dependent Bayesian model for the accuracy of IFAT showed that sensitivity, specificity, prevalence of the population Pi_1 and the population Pi_2 were 0.92 (0.89–0.95), 0.88 (0.85–0.91), 0.28 (0.25–0.32) and 0.06 (0.048–0.075), respectively. The posterior inferences obtained for VNT sensitivity, specificity and conditional correlation between the tests for sensitivity (rhoD) and specificity (rhoDc) were 0.78 (0.74–0.83), 0.97 (0.95–0.99), 0.052 (−0.03–0.15) and 0.019 (−0.01–0.06), respectively. The interval estimation of conditional correlation for both sensitivity and specificity clusters around zero and thus conditional dependence between the two tests was not significant. Although accuracy measure would not be the only basis for test selection, the result of our study demonstrated that IFAT has a reasonable high accuracy to be used for the diagnosis and sero-surveillance analysis of LSD in the target population.

Keywords: Bayesian model; Cattle; Ethiopia; Lumpy skin disease; Sensitivity; Specificity; IFAT

1. Introduction

Lumpy skin disease (LSD) is an acute to subacute viral disease of cattle that can cause mild to severe signs including fever, nodules in the skin, mucous

* Corresponding author. Current address: CIRAD, Epidemiology and Ecology of Animal Diseases Unit, TA A-16/E, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France. Tel.: +33 637130848; fax: +33 467593754.
E-mail address: getachew.gari@cirad.fr (G. Gari).

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membranes and internal organs, skin oedema, lymphadenitis and sometimes death. The disease causes high economic loss as a result of decreased milk production, abortion, infertility, weight loss, poor growth and skin damage (Ali et al., 1990; OIE, 2004). Lumpy skin disease is caused by the strain of capripox virus which is genetically and antigenically close related to the strain of sheep and goat pox virus and the prototype strain is known as the Neethling Pox virus (Alexander et al., 1957; Davies, 1982).

Currently, the distribution of LSD in Africa has increased its horizon from Sub-Saharan countries to Egypt and western African countries (Davies, 1991). Davies (1991) has also emphasized that the epidemiological distribution trend of LSD has posed a considerable risk to extend its range to the northern African countries and eastern ward of the Egypt to the Middle East countries. In Ethiopia LSD was first observed in the western Ethiopia (south west of Lake Tana) in 1983 and the assumption was that it has been introduced from Sudan (Mebratu et al., 1984). The Ethiopian National Veterinary Service field report from 1999 to 2006 revealed that the occurrence of LSD outbreak has almost spread to all regions of the country including different agro-climatic zones. According to these reports we noted that the number of outbreaks reported per month increases highly during the wet season that is from June up to October (Ministry of Agriculture and Rural Development Disease Report Database). However, no epidemiological study has been done yet in different regions and ecotypes of the country which is indeed required to give a more realistic epidemiological picture than the one obtained by passive surveillance data.

Diagnostic and screening tests are the primary tools for such successful epidemiological study (Greiner and Gardner, 2000a). The OIE recommended serological tests used for LSD diagnosis are essentially IFAT (indirect fluorescent antibody test), ELISA and VNT (Virus neutralization test) (OIE, 2004). Indirect ELISA of recombinant P32 antigen from KS-1 strain has been developed previously but till today it has not been validated to replace the conventional once (Heine et al., 1999). Lack of information on the performance of the available diagnostic test is also one of the limiting factors to conduct large epidemiological studies. Understanding the characteristic of the tests is essential to know how they affect the quality of data obtained from epidemiological research and can be considered as a precursor step (Dohoo et al., 2003). The accuracy of these diagnostic tests should also be evaluated for specific target population of concern (Greiner and Gardner, 2000b).

The availability of a suitable reference test is an important requirement for the performance evaluation study. But it is difficult or sometimes next to impossible to obtain perfect (gold standard) test which can identify the true disease status of the animal (Enoe et al., 2000, 2001; Dohoo et al., 2003; Biteau-Coroller et al., 2006). However, when gold standard test is not available the performance of two tests can be estimated using latent-class approaches, provided that the error probability of the reference test is known (Enoe et al., 2000). In most cases virus neutralization test (VNT) is considered as reference test which has a strong specificity but less sensitivity for capripox virus (OIE, 2004; Bhanuprakash et al., 2006).

Diagnostic test evaluation is particularly suited to the Bayesian framework (Branscum et al., 2005). The Bayesian analysis for diagnostic test evaluation without gold standard was discussed for conditional independent and conditional dependent tests (Enoe et al., 2000; Gardner et al., 2000; Johnson et al., 2001; Georgiadis et al., 2003; Branscum et al., 2005). Bayesian approach uses prior information knowledge about the parameters of the tests under study either from other similar studies or expert's best guess. Moreover, it has an advantage to provide more stable point estimates and intervals without the necessity of large sample sizes (Enoe et al., 2000). The Bayesian inference is the combination of the beta distribution of the prior information and the maximum likelihood estimates of the observed data (Gardner et al., 2000).

The objective of this study was to evaluate the performance of IFAT for diagnosis and screening of lumpy skin disease in Ethiopia using VNT as second test for comparison. The Bayesian model and Hui and Walter (1980) model were used to analyse the test performance where there is no gold standard. The parameters used to measure the accuracy are sensitivity, specificity, prevalence and the conditional correlation between the two tests.

2. Materials and methods

2.1. Study area and study population

The study was conducted from September 2006 to March 2007. Two study areas with different farming system and expected different LSD prevalence were selected. The first study area was in Amhara Region (North Wello, South Wello and Oromia administrative zones, in northern part of Ethiopia) where the altitude range from 1400 to 2230 m above sea-level (Fig. 1). Livestock production is extensive system whereby animals of different species and age groups share common grazing land and watering point. The breed composition of the subpopulation is predominantly the local zebu breed. At the time of this study, lumpy skin disease re-occurred as an outbreak in these study areas starting from the month of July 2006 after 5–6 years elapse (personal communication with the local veterinary officers).

The second study area was in Oromia Region (Borena and Guji administrative zones in southern part of Ethiopia) which have an agro-pastoral farming system with semi-arid and sub-humid climate, respectively. Altitude range is from 1590 to 1740 m. There was no reported LSD outbreak since 2005 in the area and no vaccination program was put into place for the last 12 months.

2.2. Sampling

The purpose of the sampling design was to obtain different prevalence between the two study populations to hold the Bayesian assumption true where gold standard test is not available. All animals above 6 months old and both sex groups were subjected to random sampling.

- Population 1 (P_1): In the northern study area, at the district level, the target Peasant Associations (PA) (it is the lowest rural administrative level in Ethiopia which can hold variable number of villages in it) were those with LSD outbreak history. In seven PA selected from three districts, the herds and animals were randomly selected for sample collection. In all the places, the sampling was carried out

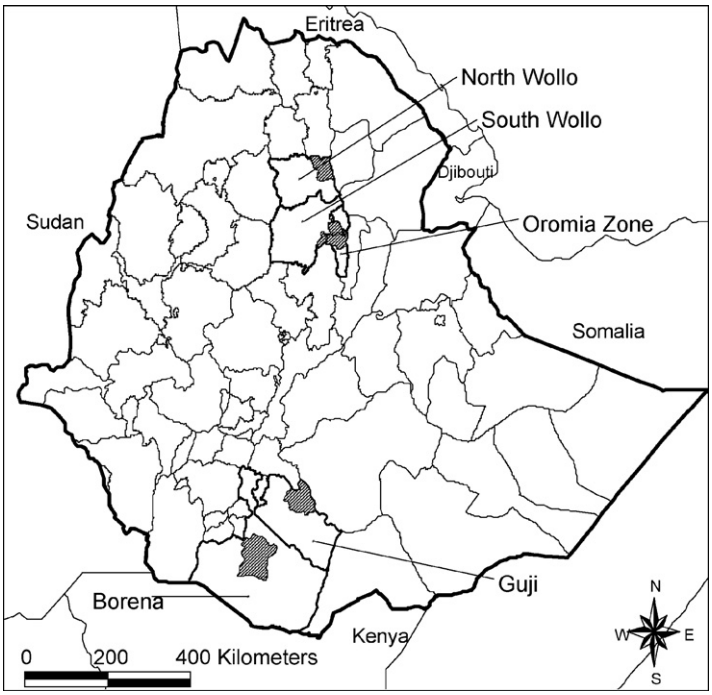

Fig. 1. Map of Ethiopia with the locations of the study areas (shaded areas) (Source: International Food Policy Research Institute, Atlas of the Ethiopian Rural Economy, 2006).

before the deployment of vaccination to control the outbreak.

- Population 2 (P_2): In southern study area, there was no recent evidence of LSD outbreak. The samples were collected by multistage random sampling technique in five PA selected from two districts. At the district level, the PA's were randomly selected and then the herds and the animals too. Thus the study design applied agrees with the complete verification approach (Greiner and Gardner, 2000b).

Sample size determination was based on Greiner and Gardner (2000b) formula using the prior estimates for sensitivity and specificity of IFAT to be 90% and 80%, respectively, with the desired precision level of 0.05. A total of 463 sera that is 263 sera from the northern area (P_1) and 200 sera from the southern area (P_2) were assigned for the study.

2.3. Serological tests

Blood samples of 5–7 ml were collected in plain vacutainer tube from the jugular vein. The samples were allowed to clot for 2–3 h at room temperature. Then the serum was extracted by spinning at 2500 rpm and the serum was preserved in −20 °C temperature until the test conducted.

2.3.1. Indirect fluorescent antibody test (IFAT)

The IFAT was used to detect serum antibody against lumpy skin disease. Antibodies of capripox virus can be detected from day 2 after the onset of clinical signs and remain detectable for about 7 months, but a significant rise in titre is usually seen between days 21 and 42 (Lefèvre et al., 2003; OIE, 2004). The serum samples were processed blindly for the test. The antigen used to detect the serum antibody against lumpy skin disease was KS1 (Kenyan sheep pox virus) strain which is recently proved to have genetically identical with Neethling virus (Gershon and Black, 1988). The KS1 strain was obtained from CIRAD Laboratory and the lamb testis cell was infected using 50 μl of 100_{TCID50} viral suspension per well cultured in 96-well flat-bottomed tissue-culture grade microtitre plate. The infected monolayer cells were fixed after 48 h using 80% acetone. The test serum was diluted in 1/25 in 0.5% lamb serum blocking buffer (blocking buffer is to avoid the non-specific background reaction) and each serum was tested in duplicate wells. The positive and negative control sera were also included in each plate. Fluorescein isothiocyanate conjugated anti-bovine gamma-globuline (IgG) of rabbit was diluted in 1/40 in 0.5% lamb serum blocking buffer and add to each well (Standard Operating Protocol of CIRAD). The plates were read using Zeiss Fluorescent microscope under 40× magnification. The positive test serum appears bright fluorescence foci where the antibody reacted with the virus and the negative serum appears as dark field or dim gray foci.

2.3.2. Virus neutralization test (VNT)

Serial dilution of the test serum was done in 1/5, 1/25, 1/125, 1/625 and 1/3125 dilutions and each serum was tested in duplicate wells. KS1 strain virus in 100 $_{TCID50}$ per wells constant titration was maintained similar for each well. The vero cell was used for the test and cultured in 96-well flat-bottomed tissue-culture grade microtitre plates (OIE, 2004). The reason for Vero cells preferred was the Vero cells are less sensitive to capripox virus and to reduce the problem of ''breakthrough'' in which the virus dissociate the antibody binding and relapse to infect the cells (OIE, 2004; Bhanuprakash et al., 2006). The plates were incubated at 37 °C, 5% carbon dioxide (CO_2) for 9 days. The plates were examined under inverted microscope for the presence of cytopathic effect (CPE) starting from day 4. The final reading was taken on day 9 and the result was recorded from the highest dilution which inhibited the CPE in both or either of the duplicate wells. The test result was recorded as the reciprocal of the log titration. The interpretation of the result is that the wells with no CPE in 1/25 and more dilutions were considered as positive serum. This indicates that the antibody against the LSD virus has reacted with the KS1 virus and inhibited the growth of the virus not to produce CPE.

2.4. Questionnaire survey

Questionnaire survey included the LSD disease status, potential risk factors and other epidemiological records using questionnaire format which was prepared based on the prior knowledge of the disease in

Table 1
Cross-classification of the IFAT and VNT results from serum samples in two cattle populations with expected high prevalence in P_1 and low prevalence in P_2

		Virus neutralization test				Total
		P_1		P_2		
		+	−	+	−	
IFAT	+	82	29	12	20	143
	−	3	149	3	165	320
Total		85	178	15	185	463

the respective sampled sites. The data was analysed by *t*-test to compare the disease prevalence between the two sampling areas.

2.5. Test evaluation

The laboratory result obtained was cross-classified for each population to calculate the test parameters (Table 1). Statistical methods and tools used for evaluation of the test performance under different specific conditions have been discussed (Enoe et al., 2000; Gardner et al., 2000; Johnson et al., 2001; Pouillot et al., 2002; Georgiadis et al., 2003; Orr et al., 2003; Branscum et al., 2005; Kostoulas et al., 2006; Van Schaik et al., 2007).

We applied methods without gold standard to analyse the accuracy since the reference test used was not gold standard in its accuracy. We used comparatively the following methods to analyse our estimates: maximum likelihood estimate (Hui and Walter, 1980) model, conditional independent and dependent Bayesian models (Branscum et al., 2005).

The maximum likelihood method assumes three conditions: (i) the studied population should consist of two subpopulations with different prevalence, (ii) in these subpopulations, the test accuracy should be constant and (iii) the two tests should be conditionally independent of each other (Hui and Walter, 1980; Pouillot et al., 2002). We used the spreadsheet model of Hui and Walter (1980) from the web site (http://www.epi.ucdavis.edu/diagnostictests/).

The Se and Sp estimates of IFAT (Se_{IFAT} and Sp_{IFAT}), the Se and Sp of VNT (Se_{VNT} and Sp_{VNT}) and the prevalence of the two populations (Pi_1 and Pi_2) were also calculated using Bayesian methods. The study populations have different disease prevalence based on information obtained from the analysis of questionnaire interview data (Table 2) and thus complies with the assumption where gold standard test is not available.

Tests based on similar biological basis might have correlated errors that cause incorrect estimation of sensitivity and specificity (Gardner et al., 2000; Georgiadis et al., 2003; Orr et al., 2003; Branscum et al., 2005). As both IFAT and VNT detect antibodies, it is reasonable to confirm that the tests sensitivity and specificity were indeed conditionally independent on disease status. Then, both conditional independent and dependent Bayesian models for two tests, two populations were applied which allowed us to estimate the Se and Sp conditional correlations (rhoD and rhoDc, respectively) between the tests and their 95% probability intervals (95% PI). We used the model recently reviewed by Branscum et al. (2005) for both conditionally independent and dependent assumptions, using Winbugs package (for more details see (Enoe et al., 2000; Georgiadis et al., 2003; Branscum et al., 2005).

The assumption of equal accuracy of the tests across subpopulations was checked by considering separate analysis of the two populations (Georgiadis et al., 2003). For each population, the model and prior

Table 2
Lumpy skin disease prevalence estimation based on farmers' opinions collected in the two study areas

Studied population	Northern study area	Southern study area	Total
Number of investigated herds	99	53	152
Number of sampled animals	615	1 097	1 712
Number of LSD diseased cattle	150	66	216
Estimated prevalence	24.4% (CI: 21, 27.8%)	6% (CI: 4.6, 7.4%)**	
LSD mortality	2.8% (CI: 1.3, 4.3%)	1.8% (CI :1, 2.6%)	

Note: Herd in this context is defined as cattle possessed by one farmer or a group of relatives, which are managed together in a similar manner.
** Significantly different at $p < 0.05$.

information used were identical to the model and prior used in the two-population case.

2.6. Prior information

In Bayesian analysis prior information are often specified for the unknown parameters either from published papers or experts best guess (Enoe et al., 2000; Branscum et al., 2005). Prior information on sensitivity and specificity of these current tests were obtained from scientists working on capripox research in CIRAD and Institute for Animal Health Pirbright (IAH) Laboratory. We could not get any scientific publication data relevant to the determination of the accuracy of tests for lumpy skin disease except the general recommendations on the available diagnostic tests currently in use. The prior information of disease prevalence in the two populations (Pi_1 and Pi_2) were estimated on the basis of the results of the farmers' interviews conducted during the sample collection (Table 2).

The uncertainty of prior information are often modelled through the use of beta distributions (Enoe et al., 2000). The modal value of the prior information was transformed to beta distribution model using Betabuster free software from the website (http://www.epi.ucdavis.edu/diagnostictests/). For conditional independent Bayesian model the prior information for sensitivity of IFAT was mode 0.90 and the transformed beta (a,b) was beta (130.71,15.41) with 5th percentile equals to 0.84. Prior mode for specificity of IFAT was 0.85 beta (152.9,27.8) with a 5th percentile 0.79. The sensitivity prior for VNT was mode 0.75 beta (174.5,58.8), 5th percentile 0.69 and specificity prior mode was 0.95 beta (99.7,6.2) and 5th percentile 0.89. The beta prior distributions for prevalence of population 1 (Pi1) and population 2 (Pi2) were mode 0.24 beta (118.8,374) and 0.06 (66,1032), respectively, and 95th percentiles of 0.28 and 0.075, respectively. For conditional dependent model we used similar prior information as indicated above for Se_{VN}, Sp_{VN} of VNT, Pi1 and Pi2. Georgiadis et al. (2003) discussed reparameterization of the second test parameters since prior information is not usually available for the new test during new test validation. However, in this study the prior information obtained from expert's best guess for IFAT sensitivity and specificity were applied instead of reparameterization. Thus we assigned uniform priors for λ_D and γ_D a modal value of 0.90 beta (130.7,15.4) with 5th percentile 0.84 and in the same way a uniform prior for λ_{Dc} and γ_{Dc} with a mode of 0.85 beta (152.9,27.8), 5th percentile of 0.79 (Branscum et al., 2005).

2.7. Test agreement

McNemar's χ^2-test and the Kappa statistic (κ) were used to test the level of agreement between the IFAT and the VNT. McNemar's χ^2 was carried out first to test whether there was test bias (i.e. the difference in proportion positive result in each test) (Dohoo et al., 2003). Kappa and its 95% CI, was used further to measure the degree of agreement between the two tests after taking into account the probability of agreement by chance alone. Strength of agreement based on κ was judged according to the following guidelines—<0.2: slight agreement; 0.2–0.4: fair; 0.4–0.6: moderate; 0.6–0.8: substantial; >0.8: almost perfect (Dohoo et al., 2003). The software Intercooler Stata 8.2 (StataCorp LP, College Station, TX) was used for these analyses.

Statistical and model analysis were computed using STATA 8.0 (Stata Corporation © 1984–2003), Winbugs®, Betabuster and H&W model Excel spread sheet from online at http://www.epi.ucdavis.edu/diagnostictests/.

3. Results

3.1. Descriptive epidemiology

In both study areas LSD occurrence showed to have seasonal pattern and frequently associated with high moisture climate and high insect population dynamics (Fig. 2). About 90% respondents replied that the disease occurs from July to November which is the season of high moisture and also extends up to December. In the northern study area the LSD outbreak was commenced in July 2006 and continued up to the end of December 2006 which covered a wide extensive area (in four administrative zones of Amhara Region). Retrospective data analysis of LSD outbreak pattern from year 1999 to 2006 also revealed that the temporal distribution graph peaks high at the end of

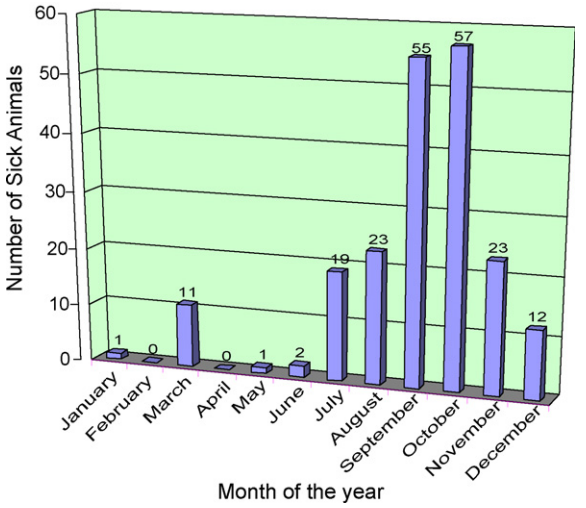

Fig. 2. Seasonal occurrence of LSD in the studied areas based on the data from questionnaire interview.

high rainy season (September) and gradually drops down up to the end of December (National disease outbreak report database) (data not shown).

