THE ROLE OF ARTHROPOD VECTORS IN THE TRANSMISSION OF LUMPY SKIN DISEASE IN CATTLE

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Declaration

This thesis is my original work and has not been presented for a degree in any other University or any other award.

Eunice Atieno Gai Misiani 18/06/04

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DEDICATION

Dedicated to my son Allen Yesutober Agalo Misiani who arrived on this planet just after this work was started and has brought so much joy into my life.

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LIST OF ABBREVIATIONS AND ACRONYMNS USED IN THIS REPORT

ANOVA – Analysis of Variance

ARPPIS _ African Regional postgraduate Programme in Insect Science

BAPBS – Phosphate Buffered Saline containing bovine serum 0.25 % w/v

BLV – Bovine leukaemia virus

BMA – Blood meal analysis

^{0}C – Degrees Centigrade

CDC – Centres for Disease Control

Chi^2 – Chi square

Conf. – Confidence Limit

CPE – Cytopathic Effect

CPV – Capripox Virus

DAAD – German Academic Exchange Programme

DF – Degrees of Freedom

DFID – Department for International Development

DMSO – Dimethly sulphur oxide

DNA – Deoxyribonucleic Acid

EAIV - Equine Infectious Anaemia virus

EDTA – Ethylenediaminetetra acetic acid

EEC – European economic community

F – F Statistic

g – Grams

ICIPE – International Centre of Insect Physiology and Ecology

ID – Identification

ILRI – International Livestock Research Institute

KARI - Kenya Agricultural Research Institute

Kb – Kilo base

KEMRI –Kenya Medical research Institute

KETRI – Kenya Trypanosomiasis Research Institute

LN – Lymph node

LSD – Lumpy Skin Disease

LSDV - Lumpy Skin Disease Virus

LT – Lamb testis

Mg/h – Milligrams per hour

Ml – Millilitre

MS – Mean sum of Squares

NARP – National Agricultural Research Programme

NS – Non Significant

NVRC –National Veterinary Research Centre

Obs – Observations

OIE – Office International des Epizooties

P, Prob., Pr – Probability

PCR – Polymerase Chain Reaction

PLF – Post-lesion Feeding

PMF – Post-membrane Feeding

PSN – Penicillin, Streptomycin, Neomycin

RH – Relative Humidity

SNK – Student Newman Keul's Test

Std. Error – Standard Error

TCID – Tissue Culture Infective Dose

VNT – Virus Neutralization Test

Z – Z value

ABSTRACT

Lumpy skin disease (LSD) is an economically crippling disease of cattle with epizootic occurrence particularly after the onset of the rainy season. Biting arthropods have been implicated as vectors of lumpy skin disease virus (LSDV) due to observations that there is no transmission of LSD when cattle are confined to insect-proof houses. Nevertheless, the method by which LSDV is transmitted under field conditions is not clear and no specific vector has been conclusively pinpointed. The main objectives of this study were to identify and incriminate biting insects as possible vectors of LSDV in outbreak areas and also determine the capacity of vectors thus implicated, in the transmission of LSDV, under laboratory conditions. Biting arthropods that are closely associated with livestock were trapped in three outbreak areas, namely Machakos, Kiambu and Kajiado Districts of Kenya. The insects were identified, dissected and inoculated onto prepubertal Lamb testis cell cultures to isolate virus from them. Blood meal analysis was carried out on engorged insects. LSD experimental transmission was done using Zebu cattle (*Bos indicus*) and insects of the species *Glossina morsitans morsitans, Glossina morsitans centralis, Stomoxys calcitrans, Phlebotomus dubosqui and Aedes aegypti*. The Neethling strain of virus was used in challenging the animals, feeding the insects and in the virus neutralisation tests. Time series dissections were performed on the insects to isolate virus from various insect parts. Meteorological data from the study areas and other Kenyan districts were recorded. The distribution of biting arthropods in the study districts revealed a total of more than twenty-nine species of insects. *Stomoxys niger* species had the highest frequency of occurrence (18 %) while the tabanids species were the least frequent (0.2 %). The average number of females trapped at any given time was significantly greater than that of the male insects (27 compared to 18, $P < 0.0001$). The blood meal analysis showed that various insect species had fed on human (14.9 %), bovine (20.7 %), goat (14.9 %), sheep (4.1 %), and lizard (33.6 %,) blood. A female field caught *Prostomoxys* species insect yielded a positive result for the presence of LSDV. There was a strong association between the insect species and insect parts from which virus was isolated ($P = 0.000$ Cramer's $V = 0.5596$). The largest proportion of the virus was recovered from the heads. The crops and hindgut pools had the smallest proportion of virus recovered from them. The source of virus had an effect on seroconversion of the animals (Pearson chi^2 (3) = 8.6152, $P < 0.035$). There was a significant difference between the different species of insects as far as seroconversion of the animals they fed upon was concerned-$P < .043$. The association between days post virus feeding by the insects, and seroconversion was statistically not significant (P- value = 0.321). Climatic variables had different effects on LSD outbreaks in the areas studied ($P < 0.05$); however, relative humidity, maximum temperature and wind-speed had significant effects on