In the northern study area only 10% herd owners used their own grazing plots but they shared the same watering point with animals in the surrounding community. The farmers are sedentary in their occupation. However, in the southern part all the community in the peasant association shares the same grazing land and watering point. Moreover, about 50% of the herd owners in the southern part responded that they have transhumant mode of life in which they move their herd seasonally to other grazing places in search of better feed and water for their animals.

3.2. Maximum likelihood estimates

The maximum likelihood estimates (MLE) of Hui and Walter model highly over-estimated the Se_{IFAT}, Se_{VN} and Pi1 as compared to the estimates of Bayesian models. The point estimates of MLE were not included in the 95% probability interval ranges of the Bayesian posterior inference (Table 3). However, specificity of both tests and Pi2 were not significantly different from the estimates of Bayesian models in which the estimates were in the 95% probability interval range of the respective parameters.

3.3. Bayesian conditional independent and dependent models

The posterior inferences obtained by conditional independent and dependent Bayesian models were consistently similar in all estimated parameters. The analysis of conditional correlation between the two tests showed the conditional dependence between the

Table 3
The sensitivity and specificity estimates of indirect fluorescent antibody test (IFAT) (Se_{IFAT} and Sp_{IFAT}) and virus neutralization test (VNT) (Se_{VNT} and Sp_{VNT}), for the detection of antibodies against lumpy skin disease in serum samples by the Hui and Walter and the Bayesian models with prevalence estimates for the two study populations and conditional correlation estimates

Parameters	Median (%) and (95% probability interval)				
	Model 1[a]	Model 2[b,c]	Model 3[c,d]	Model 4[d,e]	Model 5[d,f]
Pi_1	0.36 (0.27–0.44)	0.28 (0.25–0.32)	0.28 (0.25–0.32)	0.27 (0.24–0.31)	0.265 (0.23–0.30)
Pi_2	0.07 (0.03–0.10)	0.06 (0.05–0.08)	0.06 (0.048–0.075)	0.058 (0.045–0.07)	0.058 (0.05–0.07)
Se_{IFAT}	0.99 (0.93–1.0)	0.93 (0.89–0.96)	0.92 (0.89–0.95)	0.89 (0.75–0.98)	0.95 (0.84–0.99)
Se_{VN}	0.87 (0.72–1.0)	0.79 (0.74–0.83)	0.78 (0.74–0.83)	0.77 (0.71–0.82)	0.94 (0.79–0.99)
Sp_{IFAT}	0.90 (0.85–0.95)	0.88 (0.85–0.91)	0.88 (0.85–0.91)	0.85 (0.8–0.90)	0.85 (0.80–0.89)
Sp_{VN}	0.98 (0.96–1.0)	0.97 (0.96–0.99)	0.97 (0.95–0.99)	0.94 (0.91–0.97)	0.96 (0.91–0.99)
RhoD			0.052 (−0.03–0.15)	0.46 (−0.04–0.90)	0.38 (−0.016–0.88)
RhoDc			0.019 (−0.01–0.06)	0.43 (0.17–0.61)	0.28 (−0.015–0.55)

[a] Model 1 is a maximum likelihood estimate (MLE) based on the Hui and Walter (1980) model.
[b] Model 2 is based on the assumption of conditional independent model.
[c] Models 2 and 3 used the following priors—Se_{IFAT}: 0.90, β(130.7,15.4); Sp_{IFAT}: 0.85, β(152.9,27.8); Se_{VN}: 0.75, β(174.5,58.8); Sp_{VN}: 0.95, β(99.7,6.2); Pi_1: 0.24, β(118.8,374); Pi_2: 0.06, β(66,1032).
[d] Models 3, 4 and 5 are based on the assumption of conditional dependent model.
[e] Model 4 used non-informative priors β(1,1) for Se_{IFAT}, Sp_{IFAT}, the other parameters are the same priors as in models 2 and 3.
[f] Model 5 used non-informative priors β(1,1) for Se_{IFAT}, Sp_{IFAT}, Se_{VN}, Sp_{VN} and the same priors as in models 2 and 3 for Pi_1 and Pi_2.

tests were significantly minimum, which was less than 0.1 for both sensitivity (rhoD) and specificity (rhoDc) (Table 3). The 95% probability interval of conditional correlation estimate for sensitivity and specificity included zero in which the hypothesis for the conditional dependence could be rejected (Gardner et al., 2000).

The Sp_{VN} obtained from Bayesian estimates was nearly perfect for LSD diagnosis which is in the contrary to its low sensitivity estimate 0.78 (0.74–0.83). In our finding, the Se_{IFAT} was found to be high at 0.92 (0.89–0.95) as expected. Similarly the specificity was also fairly good at 0.88 (0.85–0.91) as it was considered to have lower specificity due to the possible crossreactions of parapox and orthopox virus with capripox virus.

The Se and Sp estimates calculated separately for each population showed that one-population analysis were consistent with the second population analysis and with the two-population case, indicating that our assumption of similar accuracy of the tests across the two populations was valid (Table 4). The precision of point estimates for sensitivity and specificity of both tests were within the range of 0.03 and 0.01, respectively.

3.4. Analysis of Bayesian model sensitivity

We used three sets of prior information for model sensitivity analysis (Table 3): (1) Non-informative priors for all parameters of the two tests showed that the posterior inferences for Se_{IFAT} and Se_{VN} were largely over-estimated while the rest parameters were remained almost similar estimation (result not shown). (2) Using informative priors for the two prevalences only, the median estimate for Se_{VN} was still over-estimated although its interval estimate included the true value and the remaining estimates were seemed not significantly affected (Table 3, model 5). (3) Additional model sensitivity analysis using informative priors for Pi1, Pi2, Se_{VN} and Sp_{VN} showed that the model estimates were not distinctly different from the analysis obtained using prior information for all parameters (Table 3, model 4). But the conditional correlation for specificity resulted significant test dependence. The model converged fairly for all parameters in models 4 and 5 (Table 3) prior information than when non-informative priors were assigned for all the parameters.

The convergence of the Bayesian models were analysed by observing kernel density and trace plots of the model visually and the plots stabilized consistently for all the parameters. The first 5000 iterations were discarded as burn-in phase and the posterior inferences were based on 100,000 iterations. Autocorrelations were also checked and there was no meaningful autocorrelation observed.

3.5. Test agreement

The difference in the proportion positive tests calculated for McNemar's χ^2-test showed significant difference (McNemar's $\chi^2 = 33.62$, $p < 0.000$) between the tests. Test agreement between the two tests using Kappa statistics was Kappa = 0.70 (0.61–0.78) showing that the two tests have substantial agreement according to the interpretation of Kappa result (Dohoo et al., 2003).

4. Discussion

In both study areas we noted that extensive livestock production system allows maximum chance

Table 4
Estimates of one-population two tests and combined two-populations two tests Bayesian analysis for the evaluation of test accuracy similarity across the two populations

Parameters	P_1	P_2	$P_1 + P_2$
Se_{IFAT}	0.93 (0.89–0.96)	0.90 (0.85–0.94)	0.92 (0.89–0.95)
Se_{VNT}	0.78 (0.74–0.83)	0.75 (0.70–0.80)	0.78 (0.74–0.83)
Sp_{IFAT}	0.86 (0.82–0.90)	0.87 (0.84–0.91)	0.88 (0.85–0.91)
Sp_{VNT}	0.97 (0.94–0.97)	0.97 (0.94–0.98)	0.97 (0.95–0.99)
Pi	0.28 (0.25–0.32)	0.061 (0.049–0.076)	

In parenthesis, 95% probability interval.

for different herd mixing during utilization of communal grazing lands and watering points. Under this prevailing system it is likely to speculate that the introduction and spread of LSD infection could have favourable environment. Uncontrolled cattle movements due to trade, pastoralism, vector insects population and dynamic, wet climate which favours insect multiplications and other reasons of cattle movement from place to place could render potential risk factors for the transmission of the disease from herd to herd and from place to place as it is true for other infectious disease too (Toma et al., 1999).

Seasonal characteristics of LSD occurrence implies that the transmission of the disease might linked with the optimum season for the development of vector insects population (Kitching and Mellor, 1986; Chihota et al., 2001, 2003). However, there are still little hard evidences for the specific insect vectors incriminated in the transmission of LDSV and may deserve further study to elaborate the principal vectors.

The immune response against LSD involves predominantly cell mediated immune response and the humoral immune system would last short period of life mostly for 7 months (Lefèvre et al., 2003; OIE, 2004). Hence studies based on serological detection of the disease should take into consideration the short lifespan of detectable antibody in the blood. For sample collection, we selected the natural infected population under active disease outbreak situation as P_1 and the other population with unknown disease status but which could have had exposure to the infection as P_2. This approach has greatly enabled to get significantly different prevalence between the two subpopulations which might be the ideal assumption for epidemiological approach of diagnostic test evaluation for lumpy skin disease.

An optimum consideration was taken during the laboratory techniques to limit the possible cross-reaction of parapox and orthopox virus with LSD virus and the information obtained through epidemiological disease investigation records was also used to understand the clinical disease situation in the study population. The members of capripox virus genus are antigenically very close related, which makes not possible to distinguish them by serological tests (Davies and Otema, 1981). However, capripox virus is highly host-specific under natural environment (Capstick and Coackley, 1961) and there has not been recorded incidence of lumpy skin disease occurrence from sheep pox or goat pox disease outbreak. This has been clearly evidenced that the Republic of South Africa had LSD but having huge number of sheep and goats there was no incidence of sheep and goat pox disease (Capstick and Coackley, 1961). In Middle East countries where sheep pox is endemic, LSD incidence has never been reported except the case reported in Israel and eradicated soon in 1989 (Yeruham et al., 1995). In Kenya sheep was found infected where the first outbreak of LSD occurred, which was the first in kind (Davies, 1991). But in an other study this Kenyan sheep and goat pox strain was proved to have more genetic similarity to Neethling virus than classical sheep pox or goat pox virus which maybe due to some genetic mutation enabled for adaptation to cattle (Gershon and Black, 1988). In our study sheep pox did not occur concurrently with LSD in the outbreak areas and it had never been noted to occur as a multi-host outbreak in the same place unless they coincided due to accidental overlap (personal communication with vet officers in study area).

Crossreaction of cowpox virus was observed to occur with LSD virus at lower dilution ($\leq$1/8) (Davies and Otema, 1981). But we diluted the test sera for IFAT at 1/25 concentration that might help us to reduce the possibility of cross-reacting globulins and non-specific background reactions. As a result it might have contributed to get better specificity test result which was 0.88 (0.85–0.91). However, the cross-reaction of cowpox with LSD virus observed in IFAT had not been demonstrated in VNT (Davies and Otema, 1981) which is in congruent with the high specificity estimated for VNT in our finding.

Maximum likelihood estimate (MLE) over-estimated three parameters out of six which did not fall in the 95% probability interval of the Bayesian estimates. The 95% confidence interval of MLE was also wider than the interval estimates of Bayesian models. The variation in the point estimates and the wider range for the interval estimates might reveal the uncertainty of MLE that assumes large sample size. Thus the method might not be applicable in our case due to small sample size (Enoe et al., 2000; Orr et al., 2003).

As both tests measure the same biological factor we expected certain degree of dependence between the two tests. However, both conditional independent and

dependent models had similar estimates for all parameters which indicated that the two tests are conditionally independent. The 95% probability interval of conditional correlation estimates were clustered around zero for both sensitivity and specificity which showed that the dependence of the tests were not significant (Georgiadis et al., 2003). This implies also the two tests could be used in series and parallel test combinations for a maximum test efficiency (Gardner et al., 2000; Greiner and Gardner, 2000b; Dohoo et al., 2003). Although the estimates we found from conditional independent and dependent models did not vary, we preferred to use the conditional dependent model for discussion to elucidate the information regarding the magnitude of test dependence (Enoe et al., 2000; Gardner et al., 2000; Branscum et al., 2005).

The posterior inferences estimated across the two populations separately and jointly showed insignificant difference (Table 4). The point estimates of each parameter obtained from separate analysis of each population lies within the 95% probability interval ranges of respective parameter in the combined population analysis. This supported the model assumption for similar test accuracy in the two populations. The slight variation observed in the precision of point estimates of Se_{IFAT} and Se_{VN} in population 2 might be due to small sample size coupled with lower disease prevalence in this population. The results could also reveal that Bayesian method has superior approximation to give reasonable posterior inference even under a small sample size condition (Branscum et al., 2005).

In the analysis of model sensitivity using non-informative priors for all the parameters, we found that the Se_{IFAT} and Se_{VN} estimates were unlikely over-estimated which might be due to non-identifiable model where the unknown parameters are greater than the degree of freedom. Whereas the posterior median obtained by using informative prior for Pi1, Pi2, Se_{VN} and Sp_{VN} (Table 3, model 4) and for Pi1, Pi2 (Table 3, model 5) resulted better estimation except the overestimated value of Se_{VN} in the latter model analysis. This indicates that the availability of prior information for the prevalences and the accuracy of one test would be necessary to get optimum posterior inferences (Georgiadis et al., 2003; Branscum et al., 2005). In general we can conclude that the posterior inferences of the Bayesian models did not vary distinctly for the changes in the prior information which indicates that the models were not significantly influenced by the prior information.

The substantial agreement between the tests observed from Kappa statistics was not supported by McNemar's χ^2-test. The difference in the proportion positive test results was significantly different for the two tests (McNemar's $\chi^2 = 33.62$, $p < 0.000$). This significant difference in the proportion of positive test results might be explained by the low sensitivity in virus neutralization test and a minimum conditional dependence between the two tests (Dohoo et al., 2003). But in reality this empirical difference might not justify the presence of a test bias from a biological point of view since it reflects the existence of significant difference in the sensitivity estimates of the two tests.

5. Conclusion

In this study we observed that the accuracy of indirect fluorescent antibody test was fairly good in both sensitivity and specificity parameters indicating that it can be used for LSD diagnosis and screening with low misclassification. Its capacity to run large number of samples per plate (45 samples per plate) could be also taken as an advantage to use for large epidemiological studies of LSD. However, to undertake similar epidemiological study on sheep pox and goat pox, we suggest further evaluation study to determine the accuracy of these tests for sheep pox and goat pox diseases. Test accuracy may vary according to the target population of concern and extrapolating directly the result of current study on LSD might lead to unwise conclusion for sheep pox and goat pox diseases.

The conditional correlation estimates between the two tests revealed that the tests are conditionally independent on the disease status of the animal. This implies that the two tests could be used especially in parallel test combinations with maximum sensitivity efficiency.

The drawback in using IFAT is that the test requires longer time and may be more costly as compared to ELISA technique. We recommend more efforts and studies should be done towards the development and

validation of ELISA test which may outmatch the limitations of the currently in-use diagnostic and screening tools.

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CHAPTER III.

ARTICLE 2: Risk factors associated with observed clinical lumpy skin disease in Ethiopia

Facteurs de risque associés aux cas cliniques de Dermatose Nodulaire Contagieuse Bovine en Ethiopie.

G. Gari, A. Waret-Szkuta, V.Grosbois, P.Jacquiet and F. Roger

Résumé

Très peu d'études épidémiologiques ont été réalisées depuis l'apparition de la DNC bovine en Ethiopie. De plus, très limitées géographiquement, elles ne prenaient pas en compte les différentes zones agroécologiques du pays. C'est pourquoi cette étude a eu pour but d'évaluer la prévalence des cas cliniques de DNC dans les trois grandes zones agroécologiques (haute, moyenne et basse altitude) du pays et de préciser les principaux facteurs de risque associés avec cette maladie. Un total de 330 questionnaires a été obtenu : 103 questionnaires dans les zones de haute altitude, 165 pour les zones de moyenne altitude et 62 pour les zones de basse altitude. L'étude était basée sur l'identification des signes cliniques de la maladie par les éleveurs eux-mêmes dans trois années précédant l'enquête. Les autres maladies cutanées des bovins ont été prises en compte dans le questionnaire pour établir un diagnostic différentiel et minimiser les erreurs de diagnostic. A l'échelle du troupeau, la prévalence de la DNC était significativement supérieure dans les zones de moyenne altitude (55,2%) que dans les zones de haute et basse altitude (respectivement 22,3 et 43,5%). La prévalence de la DNC à l'échelle de l'animal était de 8,1% et le taux de mortalité de 2,12%. De même, la prévalence et la mortalité de la DNC à l'échelle de l'animal était significativement supérieure dans les zones de moyenne altitude que dans les zones de haute et basse altitude. Un modèle de régression logistique multiple a permis de déterminer l'importance de trois variables : la zone agroécologique, la conduite de troupeaux sur des pâturages et abreuvements communs et l'introduction d'animaux. L'importance de la zone agroécologique est mise en relation avec l'abondance particulière des insectes

hématophages dans cette zone, en particulier pendant la saison des pluies. Partager les mêmes pâturages et les mêmes lieux d'abreuvement est un facteur de risque important de la DNC car cela favorise les contacts directs entre animaux de troupeaux différents et la transmission mécanique par les stomoxes ou les moustiques. L'introduction d'un animal infecté dans un troupeau sain est clairement un facteur de risque important comme pour d'autres maladies infectieuses comme la tuberculose et la paratuberculose. Les autres facteurs de la conduite du troupeau, la taille du troupeau et le contact avec des moutons et des chèvres ne sont pas associés avec la DNC bovine. La connaissance de ces facteurs de risque par les éleveurs et les services vétérinaires peut être très utile dans le contrôle de la maladie en Ethiopie mais aussi dans les autres pays africains affectés par cette pathologie. Le rapport coût/bénéfice des méthodes de contrôle que l'on peut déduire de cette étude doivent être analysées, c'est l'objet du chapitre suivant de la thèse.

NB : des informations relatives à la dynamique saisonnière des populations de trois espèces de stomoxes (*S. calcitrans, S. sitiens* et *S. niger niger*) dans les trois grandes zones agroécologiques sont présentées en complément de l'article paru dans Epidemiology and Infection en 2010.

Risk factors associated with observed clinical lumpy skin disease in Ethiopia

G. Gari, A. Waret-Szkuta, V.Grosbois, P.Jacquiet and F. Roger

Summary

Few epidemiological studies have been carried out since LSD has established in Ethiopia and that with limited scopes as compared to the diverse agro-ecological and production systems prevailing in the country. This study was aimed to address important knowledge gaps regarding the prevalence of LSD occurrence in different agro-climatic conditions and the associated risk factors. A cross-sectional study based on a questionnaire survey was conducted along with retrospective data investigation. Of the 330 questionnaires administered, 103 were collected from highlands, 165 from midlands and 62 from lowlands. The retrospective questionnaires were limited to the last 3 years period because the farmers might fail to memorize numerical estimates of reported facts, thereby introduce potential recall bias beyond that time period. Serological sampling was conducted concurrently with the risk factor study, but the results of the sero-prevalence analysis by IFAT and VNT could not be incorporated in the thesis because of time limitation.

The study represents the majority crop–livestock production system prevailing in the highland and midland agro-climates and has also included classical areas of semi-pastoral production system in the lowlands. The study approach was based on the symptomatic disease identification experience of the herd-owners complemented by epidemiological records of veterinary offices at different levels. This epidemiological surveillance method has allowed us to obtain preliminary disease information to estimate the prevalence and distribution of LSD. Endemically occurring skin diseases of cattle were taken into consideration to rule-out the differential diagnoses while the questionnaire interview was conducted.

Across the agro-climate zones, herd-level LSD prevalence in the midland agro-climate was significantly higher 55.2% (95% CI: 47.5–62.6) than in lowland and highland agro-climate zones (22.3% and 43.5%, respectively). The average herd level LSD prevalence was 42.8% (95% CI: 37.5–48.3). The observed LSD prevalence at animal level was 8.1% which was close to the 10% reported previously (Davies, 1991; Babiuk et al., 2008a) in endemic areas of Africa and observed mortality was of 2.12%. Observed prevalence and mortality at animal level were still higher in the midland zone (10.4% and 3.2%, respectively) than in lowland and highland zones ($P<0.05$).

The final multiple logistic regression model showed that three variables: the effect of agro-climate, communal grazing/watering management and introduction of new animals were significantly associated with LSD occurrence. The potential risk of agro-climate variations to LSD occurrence showed that herds in midland and lowland agro-climates were more likely infected by LSD than in the highland agro-climate. This association might be attributed to the availability and abundance of effective mechanical vector insects. The temporal association between LSD occurrence and increase in the biting-fly population was also positively correlated and significant by Spearman rank correlation coefficient. Both biting-flies activity and disease outbreak frequencies begin to increase from April reaching a maximum in September which suggested that mechanical vector insects might play a major role in the epidemiology of LSD (Figure 11and 12). Agro-climate variation is the basis for the type and abundance of speculated mechanical vector insects. The warm and humid climate in midland agro-climates might be a more favorable environment for the occurrence of large populations of biting flies than the remaining two agro-climates (Table 3) (Zumpt, 1973; Kettle, 1990; Troyo *et al.*, 2007).

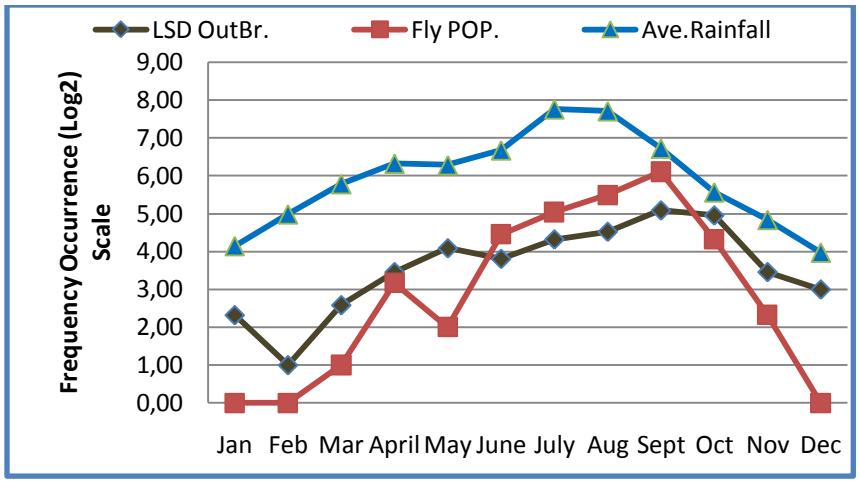

Figure 11: Questionnaire survey results of seasonal increase in biting-fly activity vs. lumpy skin disease (LSD) occurrence.

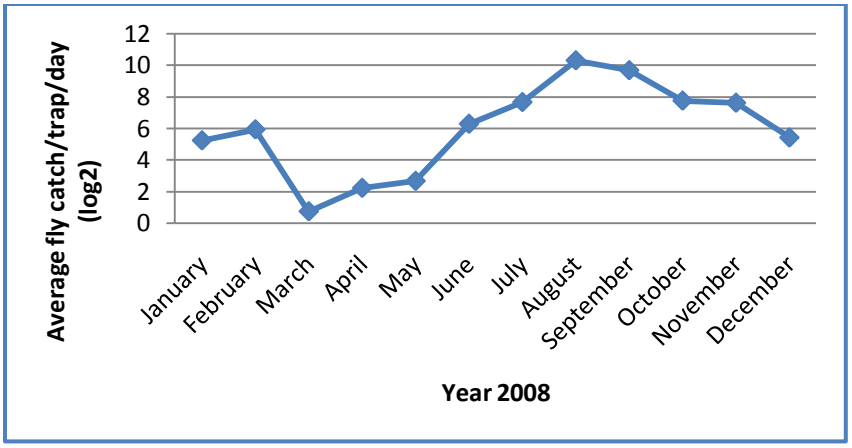

Figure 12: Biting fly population density through the year in 2008/2009 based on fly catchment.

Communal grazing and watering point utilization were found to be significantly associated with LSD occurrence. Sharing common watering points and grazing plots would allow contact and intermingling of different herds that would probably increase the risk of exposure and enhance the virus transmission through contamination and/or the speculated mechanical vectors such as Stomoxys spp. and mosquitoes (Aedes aegypti) (Kitching and Mellor, 1986; Chihota et al., 2001;

Waret-Szkuta et al., 2010). In private grazing managements, herd mixing is assumed to be less likely to occur.

The introduction of new cattle to the herd was also associated with an increase in the risk of disease transmission to the herd, as already reported for infectious diseases such as tuberculosis and paratuberculosis (Tiwari *et al.*, 2009; Tschopp *et al.*, 2009). Farming system, herd size and contact with sheep and goats were not significantly associated with LSD occurrence.

Hence it is likely that improved awareness by farmers and veterinary services on the potential disease transmission associated with shared use of grazing areas and watering points as well as promotion of biosecurity consciousness in the management of the introduction of new animals may assist in the control and prevention of infectious diseases in Ethiopia. The result from this risk factor analysis may shed light on the epidemiology of LSD in other African countries suffering from the disease. The control options proposed in this study should be complemented with economic feasibility investigations for the cost-effective way of controlling the disease which would certainly provide guidance for the eventual knowledge-based decision process for the control of the disease or to mitigate the risks to an acceptable level. The next study underpin on the financial impact of clinical LSD and the financial benefit of its control by annual vaccination.

NB: The biting flies population dynamics in the article was actually determined from herd-owners observations. But this data was also compared with the data obtained from the study on biting flies dynamics (particularly *Stomoxys spp*) based on modified Vavoua traps through a one year caught in the three different agro-climatic zones. I incorporated the summary of fly catch data as a supplement resource in this section.

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Risk factors associated with observed clinical lumpy skin disease in Ethiopia

G. GARI[1,2*], A. WARET-SZKUTA[2,3], V. GROSBOIS[2], P. JACQUIET[4] AND F. ROGER[2]

[1] *National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia*
[2] *CIRAD, AGIRs, TA C-22/E, Campus International de Baillarguet, Montpellier, France*
[3] *Veterinary Epidemiology and Public Health Group, Department of Veterinary Clinical Sciences, The Royal Veterinary College, Hatfield, Herts, UK*
[4] *UMR INRA/DGER 1225, Interactions Hôtes-Agents Pathogènes, École Nationale Vétérinaire, Toulouse, France*

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SUMMARY

A cross-sectional study based on a questionnaire survey was conducted to determine the distribution of lumpy skin disease (LSD) and associated risk factors in three main agro-climatic zones of Ethiopia. A total of 330 questionnaire surveys were collected from 44 peasant associations (PA) distributed in 15 districts. Across agro-climate zones, herd-level LSD prevalence in the midland agro-climate was significantly higher 55·2% [95% confidence interval (CI) 47·5 62·6] than in highland and lowland agro-climate zones. Overall observed LSD prevalence at animal-level was 8·1% (95% CI 7·3 8·9) and observed mortality was 2·12% (95% CI 1·73 2·6). The odds ratio (OR) of LSD occurrence in midland *vs.* highland and lowland *vs.* highland zones was 3·86 (95% CI 2·61 5·11) and 4·85 (95% CI 2·59 7·1), respectively. Significantly high risk of LSD occurrence was associated with communal grazing and watering management (OR 4·1, 95% CI 2·02 6·18) and introduction of new cattle (OR 8·5, 95% CI 6·0 11·0). Our findings describe the distribution of LSD in different agro-climates in Ethiopia along with associated risk factors, and can help shed light on the epidemiology of LSD in other African countries suffering from the disease.

Key words: Agro-climatic zone, Ethiopia, logistic regression, lumpy skin disease, risk factor.

INTRODUCTION

Lumpy skin disease (LSD) is one of the most serious poxvirus diseases of cattle caused by lumpy skin disease virus (LSDV) within the genus *Capripoxvirus*. The prototype strain is Neethling virus. It causes acute to subacute 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 [1, 2]. The skin nodules are painful and could involve tissues up to the musculature. Where extensive generalization occurs, animals may become lame and reluctant to move mainly because of severe oedema in the brisket and around the legs. Superficial lymph nodes draining affected areas of skin become enlarged, up to 4 10 times normal size. Abortion may occur as a result of prolonged fever [3, 4]. The disease

* Author for correspondence: Dr G. Gari, National Animal Health Diagnostic and Investigation Centre, PO Box 04, Sebeta, Ethiopia.
(Email: getachewgj@yahoo.com)

has high economic costs due to production losses and chronic debilitation. Milk production ceases and permanent damage can occur to the hides [5, 6].

Laboratory diagnosis to confirm the disease can be made either through the isolation and identification of the virus, or by using serological tests such as the virus neutralization test and indirect fluorescent antibody test (IFAT) [4, 6, 7]. Under experimental infection the incubation period is 6 10 days. Clinically sick animals shed the virus through saliva, nasal and lachrymal discharges, blood and semen. Skin lesions have high viral concentration from days 7 18 post-infection. However, the virus can persist in skin plugs for about 42 days [8].

Morbidity and mortality of the disease vary considerably depending on the breed of cattle, the immunological status of the population, insect vectors involved in the transmission and isolates of the virus. In endemic areas morbidity is usually around 10% and mortality ranges between 1% and 3% [4, 9]. The most effective method of transmission is mechanically through biting flies [10 12]. The incidence of LSD occurrence is high during wet seasons when biting-fly populations are abundant and it decreases or ceases during the dry season [12]. LSD has been endemic in Africa for more than 70 years occurring in a wide range of ecotypes. The first outbreak in Egypt was reported in 1988 [5] but the disease has never reached northern African countries. Outside the African continent Israel has reported two outbreaks, the first in 1989 which was eliminated by the slaughter of all infected and contact animals [11], and the other more recently in 2006 [13].

Ethiopia has the most abundant livestock population in Africa [14] and the cattle population is estimated to be 41·5 million [15]. The livestock subsector accounts for 40% of the agricultural gross domestic product (GDP) and 20% of the total GDP without considering other contributions, e.g. traction power, fertilization and transportation [16]. In 2004 the livestock sector contributed around 12% of total foreign currency earnings [17]. About 99% of cattle populations are of local Zebu breed [15]. Genetically and geographically the main breed classifications in Ethiopia are Arsi, Fogera, Horo, Borana, Nuwer, Sheko and Afar breeds. The remaining 1% of exotic breeds are kept mainly for dairy production in and around urban areas. Traditional cattle management in the rural part of the country is extensive. Animals are free-ranging in communal grazing fields and different age groups are herded together. Natural grass, post-harvest crop residuals and straw are the main source of feed. Concentrate feeds and feed additives are seldom used [18].

LSD was first observed in the western part of Ethiopia (southwest of Lake Tana) in 1983 [19]. It has now spread to almost all the regions and agro-ecological zones [20]. Vaccination is classically used to control outbreaks whenever they occur. Few epidemiological studies have been carried out since the disease has become established in the country, with limited scope in terms of the diverse agro-ecological and production systems. Studies based on clinical disease observation around Nekemt town have reported a prevalence of 7·02% [21]. Another study based on seroprevalence in southern Ethiopia reported a prevalence of 6% [7]. Targeted sampling from outbreak areas around Southern Range land, Wolliso town and north Ethiopia reported prevalences of 11·6%, 27·9% and 28%, respectively [7, 22, 23]. Taking into account the countrywide distribution of the infection and the size and structure of the cattle population in Ethiopia it is likely that LSD is one of the most economically important livestock diseases in the country. This study aimed to address important knowledge gaps regarding the magnitude of LSD occurrence in different agro-climatic conditions and the associated risk factors. A cross-sectional study based on a questionnaire survey was conducted along with retrospective data investigation (the questionnaire is available online).

METHODS

Administrative and agro-climate structure in Ethiopia

The Ethiopian administrative structure encompasses 11 regions that include about 546 districts. Each district is composed of a different number of peasant associations (PA) [15]. Ethiopia's topography consists of a central high plateau bisected by the Great Rift Valley into northern and southern highlands and surrounded by lowlands. The lowland areas are more extensive on the east and southeast of the country than on the south and west (Fig. 1). The diverse topographic structure of the country forms the basis for several agro-climatic zones. The highlands range from 2300 to 3500 metres above sea level (m.a.s.l.) surrounded by a temperate transition zone between 1500 and 2300 m.a.s.l. Those areas having an altitude <1500 m.a.s.l. are classified as lowlands and include the Rift Valley, the southeast, southern and western

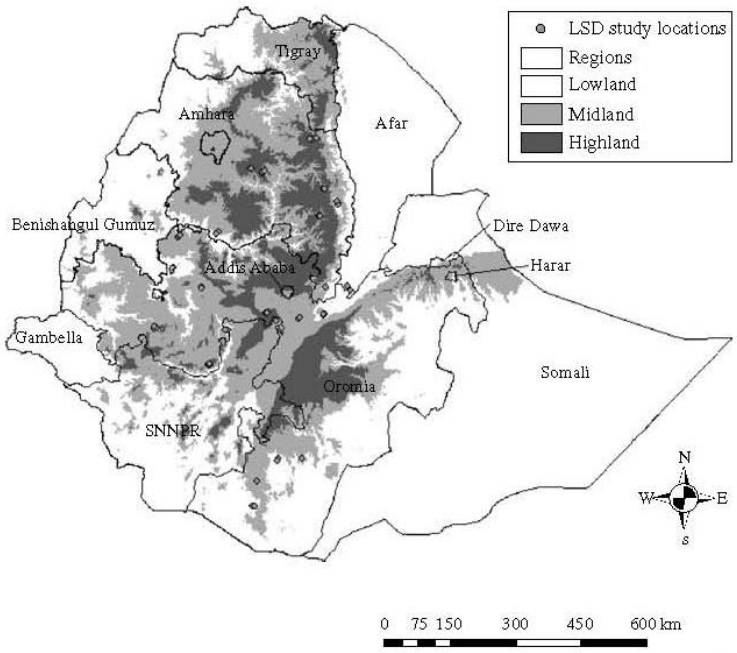

Fig. 1. Ethiopian topography and regional administrative divisions and lumpy skin disease (LSD) study sites.

border lands. The daytime temperature in the lowlands varies from 30 °C to as high as 50 °C in Denakil depression [24]. The rainfall has two major seasons: a long rainy season that occurs between mid-June to mid-September representing around 75% of the annual rainfall and a short rainy season from mid-February to end of April. In general, relative humidity and rainfall decrease from south to north and are always meagre in the eastern and south-eastern lowlands ranging from 50 to 300 mm per year [25]. The agricultural production system is mainly a sedentary crop–livestock production system in midland and highland altitudes whereas in most lowland parts semi-pastoral and pastoral production systems are dominant (herd-owners move their animals seasonally in search of feed and water, sometimes over long distances) [18].

Sampling technique and data collection

A cross-sectional study based on the administration of questionnaires to 330 herd-owners was conducted from April 2007 to July 2008 in 44 PAs distributed in 15 districts which were selected from 4/11 regions [Amhara, Oromia, Southern Nations, Nationalities and People region (SNNPR), Afar] as illustrated on Figure 1 and Table 1. The selection of the 44 PAs was performed using a multi-stage sampling strategy with four hierarchical stages. Regions followed by districts were purposively selected to include the main agro-climatic variations and different farming systems. At the third level, the selection of three PAs from each district was based on geographical representation and accessibility in consultation with district experts. The sample frame for PA was obtained from respective district agricultural offices. Finally individual herd-owners and their respective herds were selected for interview based on willingness to participate in the study. The questionnaire was administered by the first author during face-to-face interviews with the farmers using the local language.

In addition to the data produced from the questionnaires, data relating to LSD occurrence in the study area and countrywide for years 2000–2007 were obtained from district agricultural office documentation and from the national disease outbreak report database. Annual rainfall and temperature data for the same period were obtained from FAO Cropwat (CLIMWAT-database) [26].

Questionnaire

The questionnaire was designed based on previous knowledge of the disease. Sixteen questions were structured under three main sections. The section on 'The history of LSD occurrence' included questions

Table 1. *Administrative hierarchy of study locations, official reported and non-reported lumpy skin disease outbreaks in the selected districts and number of PA affected for years 2000 2007*

Region/district	No. of PAs in district	Selected PAs	Agro-climate	Number of PAs affected in the year							
				2000	2001	2002	2003	2004	2005	2006	2007
Amhara											
Laygayint	29	3	H		5						
Farta	28	1	H	5							2
Gozamen	35	3	H	1	5						1
Kobo	34	3	H, L							5	
Oromia											
Adola	26	3	M					1	14	3	
Yabello	23	3	L, M		1	3	13	8	6	4	1
Sebeta-Awas	42	3	L, M		1		1	2	1	1	2
Bako-Tibe	32	3	L, M		2	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Amuru	21	3	L, M			n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Chora	32	3	L, M				10			n.r.	
Sokoru	38	3	L, M							n.r.	
Ade'a	27	3	M				n.r.				
Fentale	18	3	L				3				
SNNP											
Kabiena	23	3	M							1	
Afar											
Awash-Fentale	6	4	L			1					
Total	15	44									

H, Highland; L, Lowland; M, Midland; n.r., Non-reported outbreaks; PA, peasant association; SNNPR, Southern Nations, Nationalities and People region.
Source: MoARD, Veterinary Service Department and respective district agricultural offices.

about year and month of LSD occurrence, number, sex and age of affected and/or animals that had subsequently died. The farmer's ability to identify LSD infection was cross-checked by enquiring about the clinical signs and local vernacular name for LSD. Sometimes the description of the disease was necessary in order to avoid confusion with other possible skin diseases such as bovine herpes mammilitis, dermatophilosis, demodecosis, and ringworm. Respondents describing the occurrence of a case with a clinical sign of generalized skin nodules, fever, peripheral lymph nodes swelling and discharge from eyes, nostrils and mouth were tentatively diagnosed as LSD by the authors. Moreover, epidemiological records from district public veterinary clinics were consulted to verify the occurrence of LSD in the time and place specified by the respondents. Recorded evidences of LSD occurrence both at district and central government levels were fully based on clinical disease observation reports.

The 'Herd management' section included questions about sedentary/transhumant farming system, herd size, vaccination against LSD, grazing/watering point management, contact with sheep and goats and introduction of new animals. Under the section entitled 'Biting flies of cattle', a question relating to the months during which the activity of biting flies was at its highest was recorded. In addition to the information obtained from the herd-owners, we recorded administrative hierarchies (region, districts, PAs) and their agro-climate classification for each questionnaire. 'Herd' was defined as a collective group of cattle from a single farmer ownership or family members. When at least one animal in a herd was reported as having had the infection in the past, the entire herd was considered infected. All the data was entered and stored electronically in Microsoft Excel XP 2003.

Data analysis

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

explored to investigate the disease distribution pattern in the country and LSD occurrence history in the selected study districts [20].

Descriptive statistics of the studied variables were obtained. Herd-level and animal-level prevalence, as well as the frequencies associated with four binomial variables (farming system, grazing and watering point management, introduction of new animals, and contact with sheep and goats) were estimated for each of the three agro-climate zones using back-transformed point estimates and confidence intervals from logistic regressions with agro-climate as a single explanatory variable. Statistical significance of the variation in agro-climate zones was assessed using homogeneity χ^2 test for these prevalences and frequencies.

Spearman rank correlation tests were used to assess the temporal association between LSD occurrence and increase in the biting-fly population. For these correlation tests, the different months were considered as different statistical units. The number of respondents in each month for which the month was designated as a time when LSD occurred was considered as one variable. The other variable considered for the correlation test was the number of respondents for which this month was designated as a time when biting-fly density was increasing. One distinct test was performed for each type of agro-climatic zone.