occurrence of LSD in all the areas. Vaccination and quarantine significantly reduced LSD occurrence. The results showed that that several species of insects are able to transmit the LSD virus. Integrated vector management and application of meteorological information in planning LSD control programmes may have an effect on reduction of LSD outbreaks.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

Lumpy skin disease (LSD) is an economically crippling infectious disease of cattle with epizootic occurrence particularly after the onset of the rainy season. It is characterized by swelling of the superficial lymph nodes, and cutaneous nodule formation. The nodules also form on the oral, nasal and genital mucous membranes. The aetiological agent of the true LSD is the Neethling type virus, a capripoxvirus (CPV) (Alexander *et al.,* 1957).

Sheep poxvirus, goat poxvirus and the virus of bovine lumpy skin disease (Neethling) make up the capripox group of viruses, which cause serious disease in sheep, goats and cattle. Transmission usually occurs between susceptible and infected animals, but the route of entry is not clear (Kitching and Mellor, 1986).

Capripoxviruses infect only ungulates and most strains cause clinical disease in only one species. The capripox viruses produce the most severe pox diseases of sheep, goats and cattle, and are the most important group of animal pox viruses with serious economic consequences in terms of mortality, lost productivity, hide damage and loss of revenue associated with trade restrictions following outbreaks. They are economically the most important group of poxviruses (Kitching and Mellor, 1986).

LSD has been a disease confined to the African continent since its emergence although it has spread to other continents in recent years. LSD occurs in many countries of Africa including, Kenya, Tanzania, Zambia, Botswana, South Africa, Chad, Nigeria, Sudan, Niger, Ivory Coast, Egypt (Woods, 1988). "In 2001 Lumpy skin disease was recorded in Mauritius, Mozambique and Senegal. It has never occurred in Great Britain although it has been recorded in Israel. Lumpy skin disease is an OIE List A disease, which shows its serious socio-economic status" (Government of UK, 2003; Yeruham *et al.,* 1995).

The occurrence of capripox infections of sheep and goats (sheep and goat pox) closely mirrors that of LSD although the small ruminant disease is more widespread and it occurs in India, Bangladesh, Near and Middle East; and has also been reported in Italy (Carn, 1994; Kitching *et al.*, 1987).

The transmission of LSD seems to be closely associated with insects; outbreaks of LSD frequently follow high rainfall and move along watercourses. In nearly all the areas where there have been outbreaks of LSD the prevalence of biting flies and the conditions favourable to their breeding support the suggestion that the disease is spread by insects (Haig, 1957). Biting arthropods have been implicated as vectors of Lumpy skin disease virus (LSDV) due to observations that there is no transmission of LSD when cattle are confined to insect-proof houses (Carn, 1994).

Lumpy skin disease (LSD) is endemic in Africa south of the Sahara; but since the eighties, it has been spreading to northern Africa and even outside Africa. The transmission of LSDV has been associated with insects because the disease outbreaks usually coincide with conditions favourable to insect activity, which occur during the warm moist seasons.

Control of LSD without vaccination is extremely difficult in endemic areas. There have been attempts to carry out transmission studies using a few insects such as *Stomoxys spp.* (Kitching and Mellor, 1986) but the work has not been done exhaustively. The method by which LSDV is transmitted under field conditions remains unclear and no specific vector has been conclusively pinpointed.

Further work needs to be done to identify the method by which both mechanical and biological transmission occurs. In addition, the most important task is to identify the specific potential vectors of LSDV.