Analysis to identify the risk factors of LSD considered the following potential risk factors: agro-ecological category, type of farming system, type of grazing and watering point management, introduction of new animals, herd size, and contact with sheep and goats. The age composition of the population at risk (i.e. the herd) at the time when LSD infected the herd could not be obtained from the farmers because the farmer could not recall this quite complex piece of information. It is therefore difficult to assess whether or not age is a risk factor for LSD from these data. Each of these factors was first tested for its association with LSD occurrence at herd level by means of χ^2 association test for categorical variables and Kruskal Wallis rank test for count variable. The factors that turned out to be associated with LSD occurrence with a P value $<0{\cdot}20$ were shortlisted. All the factors shortlisted were included in a multiple logistic regression model. This model was then reduced step-wise by removing the factors with a P value $>0{\cdot}05$. Finally, the effects of pair-wise interactions between all factors retained in the final model were tested [27 29]. Confounding was considered present if the coefficients of a variable in the final model changed by $>50\%$ compared to its value in univariate regression [27]. Odds ratio estimates were computed from the coefficients of the final multiple logistic regression model using an exponential transformation. The goodness-of-fit (GOF) test for all models was assessed using Pearson's χ^2 GOF test for logistic regression.

Statistical analyses were conducted using Stata 8.0 (Stata Corp., USA, 1984 2003) and R 2.8.1 (R Development Core Team, Austria, 2008).

RESULTS

Description of LSD occurrence report data

Data investigations from the national disease outbreak report database and documentation of the respective district veterinary services showed that all selected districts had experienced at least one LSD outbreak during the period 2000 2007. A major epidemic outbreak of LSD occurred in 2000/2001 in the northern parts of the country in Amhara and West Oromia. Then it extended to the central and the southern parts of the country in 2003/2004 covering large parts of Oromia and SNNP regions. In 2006/2007 another extensive outbreak reappeared in Tigray, Amhara and Benishangul regions in the northern and north-western parts of the country. The frequency of occurrence of LSD was extremely variable across the study districts. Some districts reported outbreaks almost every year of the period considered (Yabello, Sebeta Awas), whereas others faced a limited number of outbreaks as shown in 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 selected study districts (Adola and Yabello districts) in the years 2003 2005; other districts (Amuru and Bako-Tibe) experienced small foci of cases involving few animals. An outbreak record discrepancy between national disease outbreak data and district documentation report was noted in five districts (Bako-Tibe, Amuru, Chora, Sokoru, Ade'a) for which the cases had not been declared at national level although LSD had been reported at district level involving a different number of PAs.

Variation of LSD occurrence and its potential risk factors in agro-climatic zones

Of the 330 questionnaires administered in 44 PAs, 103 were collected from highlands, 165 from midlands

Table 2. *Descriptive statistics of potential risk factors assessed by the questionnaire survey*

No.	Events recorded	Highland	Midland	Lowland	Pooled
I	Agro-ecology and herd management				
	• Farming system, transhumant (%)***	1·9 (0·5–7·4)	5·5 (2·8–10·2)	51·6 (39·3–63·7)	13 (9·8–17·1)
	• Grazing/watering resource management, communal (%)**	81·5 (72·9–87·9)	94·5 (89·8–97·1)	80·6 (68·9–88·7)	87·9 (83·9–91)
	• New cattle introduction (yes) (%)**	25·2 (17·8–34·5)	40·6 (33·4–48·3)	21 (12·6–32·8)	32·1 (27·3–37·3)
	• Contact with sheep and goats (yes) (%)	39·8 (30·4–49·26)	38·8 (31·4–46·2)	22·6 (12·2–33·0)	36·1 (31–41·24)
II	Lumpy skin disease (LSD) status				
	• Total herds investigated	103	165	62	330
	• Total head of cattle declared	533	2478	1427	4438
	• Number of infected herds	23	91	27	141
	• Total head of cattle declared as affected	46	258	55	359
	• Apparent LSD prevalence at animal level (%)***	8·6 (6·5–11·3)	10·4 (9·3–11·7)	3·8 (3·0–5·0)	8·1 (7·3–8·9)
	• LSD prevalence at herd level (%)***	22·3 (15·3–31·4)	55·2 (47·5–62·6)	43·5 (31·9–56)	42·8 (37·5–48·3)
	• Estimated prevalence in infected herds (%)	31·7 (24·7–39·7)	16·8 (15–18·8)	9·4 (7·3–12·1)	15·9 (14·4–17·4)
	• Apparent mortality due to LSD (%)***	0·19 (0·0–1·3)	3·2 (2·6–4)	0·91 (0·5–1·6)	2·12 (1·7–2·6)

Values in parentheses are 95% confidence intervals.
***$P = 0·001$, ** $P = 0·01$.

and 62 from lowlands. Different vernacular names of LSD were recorded from different sampling locations, e.g. Suki, Kodhobo, Guribrib, Gifir.

Farmers in highland and midland agro-climate zones described their farming system as sedentary. In the lowland agro-climate, about 51·6% of herd-owners reported exercising a transhumant mode of life (Table 2). Communal grazing and watering point resource utilization was dominant in all highland, midland and lowland agro-climate zones (81·5%, 94·5% and 80·6%, respectively) and significantly higher in midland agro-climate zone.

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. 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).

Of the interviewees 42·8% reported occurrence of LSD in their herd. This proportion was higher in the midland zone (55·2%) than in the highland and the lowland zones (22·3% and 43·5%, respectively). Out of the total 4438 animals that farmers had owned, 8·1% were declared as having had LSD (observed prevalence) and 2·12% as having subsequently died (observed mortality). Observed prevalence and observed mortality were significantly higher in the midland zone (10·4% and 3·2%, respectively) than in lowland and highland zones ($P < 0·05$) (Table 2).

The temporal association between LSD occurrence and increase in the biting-fly population was positive and significant (Spearman rank correlation coefficient was 0·88, 0·79 and 0·79 for highland, midland and lowland zones, respectively, and corresponding P values were 0·0001, 0·002 and 0·002). The time at which the biting-fly population begins to build up and reach its highest peak size was found to follow a similar pattern to the temporal pattern of LSD occurrence as shown on Figure 2. Both biting-fly activity and disease outbreak frequencies begin to increase from April reaching a maximum in September.

Logistic regression analysis

The final multiple regression model included effects of agro-climate, type of grazing/watering management and introduction of new animals as shown in Table 3. Communal grazing/watering point utilization increased the risk of LSD compared to privately owned resources [odds ratio (OR) 4·1]. Herds with new cattle introduced were also more likely to be infected by LSD than herds where no new animals had been added (OR 8·5). Agro-climate was significantly

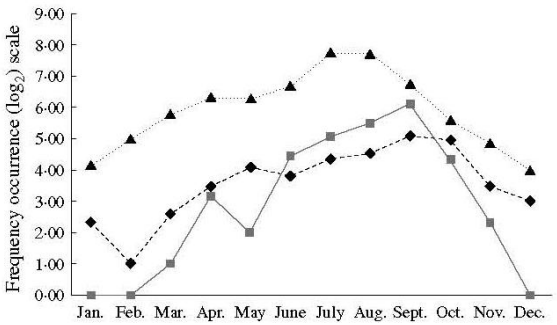

Fig. 2. Questionnaire survey results of seasonal increase in biting-fly activity *vs.* lumpy skin disease (LSD) occurrence. Data source for rainfall data: FAO Cropwat database. ···▲···, Average rainfall; --◆--, LSD outbreak; —■—, fly population.

associated with LSD occurrence and the odds ratio in the final model for midland vs. highland and lowland vs. highland were 3·86 and 4·85, respectively. After having accounted for the effect of the two other variables included in the final model (grazing/watering management and introduction of new animals), the occurrence of LSD was much more likely in midland and lowland than in highland zones. Farming system, herd size and contact with sheep and goats were not significantly associated with LSD occurrence by both univariate tests and multivariable logistic regression model. No interaction between variables was observed. With a Pearson χ^2 GOF test for logistic regression the P value was 0·55 which meant that the final model fitted the data adequately.

DISCUSSION

Immunity to LSD infection is predominantly driven by a cell-mediated response. Humoral immunity remains detectable only for 7 months [6, 30]. Serological studies with vaccinated cattle have shown that many animals resist challenge with virulent LSDV while they have no detectable antibody [4]. Thus although LSD surveillance based on serological results would probably not have shown the long-standing infections and the true immunological status of the target population [7], the current questionnaire survey results have allowed us to evaluate at least 3 years of retrospective disease information in the studied areas. The retrospective questionnaires were limited to the 3-year period because of the possibility of memory loss regarding numerical estimates of reported facts by the farmers, thereby reducing potential recall bias beyond that time period.

The study represents the majority crop livestock production system in the highland and midland agro-climates and has also included classical areas of semi-pastoral production system in the lowlands. Probabilistic sampling is a challenging task in a country with an infrastructure such as Ethiopia, since large areas have to be covered which are not easily accessible. Moreover, sampling frames of lower administrative units are not often available at central level. Under these circumstances, multi-stage sampling is the preferred technique to limit selection bias since the decision is then made with closer insight to the target population [31]. Nevertheless, our study might still have some degree of selection bias influencing the results.

The study was based on the symptomatic disease identification experience of the herd-owners complemented by veterinary office epidemiological records at different levels. Endemically occurring skin diseases of cattle such as bovine herpes mammilitis, dermatophilosis, demodecosis, and ringworm were taken into consideration in the differential diagnosis but we cannot exclude the possibility of some degree of misclassification. In the same way confusion with pseudo-LSD cases might have occurred although its presence has not yet been confirmed in Ethiopia. The discrepancy on LSD outbreak between the selected study districts and the national level reports was presumed to be the combination of errors committed at each level of reporting from grassroots to national level. The data obtained from the selected districts were considered to be a more credible source of information than from the national level [32].

The magnitude and frequency of LSD occurrence varied across districts during the study period. The observed LSD prevalence at animal level found in this study was 8·1% which was close to the 10% reported by Davies [4] and Babiuk *et al.* [9] in endemic areas of Africa. Prevalence of LSD in southern Ethiopia was less than the estimate reported here. This difference might be attributable to the fact that the seroprevalence method could underestimate the prevalence of LSD [7]. However, the study conducted around Nekemt town reported a similar result and it was in the 95% confidence interval range of our finding [21]. Apparent mortality in the present study was 2·12% which agreed with the previous reports by Davies and Babiuk *et al.* [4, 9], whose results ranged between 1% and 3% and with the previous studies done in Ethiopia.

Table 3. *Multivariable logistic regression model analysis of potential risk factors for lumpy skin disease occurrence at herd level*

Explanatory variables	Variable categories β	OR	95% CI	*P* value
Agro-ecology				<0·001
	Highland *vs.* midland	3·86	2·61–5·11	
	Highland *vs.* lowland	4·85	2·59–7·1	
	Midland *vs.* lowland	1·26	0·74–1·78	
Grazing/watering	Communal *vs.* private	4·1	2·02–6·18	0·003
New cattle introduction	Yes/no	8·5	6·0–11·0	<0·001

OR, Odds ratio; CI, confidence interval.

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. This association might be attributed to the availability and abundance of effective mechanical vector insects. The temporal association between LSD occurrence and the biting-fly population found in our study suggested that mechanical vector insects might play a major role in the epidemiology of LSD, and agro-climate variation could be the basis for the type and abundance of speculated mechanical vector insects. 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 [33 35]. The use of insecticides to control biting flies is a rare practice in Ethiopia except for few areas in tsetse-infested zones where pour-on drugs are used.

Regarding the extensive livestock production system, communal grazing and watering point resource utilization was dominant in all agro-climate zones. Herd contact and mixing is likely to occur in communal grazing and watering points. However, in districts like Fentale where irrigation-fed agriculture has emerged as means of subsistence in some PAs, cattle were kept on private grazing plots which relatively lowered the percentage of communal grazing and watering resource management in lowland agro-climate zones (80·6%). Herd mixing is assumed to be less likely to occur in private grazing plots.

For the midland agro-climate, introduction of new cattle was associated with an increase in the risk of disease introduction to the herd, as already reported for infectious diseases such as tuberculosis and paratuberculosis [36, 37]. Communal grazing and watering point utilization were found to be significantly associated with LSD occurrence. 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 and enhance the virus transmission through the speculated mechanical vectors such as *Stomoxys* spp. and mosquitoes (*Aedes aegypti*) [10, 38, 39]. 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 [39, 40]. Contamination of the pasture and water could be considered as a potential risk in communal grazing and watering point utilization despite the fact that contagious transmission is considered to be inefficient route of transmission [4, 8, 30].

Cattle movement due to farming system was not significantly associated with LSD occurrence in contrast with the findings of Munyeme *et al.* and MacPherson [28, 41] who reported the transhumant system as a risk factor for infectious disease transmission. This could imply that cattle movement due to farming system might not have a significant role for the spread of LSD due to the inefficient contagious transmission nature of the LSDV [38].

Our study shows that LSD has been extensively circulating across diverse agro-climatic zones with large variations between districts that could be attributed to their respective agro-ecological zones and farming practices. Moreover, factors such as virus isolates, the breed of cattle, the immunological status of the population and the vector insects involved in the transmission should also be considered for the variations observed in our findings [4, 9, 12]. Further study is required to elucidate vector insects incriminated in the transmission of LSDV and their dynamics in different agro-ecologies. Hence it is likely that improved awareness by farmers and veterinary services on the potential disease transmission associated with shared use of grazing areas and watering

points as well as promotion of biosecurity consciousness in the management of the introduction of new animals may assist in the control and prevention of infectious diseases in herds in Ethiopia. Knowledge-driven risk maps could also be built based on better knowledge of risk factors associated with LSD and could help target disease surveillance and control activities. These findings are also important to direct future studies in other countries where LSD is an important livestock disease problem.

NOTE

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/hyg).

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DECLARATION OF INTEREST

None.

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Study on biting fly population density in three distinct agro-ecological Zones, in Ethiopia.

Three geographical sites Sebeta, Ada'a-Liben and Fentale districts which were assumed to represent the three agro-ecological zones: highland, midland and lowland respectively were selected as areas where to collect flies every month over a one year period (Table 2). The type of trap used was a modified Vavoua trap to adapt the collecting cone (similar to the NGU trap) which is widely used to catch riverine tsetse flies in western Ethiopia. This trap was also reported to catch *Stomoxys* flies effectively (Gilles *et al.*, 2007). In each study location, monthly three traps were deployed for two consecutive days and the fly count was done every 24 hours. The poles were greased to prevent ants climbing the pole to prey flies. Cow urine and acetone were mixed and put near the pole to attract flies.

Identification of the caught flies at the Genus level and fly count was undertaken for each trap and recorded separately for each day. The average monthly apparent fly density (flies/trap/day) was calculated from the pooled fly counts in the two days of trap deployment. (Total fly counts divided by 6 [N° of traps/day 3*2 days]).

Systematic species identification for the Genus Stomoxys was done for a small part of the specimens and three most distinct spp were identified: *S. calcitrans, S.niger niger* and *S. sitiens.* Other types of flies caught in the trap such as a lot of house flies, few *Haematobia* spp and *Tabanids* were not recorded.

The average monthly biting fly catch per trap was highest in midland agro-climate. Pair-wise correlation of biting fly population density per month compared between agro-climate zones showed that highland vs midland, highland vs lowland and midland vs lowland were 0.77, 0.95, 0.83 respectively and statistically significant ($p< 0.05$).

Months	Highland (Sebeta-Awas)	Midland (Ada'a-Liben)	Lowland (Fentale)	Average
January	17	11	85	38
February	16	60	107	61
March	3	1	1	2
April	10	2	2	5
May	16	2	1	6
June	92 *	139 *	3	78
July	294	260	54 *	203
August	1056	1582	1166	1268
September	332 ¤	1702 ¤	429 ¤	821
October	316	136#	196#	216
November	261	102	227	197
December	56	7	65	43
AVERAGE	206	334	195	245

* The regular rain of the season started to rain in the mid-June.

¤ The Rain stopped

Unseasonal rain affected the fly catch

Table 3: Summary of monthly Biting Fly density per trap per day Records (Flies/trap/day)

CHAPTER IV.

ARTICLE 3: Epidemiological aspects and Financial Impact of Lumpy Skin Disease in Ethiopia

Epidémiologie et impact économique de la Dermatose Nodulaire Contagieuse Bovine en Ethiopie.

Gari, G., Bonnet, P., Roger, F., Waret-Szkuta, A.

(Article soumis à Prev. Vet. Med.)

Résumé

La DNC bovine cause d'importantes pertes économiques dans les troupeaux infectés : réduction de la production de lait, de viande et de travail, retards de croissance, infertilité, avortements et parfois la mort de l'animal. De plus, des dommages sévères et irréversibles sur les cuirs sont observés notamment dans les races à peau fine (Prim'Holstein ou Jersey). L'objectif de cette étude était d'évaluer l'impact économique de la DNC dans cinq districts de la région de l'Oromia en enquêtant dans des troupeaux de zébus locaux et dans de petites unités laitières où des animaux croisés Holstein sont présents. Des données d'épidémiologie descriptive ont pu être obtenues. L'incidence annuelle cumulée était plus importante chez les animaux croisés Holstein (33,9%) que chez les zébus (13,4%) comme la mortalité annuelle (7,4% contre 1,25%). Chez les zébus locaux, l'incidence et la mortalité cumulées sur une année de la DNC étaient plus importantes chez les mâles que chez les femelles suggérant que le stress et la fatigue liés aux travaux des champs peuvent affaiblir les animaux. L'impact économique annuel a été calculé en additionnant les pertes de production dues à la morbidité et à la mortalité. Les coûts annuels engendrés par animal ont été évalués à 6.43 USD pour un zébu local et à 58 USD pour un animal croisé Holstein. Dans une seconde partie, le bénéfice financier d'une campagne annuelle de vaccination a été calculé : il permettrait de réduire de 40% et 58% les coûts de cette maladie chez les zébus locaux et les croisés Holstein respectivement. Cette étude donne les bases d'un meilleur contrôle de la DNC pour les éleveurs mais aussi pour les décideurs nationaux d'Ethiopie.

Epidemiological aspects and Financial Impact of Lumpy Skin Disease in Ethiopia

Gari, G., Bonnet, P., Roger, F., Waret-Szkuta, A.

(Article submitted to Prev. Vet. Med.)

Summary

LSD is one of cattle disease which causes high economic loss due to chronic debility in infected cattle, reduced milk production, poor growth, infertility, abortion and sometimes death of the animal. Moreover, severe and permanent damage can occur to hides, decreasing their commercial value. Fine-skinned breeds like Holstein Friesian (HF), and Jersey breeds have been reported susceptible to LSD infection (Weiss, 1968; Davies, 1991; OIE, 2008).

The objective of this study was to assess the financial impact of LSD in five selected districts in the Oromia Region of Ethiopia from the farmer's perspective. The financial loss impact in traditional mixed crop-livestock system rearing local zebu cattle was compared with that of small scale dairy production (SSDP) rearing HF/crossbred in the study area. The study was conducted from September to December 2009. A pre-tested questionnaire survey addressing the period of one year production cycle (August 2008 up to September 2009) was considered and 747 questionnaires were collected.

Descriptive epidemiological analysis was obtained from the questionnaire survey data. Annual cumulative incidence of LSD infection in HF/ crossbred and local zebu cattle were 33.93% (95% CI: 30.92-36.94) and 13.41% (95% CI: 12.6-14.25) respectively which were significantly different ($p<0.05$). Annual mortality was also significantly higher in HF/crossbred 7.43% (95% CI: 5.76-9.10) than in local zebu cattle 1.25% (95% CI: 0.98-1.52). This showed that HF/crossbred herds had significantly higher odds of getting infection than local zebu breed (OR=1.27; 95% CI: 1.04-1.55). In local zebu cattle the cumulative incidence of infection and mortality were significantly higher in males than in females with the odds ratio of (OR= 1.14;

95% CI: 1.06-1.23) probably associated with the risk of exposure to stress of fatigue and exhaust due to draft works which is assumed to increase susceptibility. Case fatality rate was significantly higher in HF/crossbred 21.91% (95% CI: 17.39- 26.43) than in local zebu cattle 9.32% (95% CI: 7.28-11.18).

Annual financial cost was calculated as the sum of the average production losses due to morbidity and mortality. The variables that accounted for financial cost estimation were milk loss, beef loss, traction power loss, and treatment and vaccination costs. Annual financial costs due to clinical LSD per head were estimated of 6.43 USD in local zebu and of 58 USD in HF/ crossbred cattle in infected herds.

Secondly, the study assessed the financial benefit of controlling LSD through a one year planned vaccination. Partial budget analysis was used to estimate the economic benefit of control interventions using an annual vaccination program in both management systems (Rushton *et al.*, 1999). The variables considered for the change in the farm outputs were milk production, beef production and draft work-output. The marginal rate of return (MRR) was calculated as a financial benefit indicator and it is a proportion of net benefit divided by total investment for the vaccination program. The MRR gained from the control intervention was estimated at 76 (7600%) and the net benefit per head was 3 USD and 33 USD in local zebu and HF/crossbreds cattle respectively. Thus annual vaccination enabled to reduce the financial costs due to LSD by 40% and 58% per head in local zebu and HF/crossbreds respectively. The analysis of the planned vaccination as compared to a non vaccination scenario for a one year time horizon have shown that the livestock producers would get substantial benefit not only from financial gain perspective but also to secure and maintain sustainable farm business. This high MRR reflected that the investment that incurred by the farmers for the annual vaccination was only the cost of

vaccine. The veterinary service delivery is mainly by public veterinary clinics in the rural Ethiopia. To our knowledge this study on the economic impact of LSD is of its first kind and could provide support primarily to the producers and but also to the policy-makers to rationally justify their decisions in order to promote the control of the disease in Ethiopia.

Epidemiological aspects and Financial Impact of Lumpy Skin Disease in Ethiopia

Gari, G[*1,2]., Bonnet, P[3]., Roger, F[2]., Waret-Szkuta, A[2]

[1]National Animal Health Diagnostic and Investigation Center, P O Box 04, Sebeta, Ethiopia.

[2]CIRAD-ES, AGIRs, TA C-22/E, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.

[3] CIRAD-ES, UR 18 SEPA, Campus International de Baillarguet, 34398 Montpellier Cedex, France.

*Corresponding Author: Getachew GARI,

Mail address: NAHDIC, P O Box 04, Sebeta, Ethiopia

Email: getachewgj@yahoo.com

Abstract

The study was conducted from September to December 2009 with the objective to assess the financial cost of clinical Lumpy Skin Disease (LSD) and the financial benefit of its control through annual vaccination in five selected districts in Oromia Region from the farmer's perspective. A pre-tested questionnaire survey addressing the period of one year production cycle was considered and 747 questionnaires were collected. Production losses in local zebu cattle were compared with that of Holstein Friesian (HF)/ crossbred cattle in the study area. Annual cumulative incidence of LSD infection in HF/ crossbred and local zebu cattle were 33.93% (95% CI: 30.92-36.94) and 13.41% (95% CI: 12.6-14.25) respectively which were significantly different ($p<0.05$). Annual mortality was also significantly higher in HF/crossbred 7.43% (95% CI: 5.76-9.10) than in local zebu cattle 1.25% (95% CI: 0.98-1.52). In local zebu the cumulative incidence of infection and mortality were significantly higher in males than in females probably associated with the risk of exposure to stress of fatigue and exhaust while engaged in draft works that might increase susceptibility. Case fatality rate was significantly higher in HF/crossbred (21.91% (95% CI: 17.39- 26.43)) than in local zebu 9.32% (95% CI: 7.28-11.18).

Annual financial cost was calculated as the sum of the average production losses due to morbidity and mortality. The variables accounted for financial cost estimation were milk loss, beef loss, traction power loss, and treatment and vaccination costs. Annual financial costs due to clinical LSD per head were 6.43 USD in local zebu and 58 USD in HF/ crossbred cattle in infected herds. Partial budget analysis was used to estimate the economic benefit of control intervention using an annual vaccination program in both farm managements. The marginal rate of return (MRR) gained from the control intervention was estimated at 76 (7600%) and the net

benefit per head was 3 USD and 33 USD in local zebu and HF/crossbred cattle respectively. Thus the annual vaccination enabled to reduce the financial costs due to LSD by 40% and 58% per head in local zebu and HF/crossbreds respectively. This result on the financial cost of LSD and the financial benefit of its control by annual vaccination provides an insight for the producers and the government to endeavour the control of the disease.

Key words: Cumulative incidence, financial cost, financial benefit, Holstein Friesian, Local Zebu, Lumpy skin disease

Introduction

Lumpy Skin Disease (LSD) is a severe pox viral infection of cattle caused by the genus *Capripoxvirus*. Morbidity and mortality vary considerably depending on the breed of cattle, the immunological status of the population, insect vectors involved in the mechanical transmission, and isolates of the virus. In endemic areas morbidity is usually around 10% and mortality ranges between 1 and 3% (Davies, 1991; Babiuk et al., 2008a). A recent cross-sectional study across different agro-ecological zones in Ethiopia showed an overall observed LSD animal-level prevalence of 8.1% and a mortality of 2.12% (Gari *et al.*, 2010).

LSD causes high economic losses due to chronic debility in infected cattle, reduced milk production, poor growth, infertility, abortion, and sometimes death. Moreover, severe and permanent damage can occur to hides, decreasing their commercial value. Fine-skinned breeds like Holstein Friesian (HF) and Jersey breeds have been reported susceptible to LSD infection (Davies, 1991; Barnard et al., 1994). Control of LSD in Ethiopia relies mainly on ring vaccination carried out at the onset of an LSD outbreak using the KSGP-0180 strain vaccine

produced by the National Veterinary Institute (NVI, Ethiopia). The vaccine protection lasts for a minimum of three years (Capstick and Coackley, 1961b).

In Ethiopia, the cattle population is estimated to be 41.5 million heads or 31.91 LU[1] (CSA, 2006). Approximately 80% of the cattle are found in the highlands (≥1500 meters above sea level (m.a.s.l.)) which are estimated to cover 40% of the country's land area. The remaining 20% of the cattle population is found in the lowlands (<1500 m.a.s.l.) characterized by low precipitation (mostly under 300mm per annum) and covering the rest 60% land area of the country (Nigussie, 1995). A majority of farmers (78%) operate traditional mixed crop-livestock production systems, the remainder engage exclusively in either crop or livestock production (19.6% and 2% respectively) (Tegegne, 1997). Draught power accounts for 60% of the value of products derived from cattle because of its substantial role in food production, and is one of the primary reasons for keeping cattle (Tegegne, 1997). In the highlands, 51% of the cattle are draught animals (CSA, 2006). Draught oxen are estimated to work an average of two months, or 60 working days, in Ethiopia compared to 10 months in India. The difference is due to the lengths of the cropping seasons in these two countries (Tegegne, 1997).

The objectives of this study were to assess the financial impact of LSD from the perspective of the producers in the study areas, and to compare the financial impact the disease had on traditional cattle production systems with that on small-scale dairy production (SSDP) systems. The study also assessed the financial benefit of controlling LSD through an annual vaccination program. To our knowledge, the study is the first of its kind and may serve to support primarily producers, as well as policy-makers, in decision making to control the disease in rural areas.

[1] *1 LU= 1 cow, 1 bull, 0.5 replacement heifer, 0.25 calves* is converted based on 1LU is equivalent to 250kg live weight of animal (Source: FAO, 2004)

Materials and Methods

Study site and sampling method

The Ethiopian administrative structure encompasses 9 regions and 2 city administrations that include about 546 districts. Each district is composed of a different number of *Kebeles* (the lowest administrative level in Ethiopia) (CSA, 2006). The study was conducted in six districts (Walmera, Ada'a-Barga, Yaya-Gulale, Liben-Chukala, Jimma-Arjo and Seka) in the highlands (1800-2700 m.a.s.l) of the Oromia region. The main cattle breeds raised in the study area are local zebu. HF/crossbreds constitute about 1-2% of the total population and are used for milk production and genetic improvement in cross breeding with indigenous cattle (CSA, 2006). The study districts were selected because of their proximity to Addis Ababa (40 to 450km from the city (Figure 1)) and based on LSD outbreak occurrence records from the year preceding the start of the study. Seka district was the site of the pilot study in which the study protocol and questionnaire were evaluated; the remaining five districts were the sites of the main body of research. The geographical location ensured that semi-intensive, peri-urban, SSDP based on HF/crossbred cattle would be included in the study alongside the traditional mixed crop-livestock production systems based on local zebu. In each district the *Kebeles* included in the study were selected in consultation with local agriculture office district experts based on LSD occurrence and accessibility. Individual herd owners were selected to be interviewed based on the occurrence of LSD in their herds and their willingness to participate in the study.

All districts had mixed crop-livestock production systems in which livestock production is highly integrated with crop production. Draft oxen power is used for the production of agricultural crops, animal manure for fertilizer, and milk and milk products for household consumption and sale. Farmers usually generate income by selling milk and milk products, fattened mature male

animals, and barren and culled females (Negassa and Jabbar, 2007). Market demand for beef during the months of December and January is relatively high. A study of milk and milk product consumption in the study area found that about 50% of the milk produced goes to household consumption with the remainder sold on markets either as raw milk or as milk-products like butter and cheese (Table 1) (CSA, 2006). Beef production mainly is for sale, with only 38% consumed by farm households (CSA, 2006). The off-take rate of cattle in the central highlands of Ethiopia was estimated at 8% (Aklilu et al., 2002; Negassa and Jabbar, 2007).

The average duration of lactation of 240 days for local zebu cows (with a range of 210-270 days) and of 305 days for HF/crossbreds (with a range of 305-408 days) were used as the average lactation lengths in this study (Goshu, 2005; CSA, 2006; Lobango, 2007). Based on these figures, the average milk off-take per lactation is estimated to be 323 liters (L) (range of 276-376 L) for local zebu cattle and 3694 L (range of 3473-3915 L) for HF/crossbred cattle (Goshu, 2005; CSA, 2006; Lobango, 2007).

Field data collection

The study was conducted from September to December 2009 by three interviewers using the local regional language. The questionnaire was designed and tested in a pilot district selected for this purpose. It was administered to individual herd owners, with a "herd" assumed to be a single, homogenous, free mixing cattle population. The time period selected for the financial study was one year (a full production cycle covering August 2008 to September 2009 was surveyed retrospectively). The farmer's ability to identify LSD infection was cross-checked by enquiring about the clinical signs of LSD. Each farmer who reported that LSD had infected his or her herd was asked to describe the clinical signs of the disease. Those who described generalized skin nodules, fever, peripheral lymph nodes swelling and discharge from eyes,

nostrils and mouth were included in the study, while those who described signs completely different from those of LSD were excluded. For those who mentioned clinical signs shared by diseases easily confused with LSD, such as dermatophilosis, demodecosis, bovine herpes mammilitis and ring worm infections, the interviewers reviewed with the farmers the clinical signs of LSD to verify that the respondent had understood the disease correctly. The selected farmers then were asked questions related to the composition of the herd, herd dynamics, the management system used, the number, age and sex of the animals that had been affected by LSD and subsequently died, if vaccination or any other treatment had been applied during/after the course of the disease, and the estimated production losses attributable to the disease. A total of 747 questionnaires were collected from 80 *Kebeles* distributed over the five selected districts.

Information such as the price of cattle and milk on the primary markets[2], the price of draft ox power service, and district and *Kebeles* cattle populations were completed and cross-checked in discussion with district experts of the agricultural offices and veterinary clinics. The production parameters of the study population without LSD, specific to selected study areas were obtained from CSA (2006) baseline data.

Data analysis

The following descriptive statistics of the epidemiological variables were obtained from the questionnaire data:

- annual clinical cumulative incidence and mortality rate of LSD (Thrusfield, 1995)
- case fatality (proportion of animals that died from LSD out of the clinical infected cases)
- clinical severity of the disease at the herd level (mild, severe or very severe)

[2] Primary markets are those markets closest to the producers or farmers and relatively small in size (Nigussie, G., 1995).

- LSD incidence and mortality among breed types (local zebu and HF/ crossbred cattle) and sex and age groups were determined

A general linear model was used to compare epidemiological variables between breed type, sex and age groups. Risk factors associated with breed type, sex and age group were estimated and the Odds ratio (OR) estimates were computed from the coefficients of the general linear model using an exponential transformation. The level of significance for statistical tests was set at 0.05. The Stata statistical package was used for analysis (Stata Corporation © 1984-2003).

Financial impact of the LSD outbreak at farm level

The annual financial loss following an LSD outbreak from the producers' perspective was calculated as the sum of the values of the annual production losses due to morbidity and mortality and the costs for treatment and vaccination as shown in Equation 1 (Bennett and IJpelaar, 2005; Kivaria et al., 2007). Treatment cost represents the expenses incurred by farmers for medications at the local public veterinary clinic where farmers bring their clinically sick animals for treatment. LSD vaccination is given free of charge at these clinics, which generally are used by the owners of zebu herds practicing a traditional mixed crop-cattle farming system. Ring vaccination, usually applied around focal areas, is used to control outbreaks and is provided free of charge to all farmers. On SSDP farms, animals usually are confined to the farm where they receive treatment and vaccination services from private practitioners with the additional service charges covered by the farm owners.

$$C = M_d + (B + M + W_{op}) + V + T$$ ---------- Equation 1

C: Total financial costs

M_d: Mortality losses

M: Milk production losses

V: Vaccination costs

B: Beef production losses

T: Treatment costs

W_{op}: Work output losses

with: $M_d = P*Q_i*U$ and: $T = P*I*I_t*U_{tv}$

$(M + W_{op} + B) = P*I*Q*U$ $V = P*I_v*U_{tv}$

P= Size of population at risk

I= annual cumulative incidence of disease as a proportion of the affected population

Q= quantity of disease losses (liter of milk, lost working days)

Q_i= proportion of disease losses (mortality rate, off-take rate)

U= unit value of lost output (USD/L of milk lost, USD/work output lost, USD/unit animal)

I_t= proportion of affected population treated

I_v= proportion of population vaccinated

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

The average quantity of milk production loss and the time duration that an LSD infected, lactating cow was subject to milk production loss were estimated in surviving lactating cows. The annual cumulative incidence of LSD in female animals and the number of lactating cows during the study period were obtained from the survey data. The percentage of milk production loss in the study groups was calculated from an average milk off-take per lactation without LSD in the local zebu and HF/crossbred cattle as given in Equation 2.

Percentage of production losses (%) = $\boxed{(Q/D)*I*100}$ ---------- Equation 2

Q= quantity of production lost (milk (L)/ lactation, draft output in days, off-take rates)

D= Parameters of the breed types without LSD (Milk off-take/ lactation, Annual draft output)

I= cumulative incidence of LSD

The annual beef production losses were estimated as the decrease in the off-take rate in the study groups. LSD incidence interferes with normal herd dynamics, causing a reduction of surplus in the case of mortality, or a reduction in finished stock for the market in affected herds because of long term morbidity that can lower weight gain. Thus, the annual off-take rate reduction that accounted for beef production loss was calculated as the decrease in the off-take rate of the study population caused by the cumulative incidence of LSD (Equation 1).

The valuation of the draft power loss depended on the point in the crop season that an ox fell sick and on the corresponding demand for draft power during that specific season. Thus, the draft work output loss in terms of days was taken into account on two levels, when demand for draft power was high and when it was low, with demand determined by the crop calendar prevailing at the onset of the disease. The current market price for traction service per day was used to value the opportunity work output loss regardless of whether the draught animal was used by its owner or had been hired out (McDermott *et al.*, 1999). Annual percentage of draft power loss was calculated as shown in Equation 2.

Production loss due to mortality was computed based on the weighted average price, determined for each breed, sex, and age group, of animals that had died of LSD. The expenditures incurred for treatment and vaccination associated with LSD incidence were recorded from individual herd

owners and cross-checked with the cost per unit price of the items in their respective district veterinary clinics. The empirical production loss estimation was based on market prices during the year 2009-2010. Market price records were obtained from the monthly agricultural product market assessment data collected from the primary markets by the district agricultural offices (Table 2). Average estimates of the cost of different variables were considered. Production losses involving infertility, hide damage and manure losses were not considered due to the time period selected for this study.

A sensitivity analysis was performed using regression coefficient in @Risk 5.0 (Palisade Corporation) implemented on the excel spreadsheet model and assigning triangular distributions to the variables as minimum, most likely and maximum values.

Partial budget analysis: financial benefit of LSD control

Partial budget analysis was used to estimate the financial benefit of control interventions in both the traditional mixed crop-livestock and SSDP farming systems. This method is used to assess change in costs and benefits resulting from small changes such as the use of a new technology on a livestock farm (Dijkhuizen et al., 1995; Rushton et al., 1999; Rushton, 2009). If the benefits exceed the costs, then the change would be advantageous for the system. However, the most important criterion of whether or not to adopt a technology is determined by the marginal rate of return (MRR) obtained from the change (Legesse et al., 2005; Evans, 2008; Renee O'Farrell, 2010). MRR measures the increase in net benefit (ΔNB) associated with each additional investment in a new technology. It is calculated as the net benefit (ΔNB) divided by the total cost that varies (ΔTCV) only by implementing the planned vaccination as shown in Equation 3:

$$MRR = \Delta NB/\Delta TCV \quad \text{----------Equation 3}$$

A planned, annual mass vaccination program was proposed to control LSD. The cattle populations in the five selected districts were the target populations (Table 3). The production parameters of the breed types without LSD were obtained from CSA (2006) baseline data. The variables estimated in the financial loss assessment from the study groups were applied for the partial budget analysis of the target population. The animal level prevalence of LSD in Ethiopia of 8.1% (95% CI: 7.3- 8.9) was used as the parameter of LSD occurrence in the study area for this analysis (Gari *et al*., 2010).

The farm outputs considered in the model were milk production, beef production and draft work-output. The variable costs were the cost of the planned mass vaccination with the assumption that traditional farmers would pay the financial cost of the vaccine to feel actively involved in the process. SSDP producers pay the cost of vaccine and the veterinary service charges. The opportunity labor cost that the herd owner would spend to vaccinate his/her animals was not taken into account because of the relatively cheap labor cost. The benefit of LSD control was calculated as the sum of the production output that would be saved from being lost as the result of the disease control in the target population and the treatment cost saved. The model was built in a Microsoft Excel® 2007 (Microsoft Corporation, USA) spreadsheet.

Results

Description of cattle production system

In the rural areas, farmers owned local zebu; their herds were in the majority composed of males (52%) used for draught power although milk and beef productions also were cited as being important for household consumption and as a source of income (Table 4). The livestock

production system commonly was defined as extensive with more than 94% of the herd owners declaring that they used communal grazing and watering resources. In and around the towns where most of the SSDP herds were located, a majority of the owners (97%) used private grazing or zero-grazing management systems. SSDP farms were engaged in a market-oriented milk production and usually kept high milk producing HF/crossbred cattle.

The clinical severity of LSD in the past year was assessed through the questionnaire. A majority of local zebu herd owners (95%) and HF/crossbred cattle herd owners (90%) declared that their herd had experienced either severe or very severe clinical LSD based on the intensity of the lesions induced or the number of animals infected in their herd. Vaccination coverage varied across the study districts; however, on average about 6% of the local zebu cattle and more than 65% of the HF/ crossbred cattle had been vaccinated before or during the onset of the disease.

The cumulative incidence and mortality rates computed for each breed type, sex and age group based on the survey results are shown in Table 5. In the local zebu population, the cumulative incidence of infection and mortality rate were significantly higher in males than in females ($p<0.05$). A comparison of the cumulative incidence between the breeds showed that HF/crossbred cattle had significantly higher cumulative incidence of LSD infection and mortality rate than local zebu cattle ($P<0.05$). The case fatality rate also was significantly higher in HF/crossbred cattle ($p<0.05$).