1.2 LITERATURE REVIEW

Lumpy skin disease was first described in Zambia in 1929 (Macdonald, 1931). After the first diagnosis of LSD in 1929, it spread to the southern half of Africa and remained confined to that part of the world until 1988 when it was recorded in Egypt (Ali *et al.,* 1990). The establishment of LSD in Egypt caused a lot of concern about the possibility of spread outside Africa because Egypt does not have the wild ruminant population previously thought to be necessary for the disease to persist in the environment (Davies, 1982). This concern became a reality when LSD was first diagnosed in cattle in the southern part of Israel in September 1989 (Abraham and Zissman, 1991; Yeruham *et al.,* 1995).

In Kenya, it was first diagnosed in 1957 (Burdin and Prydie, 1959), probably having spread through Tanzania but the northward spread was not active for 14 years until it was seen in south western Sudan in 1971 (Woods, 1988). There has been epizootic spread of LSD in Kenya, with most cases occurring sporadically however the LSDV virus strains isolated over a twenty year period proved to be serologically identical (Davies, 1982).

1.2.1 Poxviruses

The poxviruses are distinguished from other animal viruses by their large size and cytoplasmic site of replication (Moss, 1974). The virions consist of a large dumbbell-shaped DNA genome of about 180 Kilobases. The surface of poxvirions contains a lipo-protein bilayer, of 50-55 nm thickness. Viral antigens present in the outer envelope are immunogenic. (Moss, 1987). During poxvirus development in cell cultures most of the mature virions remain within the cytoplasm, some of them being enveloped by cisternae formed from vesicles of the Golgi complex (Ichihashi *et al.*, 1971).

Poxviruses produce extensive cytopathic effects, which usually lead to host cell death. Rounding of cells is an early viral function (Appleyard *et al.*, 1962) whereas cell fusion is a later cytopathic effect in certain poxviruses, for example LSD virus. Poxvirus morphogenesis includes formation of viral membranes and local spread of infection is from cell to cell. The virus is effectively protected from circulating antibody therefore dissemination may be either through circulation of infected macrophages or extracellular virus (Boulter and Appleyard, 1973).

The poxviruses belong to the family Poxviridae, which is divided into two sub-families, the *Chordopoxvirinae* that infect vertebrates, and the *Entomopoxvirinae,* that infect insects (Mathews, 1982). Poxviruses usually

produce one or two main types of lesions, which are relevant for arthropod transmission (Bellett *et al.,* 1973). Poxvirus pathology ranges from superficial vesicle formation, epithelial proliferation and typical "pock" formation to subcutaneous tumour formation.

The various epithelial changes are typified by the now extinct disease smallpox (produced by variola virus). Viruses of some genera, e.g. the Parapoxviruses, produce papulo-pustular skin lesions, either localized or more often generalized via the blood stream; these viruses are transmitted either by contact or by the respiratory route. The others, e.g. the Capripoxviruses produce localized or generalized tumours in the skin.

1.2.2 Capripoxviruses

Capripoxvirus is one of the eight genera classified in the sub-family Choordopoxvirinae (Francki *et al.,* 1991). The other genera within this sub-family are: Avipoxvirus, Leporipoxvirus, Molluscipoxvirus, Orthopoxvirus, Parapoxvirus and Suispoxvirus (Regenmortel et al., 2000). The genus Orthopoxvirus includes the more familiar smallpox virus. Capripoxviruses are viruses of ungulates, which include sheep pox virus, goat pox virus and lumpy skin disease virus. Most of the virus strains in this genus have similar characteristics and can infect across the species barrier (Davies, 1976).

1.2.3 Clinical signs and pathology of Lumpy Skin Disease

Lumpy skin disease is characterised by firm, circumscribed skin lesions, generalized lymphadenopathy and pyrexia (Prozesky and Barnard, 1982). A "leg-swelling" form of the disease, where the affected limb had a painful oedema was described by Von Backstrom in 1945. This form was consistently followed by the "skin-nodule" form, and when the disease was first becoming established the same author noted an increasing tendency for it to manifest itself as the skin form.