The risk factor of LSD infection between breeds showed that HF/crossbreds became more likely infected than the local zebu breed [OR=1.27 (95% CI: 1.03-1.55)] which was statistically significant ($P<0.05$). In local zebu cattle, the occurrence of LSD infection was more likely in

male animals than in females [OR= 1.14 (95% CI: 1.06-1.23)]. However, the OR of LSD infection between age groups was not significantly different in either breed.

Financial impact of an LSD outbreak at farm level

The average duration of milk production loss of lactating cows that were infected by and survived LSD was estimated to be 45 lactation-days for both the local zebu and HF/crossbred cattle; the duration of milk production loss varied with the severity of the disease. The average milk production loss in a lactating cow that survived was estimated to be 51 L (95% CI: 45.5-56.5) in local zebu and 312 L (95% CI: 271-354) in HF/crossbred per lactation. The percentages of milk production loss in lactating females in the two study groups were estimated to be 1.5% (95% CI: 1.34-1.66) and 3% (95% CI: 2.6-3.4) respectively per lactation in infected herds.

The annual beef production loss was estimated to be a 1.23% (95% CI: 1.13-1.32) and 6.22% (95% CI: 5.38-7.1) reduction in off-take rates for local zebu and HF/crossbred cattle respectively.

The average duration of draft power output loss was estimated to be 16 days not worked per year for draft ox that had been infected and survived LSD, and the percentage of work output lost per ox in the study group was estimated to be 4.53% (95% CI: 4.24-4.85). The costs of annual mortality, treatment and vaccination in the affected study group are presented in Table 6 and the market prices of products and services for each type of farming system in Table 2. The overall annual financial costs incurred due to LSD infection per head was respectively USD 6.43 (95% CI: 5-8) and USD 58 (95% CI: 42- 73) for local zebu and HF/crossbred infected herds (Table 6). The sensitivity analysis of cost estimation in traditional mixed crop-livestock systems showed that mortality loss, beef loss and work output losses were contributing to the variability in the

model in descending order. Similarly, the variables subject to high variation in SSDP were mortality loss, beef loss and milk loss in descending order (Figure 2).

Financial benefit of LSD control by vaccination

The annual milk production increase calculated as a net benefit was estimated to be 1.28% and 3% of the total milk off-take per lactation for traditional mixed crop-livestock and SSDP farming systems respectively. The annual beef production in terms of the off-take rate increase was estimated to be 0.95% and 6.22% for the traditional and SSDP systems respectively. In the traditional mixed farming systems, the increase in draft power output in terms of working days was estimated to be 2.26% of the total draft work output per year. The control intervention was assumed to save the cost of treating clinical LSD cases which would be a benefit for the farmers. The cost of the planned annual vaccination program was the cost of vaccine per animal (one dose) for both farming systems.

The MRR gained from the control intervention was calculated to be 76 (7600%) and the net benefit per head was estimated to be USD 3 for local zebu and USD 33 for HF/crossbreds respectively (Table 7). An annual vaccination to control LSD would reduce the financial costs due to the disease by 40% per head in local zebu and 58% in HF/crossbreds.

Discussion

This study is the first to our knowledge to address the financial impact of LSD infection at farm level. It was implemented in the central highlands of Ethiopia where mixed crop-livestock production is the predominant farming system. Although one may assume that endemic infectious diseases, endo-parasites, ecto-parasites, and lack of feed occur concurrently during an LSD outbreak in the study groups, this underlying population health status would have the same

effect on both LSD infected and non-infected sub-populations. We therefore assumed that these external factors would not significantly change our overall comparison results.

One constraint that we faced was that herd health and productivity records are not maintained by herd owners in the study areas. To overcome this, we selected districts which recently had experienced an LSD outbreak and individually interviewed farmers as to how the disease had affected their herds the previous year. To improve the validity of the information given by the farmers and to balance the potential recall bias common to every questionnaire survey, we cross-checked the responses and considered multiple sources of information. We focused on LSD infected herds and compared them with population parameters obtained from baseline data specific to the study area. The results obtained from the study addressed the target population in the study area yet they also eventually could serve as a basis for further economic study at national level.

The incidence of LSD in HF/crossbred cattle was found to be significantly higher than in local zebu. This was in agreement with other studies, and may be due to breed susceptibility (Davies, 1991; OIE, 2008). In local zebu cattle, male animals had higher cumulative incidence than females. This might be attributable to the stress factor of exhaustion and fatigue rather than to a biological reason. The majority of male animals were draft oxen used for heavy labor, which might contribute to an increase in susceptibility (Blood *et al.*, 1983). Another hypothesis is that draft oxen cannot protect themselves well from biting flies when harnessed in the yolk, and the beat scratches on their skin induced while plowing may attract biting flies potentially capable of transmitting LSD infection. However, further study would be necessary to determine the underpinning reasons for the susceptibility of male animals.

All age groups were invariably susceptible to LSD infection in our study, which is in agreement with previous reports (Woods, 1988; Barnard et al., 1994). In local zebu cattle the cumulative incidence of LSD infection among age-groups was significant; however, this was considered to be a confounding effect due to the high incidence observed in adult male animals (Table 4). The incidence of LSD infection in calves was similar to the other age groups in both breeds which is different from previous findings (Davies, 1991). Traditional calf management practices that segregate calves from the herd might have contributed to a decreased exposure risk of calves to the source of infection (Gari *et al.*, 2010). Moreover, calves in the endemic area could have obtained a certain protective passive immunity from their dam (Prozesky and Barnard, 1982; Barnard et al., 1994).

The financial loss impact between the two breeds showed that HF/crossbreds had far higher production losses in most parameters compared with local zebu cattle, the financial loss impact thus had a linear relationship with the incidence of the disease in each breed type. Milk production losses of up to 50% per lactation have been reported in infected herd (Woods, 1988). This shows that LSD infection is very important in high producing exotic breeds and deserves appropriate disease control measure (Davies, 1991; Babiuk et al., 2008a; OIE, 2008). Moreover, LSD also may hamper the development of small-scale dairy production in rural areas because farmers are likely to place a priority on ensuring against the loss of their animals due to disease susceptibility.

The reduced work output of draft oxen due to LSD was an important loss for the mixed crop-livestock farming system. It was estimated based on the daily market price of traction services. Because the Orthodox religion is practiced widely in the study area, religious holidays and Sundays were not included in lost work days when determining the duration of draft work output

losses. Morbidity of draught oxen leads to reduced crop production through a reduction in the area that can be cultivated and lowered yields due to inefficient land preparation and timing (McDermott *et al.*, 1999).

The estimations of the financial benefit of LSD control through planned annual vaccinations show that the net benefit per animal for HF/crossbred cattle was significantly higher than for local zebu, which could imply that LSD control interventions are more profitable in SSDP than traditional mixed farming systems. The high MRR of 76 (7600%) in the current analysis could signify that the investment in planned vaccination to control LSD would result in high benefits to farmers, which would in turn lead to an efficient allocation of resources (Legesse et al., 2005; Evans, 2008; Rushton, 2009). However, this high MRR percentage clearly reflected that the cost incurred by farmers for the annual vaccination was only that of the vaccine. Veterinary service delivery, is mainly done by public veterinary clinics in rural Ethiopia. The current study considered the financial cost and benefits from the farmer's perspective; however, in a second step, it would be interesting to address the issue from the perspective of the public sector to obtain the overall economic analysis of LSD control interventions and to assess the benefit for the society. Moreover the accuracy of the results could be fine-tuned by incorporating stochasticity.

Our economic model did not take into account other possible alternative interventions (such as biosecurity measures and insecticide application) that might reduce the risk of LSD infection and that could be compared with vaccination. Infertility losses, hide damage and manure losses were not quantified because of the time period of the study and further specific studies would be needed to determine them.

In conclusion, the financial cost-benefit analysis of a planned vaccination compared with non-vaccination for a one year period and from a farmer's perspective shows that livestock producers would substantially benefit from vaccination, not only financially but also by securing and maintaining a sustainable enterprise business. Vaccination as currently practiced does not achieve the herd immunity level needed to control the incidence of LSD outbreaks in traditional mixed crop-cattle farming systems. We therefore recommend that farmers in the study area improve their vaccination coverage. The efforts of all relevant stakeholders are vital to implement a planned vaccination program that takes into account seasonal biting fly dynamics and climate variations in each study district (Gari et al., 2010).

Conflict of Interest Statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Tables

		Study Area			Oromia Region			Ethiopia		
Items	%	HH Consump.	Sale	Other	HH Consump.	Sale	Other	HH Consump.	Sale	Other
Milk	100	50	3	47	54	7	39	48	5	47
Butter	100	57	37	6	60	36	4	60	36	4
Cheese	100	87	10	3	85	10	5	84	13	3
Beef	100	38	53	9	48	39	13	46	42	12

Source: CSA data, 2006.

HH Consump. = Household Consumption

Table 1: Percentage of Milk, Butter, Cheese and Beef utilization in study area, Oromia Region and national.

No	Parameters Estimated in USD	Local Zebu	Holstein Friesian/Crossbred
1	Weighted average cattle value	148(114-188)	353(242-454)
2	Traction value of an ox/day	2.12(1.82-2.42)	0
3	Draft power (DP) value/ox/year	127(109-145)	0
4	Treatment cost/unit	1.70(1.51-2.12)	2.49(2.27-2.72)
5	Vaccination cost/unit	0.04 (0.02-0.06)	0.31(0.29-0.34)
6	Beef price/kg	2.42(2.12-2.72)	2.42(2.12-2.72)
7	Milk price/lt	0.30(0.24-0.36)	0.30(0.24-0.36)

Note: Numbers in parenthesis are minimum and maximum limits

* 1 USD= 16.52 Eth. Birr (in September 2010)

Table 2: Estimated current market prices in year 2009/2010 (in USD)

No		Name of Districts					
	Sex, Age categories	Walmera	Ada'a Barga	Jimma Arjo	A.Liban chukala	Yaya Gulale	**Sum**
1	Male	44.2	51.8	25.9	90.8	35.8	**248.4**
1.1	Male calves (<1yr)	6.9	11.1	5.82	11.7	4.9	**40.4**
1.2	Bull (1-3yrs)	6.2	6.7	3.5	9.6	4.4	**30.44**
1.3	Male (3-10yrs)	31.03	34.0	16.6	69.5	26.44	**177.6**
1.4	Male (>10yrs)	44.3	55.9	29.3	69.9	31.62	**231.0**
2	Female	9.02	12.65	7.25	12.2	5.82	**46.93**
2.1	F. Calves (<1yr)	6.95	8.03	3.9	12.65	5.7	**37.22**
2.2	Heifers (1-3yrs)	28.3	35.2	18.15	45.1	20.1	**146.86**
2.3	Female (3-10yrs)	16.7	23.13	13.0	28.7	16.33	***97.8**
2.4	Female (>10 yrs)	25.7	29.1	16.0	60.16	22.5	**153.54**
3	Lactating cows	**88.45**	**107.7**	**55.2**	**160.7**	**67.4**	**#479.4**
4	Draft animal	44.2	51.8	25.9	90.8	35.8	**248.4**
	Total (1+2)	6.9	11.1	5.82	11.7	4.9	**40.4**

Source: CSA, 2006

*Among lactating cows, the number of Exotic/ crossbred are 2647.

#Among total cattle population, Exotic/crossbred animals are **8230**.

Table 3: Cattle population by sex and age groups in the study districts (in „000 USD)

No	Descriptions	Local Zebu	H.F and crossbreds	Sum
1	Number of Herds Investigated	644	103	**747**
2	Total head of cattle	6399	955	**7354**
3	Average Herd size	10	9	10
a	Male cattle	3352 (52%)	159 (17%)	**3511**
b	Female Cattle	3047 (48%)	796 (83%)	**3843**
c	Calves	1215	234	**1449**
d	Bull	708	42	**750**
e	Heifers	710	196	**906**
f	Lactating cow	974	370	**1344**
g	Dry cow	724	105	**829**
h	Draft oxen	2068	8	**2076**

- The number in the bracket shows the percentage of male and female holdings of farmers.

Table 4: Description of the study groups by sex, age and type of breed based on questionnaire results

Description of events	Local Zebu		Holstein Friesian/ crossbred		p-value
Cumulative Incidence (CuI) in affected study group (%)	n=858	13.41(12.6-14.25)	n=324	33.93(30.92-36.94)	**0.00**
CuI in Male animals (%)	n=569	16.97 (15.7-18.24)	n=41	25.79(18.94-32.64)	**0.01**
CuI in Female animals (%)	n=289	9.48 (8.44-10.52)	n=283	35.55(32.22-38.88)	**0.00**
Age Category	p<0.05		p=0.68		
CuI in Calf (%)	n=25	2.06(1.26-2.86)	n=52	22.22(16.87-27.57)	
CuI in Bull/Heifer (%)	n=114	8.04(6.62-9.46)	n=113	47.48(41.10-53.86)	
CuI in Adult (%)	n=719	19.09(17.8-20.35)	n=159	32.92(28.72-37.12)	
Mortality rate in affected study group (%)	n=80	1.25(0.98-1.52)	n=71	7.43(5.76-9.10)	**0.00**
Mortality in Male (%)	n=53	1.58(1.16-2.00)	n=13	8.18(3.89-12.47)	**0.00**
Mortality in Female (%)	n=27	0.89(0.56-1.22)	n=58	7.29(5.48-9.10)	**0.00**
Mortality in Age Category	p=0.15		p=0.46		
Mortality in Calf (%)	n=9	0.74(0.26-1.22)	n=19	8.12(4.60-11.64)	
Mortality in Bull/Heifer (%)	n=8	0.56(0.17-0.95)	n=22	9.24(5.54-12.94)	
Mortality in Adult (%)	n=63	1.67(1.26-2.08)	n=30	6.21(4.05-8.37)	
Case fatality rate (%)		9.32 (7.28-11.18)		21.9(17.39-26.43)	**0.00**
Off take rate (%)	**n=585**	**9.14 (8.43- 9.85)**	**n=175**	**18.3 (15.9- 20.8)**	**0.003**

Note: Numbers in parenthesis are 95% confidence interval

Table 5: Cumulative incidence and mortality rate of LSD infection in the affected study group and comparison between the cattle breeds, sex and age groups.

Variables for Financial costs	Av. Prodn	Value in	Max	Min
Local Zebu cattle	losses (Q)	(USD)	(USD)	(USD)
1. Estimated milk losses	4711 lt	1.43	1.71	1.14
2. Annual Mortality Losses	80(1.25%)	14.70	18.90	11.17
3. Total work output losses	5632 days	11.94	13.65	10.24
4. Annual off-take reduction	1.23%	11.64	14.82	8.95
5. Total Treated animals & costs	841animals	1.43	1.78	1.27
6. Vaccination coverage & costs	6%	0	0	0
Total costs in Zebu cattle		**41.13**	**50.87**	**32.77**
Annual cost per head for Zebu		**0.00643**	**0.00795**	**0.00512**
Annual Financial costs				
Holstein F./crossbreds				
1. Estimated milk losses	41068lt	12.43	14.92	9.94
2. Annual Mortality losses	71(7.43%)	23.14	29.72	16.11
3. Annual Off-take reduction	6.22%	20.98	26.96	14.38
4. Total Treatment cost	312animals	0.79	0.85	0.71
5. Vaccination coverage and cost	65%	0.20	0.21	0.18
Total costs in H. F/crossbreds		**57.53**	**72.66**	**41.33**
Annual cost per head for H.F.		**0.058**	**0.073**	**0.042**
Grand total (L.Zebu+HF)		**98.66**	**123.52**	**74.09**

Exchange rate of 1USD= 16.52 Ethiopian birr

Av. Prodn losses (Q)= Average quantity of production losses

Table 6: Estimated annual LSD financial costs in the investigated herds for both local zebu and Holstein Friesian/ crossbred cattle (in „000 USD).

No	Parameters	Farm management systems		
I	**New costs**	**Extensive Husbandry**	**SSDP**	Sum
	Vaccination cost	17.41	2.59	
II	**Revenue forgone**			
	Opportunity Labor cost	0	0	
	Sub-total costs (I+II)	17.41	2.59	**20.00**
III	**New Revenue**			
	1. Milk production increase	119.16	88.78	
	2. Beef production Increase	673.72	180.88	
	3. Draft power output increase	441.54	0	
IV	**Cost Saved**			
	Treatment cost	23.04	6.66	
	Sub-total benefit (III+IV)	1257.46	276.32	**1533.78**
	Net benefit per head	0.003	0.033	
	Percentage of financial benefit from the control per head	**40%**	**58%**	

Net benefit = **1533.78 – 20.00= 1513.78 USD**

Marginal rate of Return (MRR)= **7600%**

Table 7: Financial benefit of LSD control through planned vaccination in five districts using partial budgeting model (in 000'USD).

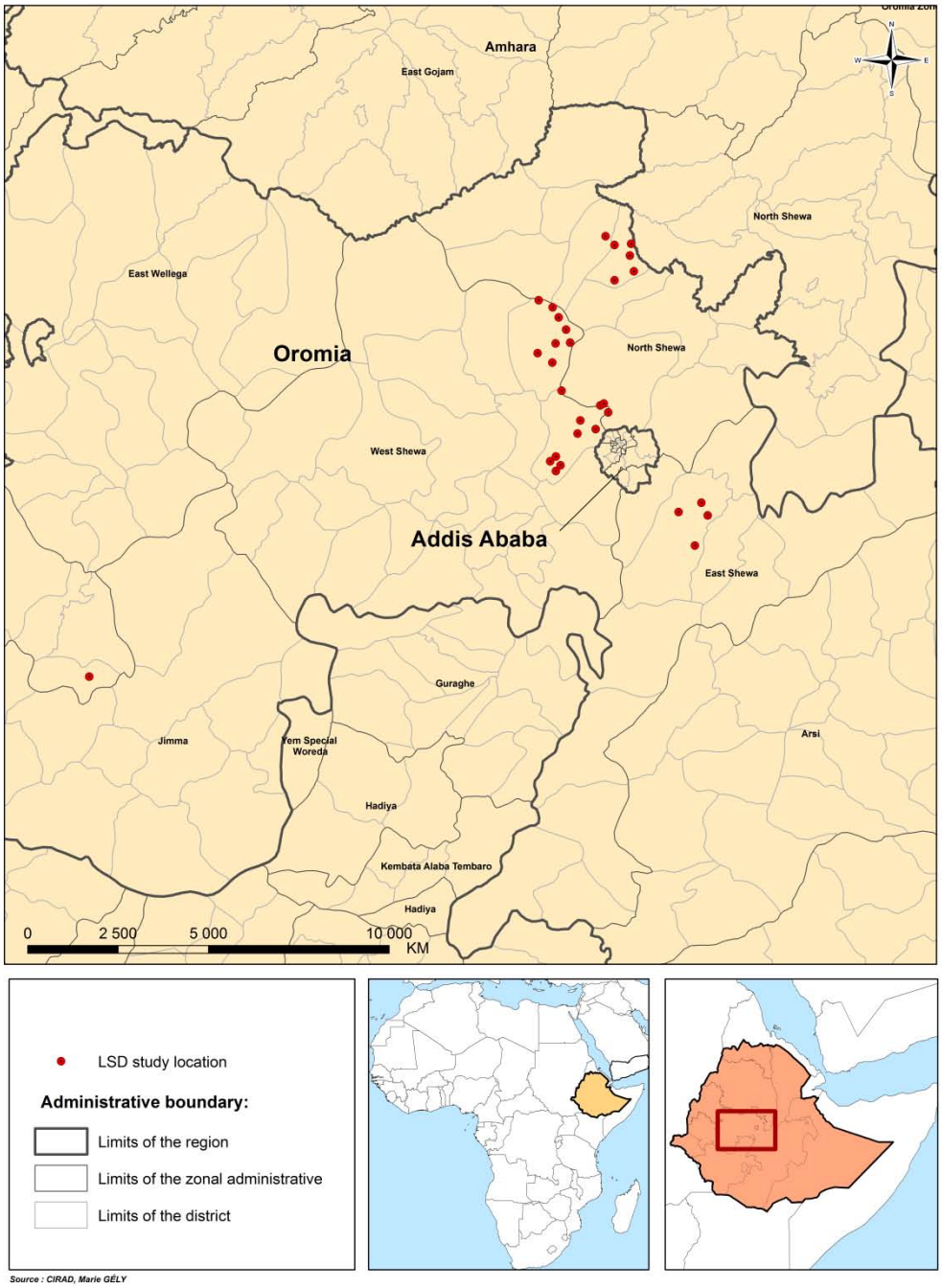


Figure 1: Study location of the five districts in Oromia Region, Ethiopia.

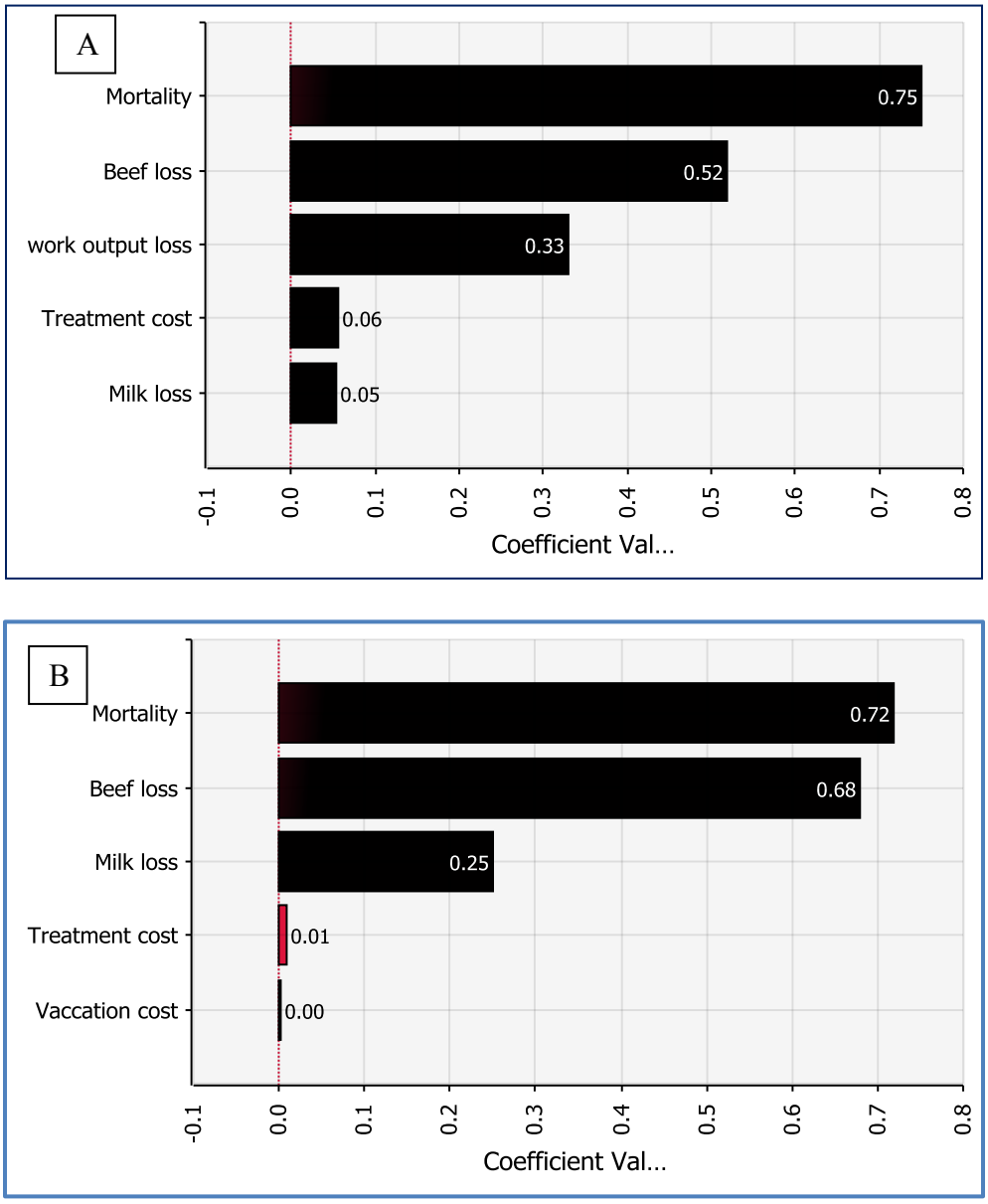


Figure 2: Sensitivity analysis of the financial cost estimates for Local Zebu cattle (A) and HF/crossbreds (B) using Regression coefficient

CHAPTER V.

General Discussion and Perspectives

General Discussion and Perspectives

Epidemiology of Lumpy skin disease in Ethiopia

The scope of the current epidemiological study of LSD in Ethiopia covers the mixed crop–livestock production system which is the most common and that prevails in the highland and midland agro-climates, and has also included classical areas of semi-pastoral production system found in the lowlands (Figure 13). The study has fulfilled substantial information gaps to our knowledge regarding the distribution and prevalence of LSD in Ethiopia. The observed prevalence of LSD at herd level and animal level was higher in midland agro-climate zone where potentially high livestock population and substantial agricultural activities are more concentrated. It is possible to imagine that LSD could play substantial role in decreasing production and productivity of cattle in this integrated system. In the highlands, estimated observed prevalence at the animal level was higher than in the lowlands. This was because the proportion of infected animals in the infected herds was much higher as compared to the proportion in the infected herd of the lowlands. The herd-size in highland zone was so small (Av. Herd size in highland ≈5; in Midland ≈ 15 and in Lowland ≈ 23) than midland and lowland zones which contributed to the inflation of prevalence at animal level. However, observed prevalence at herd level was higher in the lowlands than in the highland zone which was in agreement with the risk factor analysis results that showed higher odds ratio for midland and lowland zones. Thus, we considered the herd-level prevalence estimation would give more credible estimate of the disease distribution than animal-level prevalence for all agro-climates.

The passive surveillance through regular reports of disease outbreak for LSD has its own advantage to respond to the outbreak occurrences, to meet the basic requirements of OIE, to

locate where the disease occurs and also gives general information on the types of diseases occurring in the country if supported by Laboratory diagnosis. However, because of under-reporting and other technical shortcomings on these passive reports, an alternative approach that would enable to get more comprehensive and quality information should be designed through active surveillance (Cameron, 1999). Indeed, further comprehensive surveillance study would be required for LSD to propose effective control options, implement and monitor the control program.

The objective and importance of the surveillance study should be defined; the target population and surveillance stakeholders should be identified as a preliminary step to undertake surveillance. Then, geographical and agro-ecological representation of the surveillance study should be ensured in that the epidemiological results generated would give a comprehensive understanding on the distribution and patterns of the disease in the country. The finding in our current epidemiological study has a limitation to address a representative sampling to all regions in the country and especially to the remote lowland areas. Another pitfall in acquiring representative sampling was randomicity of the sampling process which is always a practical challenge in field conditions at the village level. The selection of regions and districts were purposive to include major agro-climate variations and farming systems with some considerations also to optimize the resources and time of the study. At the third level, three *Kebeles* were selected in consultation with district experts based on representativeness and accessibility of the *Kebeles*. Sampling of the herds and individual animal was dependent on the volunteer farmers mostly guided by the interest of *Kebeles* leaders which coordinate the villagers for the sampling process. The true random sampling approach might be influenced at different sampling strata which may cause selection bias on the final estimates. Thus, the future

surveillance study should strive to maintain the random sampling method during sampling strategy. Disease cluster variations might originate from the diverse agro-ecological patterns and the associated farming systems to be considered in the multi-stage sampling approach and should imply to consider design effects (Cameron, 1999; Dohoo et al., 2003).

Diagnostic tests are important tools for epidemiological studies in that they enable to detect and quantify non-clinical disease events in the population (Greiner and Gardner, 2000a). The results of IFAT and VNT performance test have shown that the accuracy of IFAT was good in both sensitivity and specificity parameters indicating that it can be used for epidemiological study of LSD with less misclassification. Moreover, the two tests IFAT and VNT were found conditionally independent on the disease status of the animal. This implies that if IFAT would be used for screening test, VNT can be used as a confirmation test because of its higher specificity performance. However, the drawback of these assays are that they could be run in well equipped virological laboratories and better accessibility to laboratory supplies which are not the situation in most parts of Africa where LSD is prevailing. Test precision and repeatability could also vary from one laboratory to another which could still affect the test results of epidemiological study. Thus, the development of standardized, easily run and lower cost diagnostic assay such as an ELISA test would be essential for epidemiological study of LSD.

Our study approach based on clinical disease observation has been efficiently used to estimate important epidemiological parameters and could be applied if not for all diseases but at least for those which manifest pathognomonic clinical signs like LSD. Nevertheless, this preliminary information obtained on the prevalence of LSD should be further ascertained by sero-prevalence results. The sero-prevalence study was designed to analyze the prevalence distribution of LSD at herd and animal level. The result was also intended to be used for further

analytical studies like analysis of cluster variations among different sampling strata. The serum samples have been tested by IFAT and VNT. However, the analysis result could not be incorporated here because of time limitation.

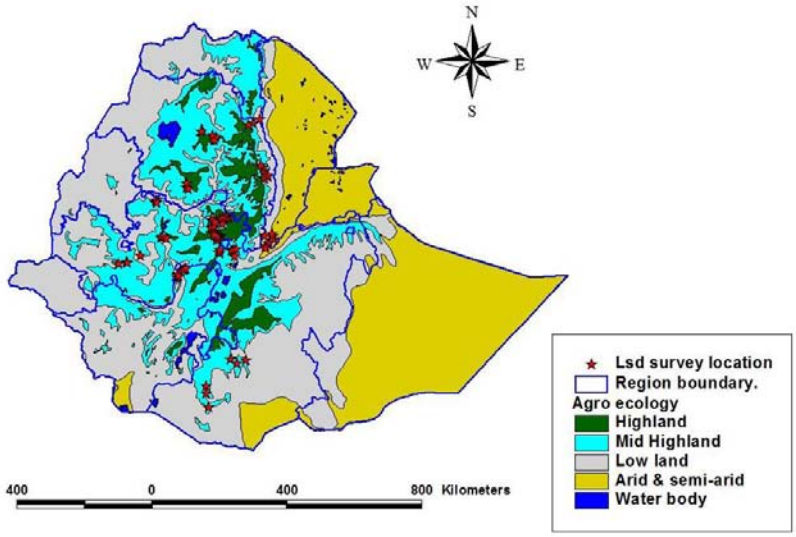


Figure 13: LSD study locations and major agro-ecological zones in Ethiopia

Risk factors associated to LSD occurrences

LSD infection causes a variable degree of clinical and pathological outcomes. The observations in the different epizootic occurrences of the disease have shown that multifaceted phenomenon dictates the pathogenicity of the virus (Woods, 1988; Davies, 1991; Barnard et al., 1994; Lefèvre and Gourreau, 2010). LSDV was also reported to exhibit different clinical response following an experimental infection and some infected cattle did not show clinical disease post infection (Carn and Kitching, 1995). This characteristic nature of the virus is particularly important in the epidemiology of the disease which allowed to exhibit quite different spreading features a combination of very slow spread and sudden extensive epidemics (Woods, 1988). On the contrary SPV and GPV showed almost uniform responses in experimentally infected animals (Babiuk et al., 2008a). Further study might be required to better understand the

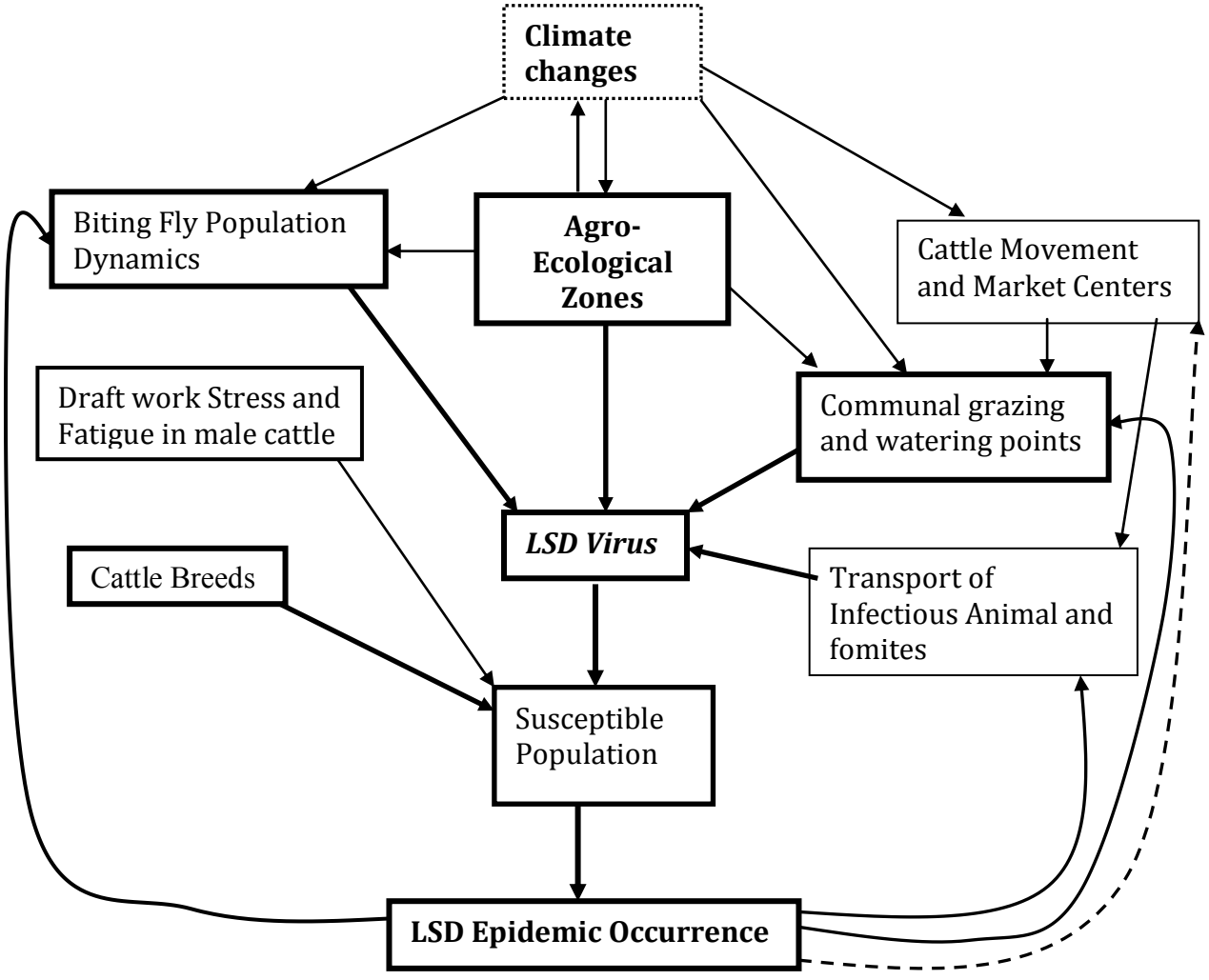
genetic characteristics of LSD virus which plays role in the variability of its pathogenecity in the future.

Important risk factors associated to LSD occurrence have been determined using targeted study design to identify the risk factors. This information would also shade light for other African countries suffering from LSD. Cattle movements for trade purpose on foot, road, rail ways or other causes of movements should also play significant role in the spread and dissemination of the virus (Woods, 1988). In the current risk factor study, cattle movement due to transhumant farming system was not associated with LSD occurrence. However, the small scale of our study in the lowland agro-ecology might need a cautious interpretation of the results and further studies addressing trade routes for example could help in a better understanding of the importance of cattle movements in the spread of the disease.

The epizootic occurrences so far observed in Ethiopia showed that LSD causes severe clinical disease in local zebu cattle as already reported for Sanga breed like Fogera cattle (Babiuk et al., 2008). However, further investigation might be required to understand susceptibility difference among local breeds in Ethiopia particularly for Shako breed which was reported to have resistance to trypanosomosis (Lemecha *et al.*, 2006).

The transmission of LSD by mechanical insect vectors has been unanimously reported by different authors. Erratic climate changes that could adversely affect the feed and water resource availability particularly in the semi-pastoral and pastoral areas would enforce the migration of people and livestock where these situations often accompanied with epidemic occurrences of infectious disease like LSD due to stress factors and increased risk of exposure. Excessive draft work stress was also found associated with susceptibility to LSD in draught male animals than female cattle. In general, taking in account the diverse agro-ecological zones and the climates

associated to each zone in Ethiopia, we developed a schematic model that represents the interactions between risk factors for the transmission and spread of LSD in Ethiopia (Figure 14). The risk factors included in the model were the most identifiable, measurable and significant ones. Five potential risk factors were described which would lead to the consequence of infection or disease events in endemic areas. These are transport of infectious cattle and fomite, biting fly populations, stress and fatigue, communal grazing and watering resources and availability of susceptible cattle populations.



Note: The Solid arrows shows the risk factors

The dash lines show the risk factors that are likely to occur but need further investigations

Figure 14: Interactions between the risk factors for LSD transmission and spread that possibly leading to the disease occurrence

Agro-ecological factors and emerging climatic changes were considered as the basis for modulating the other risk factors depending on the magnitude of their influence. For each branch of scenario, parameters involved in these risk pathways could be assigned for further qualitative risk assessment. The model would help to understand the risk factors and trigger some policy implications for risk mitigation. These policy issues are:

- The set up of regulatory policy for livestock movement control,
- Early warning and risk mitigation measures in the period of adverse climate particularly drought conditions which could predispose cattle to LSD epizootic occurrence,
- The herd owners should avoid herd mixing and contacts by using private grazing plots and watering sources,
- Implementation of quarantine system before new animals introduced to the herd and
- Implementation of planned vaccination program to increase the herd immunity level.

These control opinions should be complemented with economic feasibility investigations for the cost-effective way of controlling the disease at different scales and scenario which would certainly provide guidance for eventual knowledge-based decision process for the control of the disease or to mitigate the risks to an acceptable level.

Financial impact of LSD in infected herds and the benefit of its control

Livestock plays an important role in the livelihood of households and the impact of livestock disease on the economic wellbeing of the household could be more complex than the

financial losses apparently estimated. The multidimensional functions of cattle that contribute food, income, draft power, an asset value, and other social functions are the complex effects that may be difficult to easily enumerate (Rich and Perry, 2010). The current financial impact study for LSD is the first attempt to bring the important financial losses due to the disease into focus at the farmer's level. Comparison of annual production losses due to LSD between the breeds have shown that high producing HF/crossbred cattle had higher production losses due to high cumulative incidence and mortality rate than in local zebu cattle. The financial cost- benefit analysis of the planned vaccination as compared to a non vaccination scenario for a one year time horizon have shown that the livestock producers would get substantial benefit not only from financial gain perspective but also to secure and maintain sustainable enterprise business. This study considered the cost and benefits from farmer's perspective but the cost and benefit of LSD control from the public sectors perspective should be addressed in further study to get the overall economic analysis of control intervention for LSD. Moreover, specific losses such as infertility, hides and manure losses were not quantified in the current study because of the time horizon of the study. Thus further scientific investigations would be suggested to elucidate these losses in the future.

The accuracy of economic analysis in animal health depends on the availability and reliability of the underlying data. Even with the required data available, the quality of the data could be affected by complex factors: 1) can be influenced by other factors such as nutrition, superimposed other infections, 2) a temporal dimension of the disease impact was a complex factor in determining the losses at different stages in time, 3) production loss estimation was based on subjective approximation of the herd owners which might be subject to variability, 4) under natural condition, the severity of LSD infection could vary among individual animals and

herds because of various reasons and all these complex factors could contribute to the bias of the final estimates. 5) Another potential source of bias could be the differences among the 3 interviewers administered the questionnaire. However, before the start of the proper study, two other personnel involved in the data collection were trained and thoroughly discussed on the methodology. Questionnaire format and study design was tested and optimized in a pilot study. The interviewers took part in the testing of the questionnaire and their feedback was taken into account to reduce such biases (Thrusfield, 1995). Both closed and open questions were used to obtain the data.

Although the LSD vaccines (the Kenyan SGPV and Neethling strains) are widely used in the face of epizootic control in Africa, reports of vaccine breakdown, short duration of protection have emerged as a serious problem for efficient control of the disease (Hunter and Wallace, 2001; Kara *et al.*, 2003; Brenner *et al.*, 2009). In our observation in Ethiopia too, the problems of vaccine failure and re-infection of vaccinated animals has been getting higher magnitude particularly during epizootic occurrences in the year 2008/2009. This emanate problem should get due attention and we would suggest further research efforts should be done to evaluate the protective efficacy of currently used vaccines and to develop better immunogenic vaccines in the future. The availability of whole gene sequences now allows for the development of new vaccines by targeting genes specifically involved in virulence and host immunity system modulation. A virulent SPPV mutant with a deletion in one of its Kelch-like genes (SPPV-019) was markedly attenuated for virulence, demonstrating its potential future candidate vaccine under experiment (Balinsky et al., 2007).

Recommendations

Epidemiological trend of LSD suggests that there could be a potential risk of the disease spreading further into North Africa, into the Middle East countries and to Mediterranean regions because of the global climatic changes and trade movement in animals and animal products (Davies, 1991; Babiuk et al., 2008a). However, the epidemiological features of LSDV that it has a single serotype, no carrier state, a limited host range and vaccines are available would give a remarkable attributes for the prospect of successful implementation of regional control programs, leading to the eradication of the disease (Babiuk et al., 2008a). The eradication of sheeppox disease from Europe the latest before 1960 could give us a good lesson that CPV diseases could be eradicated from the rest of the world (Fassi-Fehri, 2010). To that effect it would envisage commitments of the governments to deploy coordinated control and prevention measures at regional and local government levels. The prevailing cross boundary cattle movements and lack of livestock movement control in the region could be a challenge that could lead to the re-invasion of the disease into the controlled area.

The main method to control LSD is through mass vaccination and control of the cattle movement. The existing vaccination coverage for LSD in Ethiopia is very low to control and prevent LSD occurrence and therefore the prophylactic vaccination scale should be improved to attain effective protection of the population. Indeed, LSD control in Ethiopia would envisage policy issues depending on the priority set of the government or global agenda for the livestock disease control. However, at least to reduce the incidence of the disease and the economic losses, we would recommend the control measure that considers a risk based approach to the agro-ecological zones and geographical locations where the control strategy could be more efficient and practical. The main reason is that the risk of LSD occurrence is more likely higher in the

midland and moist/humid lowlands than moisture stressed arid and semi-arid lowlands. Sedentary farming system in the highland, midland, humid lowlands and the Rift valley lowlands are the most affected zones with tremendous economic losses that should be focused for the control. The control measures should also extend to the peripheral lowlands to establish sufficient disease buffer zones depending on the capacity of implementation in these semi-pastoral and pastoral farming systems. A well coordinated effort should be deployed among the stakeholders at regional states and Federal government level for efficient control and prevent measures. We would suggest that further comprehensive surveillance of LSD is required to determine the scale and the scope of the control which could guide to consider different control options and strategies. It would also give an important benchmark to monitor and evaluate the control measures to ensure the achievement of the targeted control level.

The availability of efficient diagnostic tool is an essential prerequisite for effective surveillance and monitoring programs. Further research efforts are required for the development of a diagnostic assay that could have higher throughput, accuracy and can be easily run such as that of an ELISA kit for epidemiological studies of LSD.

One of the challenges for the effective control of LSD could be a lack of livestock movement control in the country particularly around the peripheries of the country which is porous for the cross boundary cattle movement. It could be a potential risk for the re-invasion of the disease into the controlled area. Thus we recommend policy considerations to establish livestock movement control which is part of livestock disease control strategy in many parts of the world.

The financial implication for implementation of the annual planned vaccination program has shown that it would be the best cost-effective method at least from the farmers' perspective.

Thus we would recommend the implementation of this control approach and efforts of farmers and all relevant stakeholders in the study area are vital to attain the intended benefit. The planned vaccination program should also take in to account the seasonal biting fly dynamics and climate variations tailored to each agro-ecology. However, the main concern of vaccine failure and breakdown of the immunity emerged in recent days could be another challenge for the effective control of the LSD. Thus, we would like to draw the attention of researchers and vaccine producing institutions to evaluate the protective efficiency and protection lifespan of currently used vaccines and avail better immunogenic vaccines in the future.

Till to date there is no hard evidence on the specific mechanical vector insects which play role in the mechanical transmission of LSD particularly during the epizootic occurrences. This would certainly pose a barrier to our epidemiological understanding of the evolution of the disease and to build targeted control measures. Further study is highly recommended to elucidate vector insects incriminated in the transmission of LSDV and their dynamics in different agro-ecologies.

Secondary infections could cause high complication to the healing process of LSD lesions. Hence antibiotic treatment at the early stage of the disease would facilitate the recovery of the affected animals and is highly recommended for high value animals.

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Appendices

Appendix I: Indirect Fluorescent Antibody Test Laboratory Protocol

I. 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 ($2*10^4$ cells/ml) to each 96 wells
3. Incubate the plates for 24hrs in 37°c and 5% CO_2
4. Prepare the KS1 virus dilution of $2*10^3$ $TCID_{50}$/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 suspension 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
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

II. Procedure to test the sera

1. Add 50 µl PBS- 0.01M to each well and keep for 30min at room temp.
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 ml 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: Anti-sheep 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.

Appendix II: Virus Neutralization Test Laboratory Protocol

- Test sera stored at -20°c were thawed and heated at +56°c for 30 minutes
- Take a record sheet and fill in the layout of the 96-plate according to the samples to be tested.
- Add in each well 100µl of cell culture medium (MEM+ 10%FCS+AB)
    - Add in wells A1&B1 25 µl of test serum no1,
    - Add in wells C1&D1, 25 µl of test serum 2 ,
    - Add in wells E1&F1, 25 µl of test serum 3,
    - Add in wells G1&H1, 25 µl of test serum 4.
    - Repeat the same operation on column 9A-9H with test sera no 5-8 (each sera in duplicate wells).

- With the multichannel pipette perform 5 fold serial dilutions (from colomn1-6, and colomn7-12) with initial dilution 1/25 and remove 25 µl of suspension from the 1/625 wells of column6 and 12.
- Add 100µl of 1000 $TCID_{50}$/ml viral suspension to each wells and incubate in 37°c for 1hr
    - ❖ The control plate should contain the positive and negative controls
    - ❖ Negative control: 6 wells in the first row are without sera and virus
    - ❖ Positive control: 4 rows of wells are filled with different dilutions of KS1 virus ($100TCID_{50}$, $10TCID_{50}$, $1TCID_{50}$ and $0.1TCID_{50}$ per 100 µl). Then the plate is incubated at 37°c for one hour similar to other plates.
- The vero cells are prepared in the $4*10^5$ cells/ml and distribute 50 µl to each wells
- The plates are incubated in 37°c with5% CO_2
- CPE reading would start on day 4^{th} and the final reading is made on day 10^{th}.
- Interpretation of the result: CPE is observed when the serum is negative (the virus is not neutralized), in the contrary, no CPE or >80% inhibition observed if the serum has antibody against LSDV.
- The test result is valid only if CPE does not occur in the Negative control wells

For the positive controls the CPE record should be accordingly to the virus titre in each four rows and expected standard protocol would be (++++, ++++, +- +-, ----).

Appendix III: Conditionally Independent and Dependent Bayesian Models

From website of http://www.epi.ucdavis.edu/diagnostictests/ (Branscum *et al*., 2005)

1. Conditionally Independent Model

```
list(n1=263, n2=200, y=structure(.Data=c(82,3,29,149),.Dim=c(2,2)),
z=structure(.Data=c(12,3,20,165),.Dim=c(2,2)), Q=2),
list(Z=1, pi1=0.244, pi2=0.06, Sevn=0.75, Spvn=0.95, Seifat=0.90, Spifat=0.85)

model;
{
y[1:Q, 1:Q] ~ dmulti(p1[1:Q, 1:Q], n1)
z[1:Q, 1:Q] ~ dmulti(p2[1:Q, 1:Q], n2)
p1[1,1] <- pi1*Sevn*Seifat + (1-pi1)*(1-Spvn)*(1-Spifat)
p1[1,2] <- pi1*Sevn*(1-Seifat) + (1-pi1)*(1-Spvn)*Spifat
p1[2,1] <- pi1*(1-Sevn)*Seifat + (1-pi1)*Spvn*(1-Spifat)
p1[2,2] <- pi1*(1-Sevn)*(1-Seifat) + (1-pi1)*Spvn*Spifat
p2[1,1] <- pi2*Sevn*Seifat + (1-pi2)*(1-Spvn)*(1-Spifat)
p2[1,2] <- pi2*Sevn*(1-Seifat) + (1-pi2)*(1-Spvn)*Spifat
p2[2,1] <- pi2*(1-Sevn)*Seifat + (1-pi2)*Spvn*(1-Spifat)
p2[2,2] <- pi2*(1-Sevn)*(1-Seifat) + (1-pi2)*Spvn*Spifat
Sevn ~ dbeta(174.48, 58.8)  ## Mode=0.75, 95% sure Sevn < 0.80
Spvn ~ dbeta(99.7, 6.2) ## Mode=0,95, 95% sure Spvn > 0.89
pi1 ~ dbeta(118.8, 374) ## Mode=0.244, 95% sure pi1 < 0.28
Seifat ~ dbeta(130.7, 15.4) ## Mode=0.90, 95% sure Seifat > 0.84
Spifat ~ dbeta(153, 28) ## Mode=0.85, 95% sure Spifat > 0.79
pi2 ~ dbeta(65.8, 1030.2) ## Mode=0.06, 95% sure pi2 > 0.0468
}
```

2. Conditionally Dependent Model

```
list(n1=263, n2=200, Q=2, y1=structure(.Data=c(82,3,29,149),.Dim=c(2,2)),
y2=structure(.Data=c(12,3,20,165),.Dim=c(2,2)))
list(pi1=0.244, pi2=0.06, Sevn=0.75, Spvn=0.95, lambdaD=0.90, lambdaDc=0.85,
gammaD=0.90, gammaDc=0.85)
model;
{
y1[1:Q, 1:Q] ~ dmulti(p1[1:Q, 1:Q], n1)
y2[1:Q, 1:Q] ~ dmulti(p2[1:Q, 1:Q], n2)
p1[1,1] <- pi1*Se11 + (1-pi1)*Sp11
p1[1,2] <- pi1*Se12 + (1-pi1)*Sp12
p1[2,1] <- pi1*Se21 + (1-pi1)*Sp21
p1[2,2] <- pi1*Se22 + (1-pi1)*Sp22
p2[1,1] <- pi2*Se11 + (1-pi2)*Sp11
p2[1,2] <- pi2*Se12 + (1-pi2)*Sp12
p2[2,1] <- pi2*Se21 + (1-pi2)*Sp21
p2[2,2] <- pi2*Se22 + (1-pi2)*Sp22
Se11 <- lambdaD*Sevn
Se12 <- Sevn - Se11
Se21 <- gammaD*(1-Sevn)
Se22 <- 1 - Se11 - Se12 - Se21
Sp11 <- 1 - Sp12 - Sp21 - Sp22
Sp12 <- gammaDc*(1-Spvn)
Sp21 <- Spvn - Sp22
Sp22 <- lambdaDc* Spvn
Seifat <- Se11 + Se21
Spifat <- Sp22 + Sp12
rhoD <- (Se11 - Sevn*Seifat) / sqrt(Sevn*(1-Sevn)*Seifat*(1-Seifat))
rhoDc <- (Sp22 - Spvn*Spifat) / sqrt(Spvn*(1- Spvn)*Spifat*(1-Spifat))
pi1 ~ dbeta(118.8, 374)
pi2 ~ dbeta(65.8, 1030.2)
```

Sevn ~ dbeta(174.5, 58.8)

Spvn ~ dbeta(99.7, 6.2)

lambdaD ~ dbeta(130.7, 15.4) ## Mode=0.90, 5th %tile=0.85

gammaD ~ dbeta(130.7, 15.4)

lambdaDc ~ dbeta(152.88, 27.8) ## Mode=0.85, 5th %tile=0.79

gammaDc ~ dbeta(152.88, 27.8)

}

Appendix IV: Questionnaire Format for Lumpy Skin Disease Risk factors Investigation

I. Study Location and Administrative levels

Region_____ Date_____

Zone_____ Owner's Name_____

Wereda_____

PA_____ Location of PA

Specific place name_____ Long _____

Latit _____

Altit _____

Livestock population in PA
Local Cattle_____
Exotic Cattle Breed_____
Sheep_____
Goat_____

LS Population in District
Local Cattle_____
Exotic Cattle Breed_____
Sheep_____
Goat_____

II. The History of LSD Occurrence

1. Have you had skin diseases of cattle in your herd? _____
2. Have you had LSD in your cattle? Yes_____ No_____

   If yes, -Skin nodules Yes_____ No_____

   - Skin nodules all over the body and transformed to plugs later on, Yes___ No____

   - Swelling around forelimbs and dewlap, Yes_____ No_____

   - Rough hair coat, Yes_____ No_____

- Enlargement of superficial lymph nodes, Yes_____ No_____

- Discharge from eyes, nostrils and mouth, yes _____ No _____

- Other sings_____

3. When did the disease commence in the area (PA)? Season _____Mon____ year____

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

4. How frequent LSD reoccurs in the area? Don't Know __Every 1yr__ Every 2yrs__ >3yrs__

5. Total cattle herd size of the farmer_____: Herd structure Ox____ Bull____ Beef____ Lactating cow_____ Dry cow_____ Heifer_____ Calf_____

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

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

III. Herd Management

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

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

Farming System
Sedentary_____
Nomadic_____
Transhumant_____

8. **Grazing/watering mgt**
   Communal_____
   Private _____
   Zero grazing

9. 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?

   _____

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

11. Do you vaccinate your cattle for LSD? Yes_____ No_____.

    If yes when? < 1year____ 1-2 Years____2-3Years____ >3Years_____

12. Do you have sheep and goats? Yes/No ____; If yes, Numbers of Sheep_____ goats_____

13. Do you have skin disease problems in them? Yes/No _____; If yes, what is the clinical signs (If possible tentative diagnosis based on the epidemiology and clinical symptoms)

    _____

14. Do your cattle herded together with sheep and goats? Yes/No ____; If yes, what about night Yes/No___

IV. Biting Flies of Cattle

In which months of the year that the biting flies of cattle become high in population?

_____

Do you consider LSD as an important disease and how do you score it? V.severe____ Severe____ Moderate____ Low____ not important_____

Appendix V : Questionnaire Format for Lumpy Skin Disease Financial Impact Investigation

V. Study Location and Administrative levels

Region_____ Date_____

Zone_____ Owner's Name_____

District_____

Kebele_____ Location of Kebele

Specific place name_____ Long _____

Latit _____

Altit _____

Herd structure and size
Total No of Cattle in **Kebele**
Ox_____ Bull_____
L.Cow_____ D. cow_____
Heifer_____ Calf_____
N° of exotic Cattle _____

No of Cattle in **Farm**
Breed _____
Ox_____ Bull_____
L.Cow____ D. cow_____
Heifer_____ Calf_____

VI. The History of LSD Occurrence

7. Have you had skin diseases of cattle in your herd? yes_____, No_____
8. Have you had LSD (Dhukkuba gogaa) in your cattle? Yes_____ No_____
9. When did the disease commence in the area (Kebele)? Season _____Mon____ year____

   - Have you seen such outbreak in the area before this time, < 1yr__ 1-2 Yrs___2-3Yrs___ >3Years___
10. How frequent LSD reoccurs in the area? Don't Know __Every 1yr__ Every 2yrs__ >3yrs__
11. Total herd size of the farmer before onset of LSD_____: Herd structure Ox____ Bull____ Beef____ Lactating cow_____ Dry cow_____ Heifer_____ Calf_____
12. How many animals had got sick and died due to LSD among the herd _____

Clinical sick animals

An.Code/name	Breed	Sex	< 1year calf	Young An	Adult
1.					
2.					
3.					
4.					
Died/slaughtered	Breed	Sex	< 1year calf	Young An	Adult
2.					
2.					

VII. Herd Management

9. Do you move your cattle to other place for grazing seasonally? Yes /No

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

10. Grazing and watering resource managements

Grazing/watering mgt
Communal_____
Private _____
Zero grazing

Farming System
Sedentary_____
Nomadic_____
Transhumant_____

15. Have you bought 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?

    _____

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

17. Did you vaccinate your cattle for LSD? Yes_____ No_____.

    If yes when? Before LSD onset____ Specify time_____ After LSD onset ____

Financial loss data

A) Do you consider LSD as an important disease and how do you score it? V.severe____ Severe____ Moderate_____ Low _____

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

b) Number of LSD died animal, and estimated current price of each?

An.Code/name	Breed	Sex	< 1year calf	Young An	Adult	Price(birr)
1.						
2.						
3.						

C) Mortality due to any other disease/case in that particular year

An.Code/name	Breed	Sex	< 1year calf	Young An	Adult	Price(birr)
1.						
2.						
3.						
4.						

D) In (♀)= Breed affected _____N° of lactating cow(s): _____; dry cow(s)_____ and heifer(s)_____.

An.Code/n°	Production stage of ♀	Parity	Lactation stage During LSD onset (mon.s)	How long days felt sick	Lactation continued or Stopped	Milk Prod. loss		
						B/re LSD	A/r LSD	Total Loss
1								

a. How many pregnant females aborted, in number _____.

b. Female animal culled due to LSD_____; other pathological pb_____

c. In (♂) affected: Breed _____ N° of draft oxen affected _____

Animal Code/N°	**When became sick**	**Estimated Bwt loss Aver, Min, Max**	**Av. number of lost-Work days**

d. Estimated cultivable land area per ox/day_____

I) Local price of draft ox service per day: max_____ mod_____ min_____

J) Extra-time expended for medical care of sick animal (in terms of hrs/day* n° of days):

max_____ min_____

K) Cost of medication in birr/animal Max_____ min_____ Total expenditure_____

L) Herd management: Indoor feeding _____; Free ranging_____

M) Feed: Price of straw/donkey pack_____; Hay/hip_____; Silage/kg _____

Concentrates/kg _____

Labor costs:

N) Estimated Labor cost per month or year for: Herdman labor _____;

Milker_____cleaner_____; Casual labor cost_____

O) Housing: Fenced stable _____; House barn_____

P) Total off-take in the year: _____ sold; _____; culled_____; Slaughtered _____;

given out for others_____

Q) Total animal brought during the past one year: _____

V. Market price data

a) Average **Cattle market** price in that month, **Ox**= Av_____ max_____ min_____, **Bull** = Av_____ max_____ min_____, **L.cow**= Av_____ Max_____ Min_____, **D.Cow** = Av____ max_____ min_____, **Heifer**= Av _____ max_____ min_____, **Calf <1year** = Av_____ max_____ min _____

b) Average price of **Milk** (lt) in that month: max _____, min_____, Beef meat (kg) max_____, min_____ Hide= max_____ min_____ Dung for feul/sack _____ compost/sack _____ .

c) LSD vaccination cost/animal_____

d) Antibiotic treatment cost/ animal _____

VI. Reproductive Parameters

a) The first calving age of heifers? Local _____ Holstein Friesian _____

b) Annual calving rate: local _____; Holstein Friesian_____.

c) Calving interval: local _____; Holstein Friesian _____

d) The average production lifespan* of local cows in year _____ Holstein Friesian _____

e) The average number of calving during the production lifespan: local cows _____ Holstein Friesian _____

f) The age at which the bull starts to work as a full time draft ox? _____

g) The average production lifespan of draft ox? _____

- * production lifespan= the length of time from the first conception up to culling or death of female animal.