LSD may present in a sub clinical, a mild, or a generalized form and if severe may result in death. Outbreaks are similarly erratic in their manifestation: sometimes they are mild with few animals affected as in the Kenyan epidemic in 1957 where morbidity was 0.5% and mortality 2% (MacOwan, 1959); other outbreaks such as the South African epidemic of 1944-1947 was more serious with morbidity rates reaching 100% and mortality rates reaching more than 50%. Cattle breeds exotic to Africa (*Bos taurus*) are most susceptible to clinical disease (Davies, 1991).

Severely affected animals are identified by the presence of numerous small plaques, especially around the head, neck, perineum, limbs and genitalia. These are usually the first observed clinical signs, although the animals may have been pyrexic for a few days preceding this. As the disease progresses, the nodules harden and necrose. They may become secondarily infected with bacteria, and if the animal survives, become "sit

fasts". These hard callous lesions may persist for more than twelve months. Eventually lesions may scar over, healing as a cicatrix, but the hide will be permanently damaged (Green, 1959).

In generalized cases the animal becomes pyrexic around ten days post infection (pi), and nasal and ocular discharges are observed. (Carn, 1994). Skin lesions may be very extensive, affecting particularly the neck, flanks and perineum. The animal may continue to eat well, but in many cases enlarged lymph nodes and mouth lesions make swallowing difficult and the animal salivates profusely and becomes anorexic.

Ulceration may also be seen in the nostrils, on the lips and on the conjunctiva. In some cases a severe keratitis may develop which can leave animals permanently blind (Henning, 1956). Severe cases may show stertorous breathing as a result of tracheal pathology, or primary (viral) or secondary (bacterial) lung lesions. Lymphadenopathy is especially obvious in the suprascapular lymph node (LN), which can reach many times its normal size (Von Backstrom, 1945). Lymphadenopathy is associated with oedema. Swollen limbs, dewlap and subcutaneous oedema elsewhere are frequently seen. Severe lameness often occurs, resulting in difficulty to move to food and water, and subsequently a further loss of condition. Pyrexia in the affected animal may last up to 3 weeks (Agag *et al.,* 1992).

Milk yields fall with the onset of pyrexia and female animals may be anoestrus for many months and may abort (Davies, 1991).

Secondary infection may increase the enlargement of the suprascapular LN, and an abscess may develop. Discharging sinuses may be produced, and may also be seen on the legs. Secondary infection is also frequently responsible for the loss, either total or partial, of the udder due to mastitis following skin lesions. Secondary infection is common in all animals without antibiotic cover: pyaemia and septicaemia are likely sequelae, sometimes resulting in fatal pneumonia. Pneumonia may also occur as a result of inhaling necrotic pseudo-membranous material from the upper respiratory tract. In some animals a partial or complete stenosis of the proximal trachea occurs 6-12 months after the acute disease (De Boom, 1947).

Recovery from LSD is often prolonged, with the potential for late complications, and young animals may never reach target weight. Convalescent cattle may remain in extremely poor condition for 4-6 months (Diesel, 1949).

1.2.4 Histological changes of Lumpy Skin Disease

Acute stage skin lesions are characterised on histological examination by a massive cellular infiltrate, vasculitis, and oedema. The cellular infiltration occurs largely around smaller blood vessels and early lesions are

characterised by marked peri-vascular cuffing. Infiltration is initially by macrophages, neutrophils and occasionally eosinophils, and as the lesion progresses by more macrophages, lymphocytes and plasma cells (Prozesky and Barnard, 1982).

Many cells have large homogenous (rarely granular) intra-cytoplasmic inclusion bodies. Vasculitis and lymphangitis are accompanied by thrombosis and infarction, which subsequently cause oedema and necrosis. Late stage lesions are characterised by fibroplasia. Epidermal changes include acanthosis and parakeratosis. Inflammatory changes in other organs are similar to those in the skin. Lesions in the buccal cavity, nasal cavities, pharynx, larynx and trachea are characterised by ulceration. Lymph nodes are oedematous and show lymphoid hyperplasia (Prozesky and Barnard, 1982).

Ultra structural studies (Prozesky and Barnard, 1982) have demonstrated virions in various stages of development in cells containing inclusion bodies. Schwann cells were seen to contain virions and show characteristic cytopathic effect. Viral particles were seen more frequently in pericytes than in endocytes. Virus particles were also seen in vessel lumens.

1.2.5 Epidemiology of Lumpy Skin Disease

Lumpy skin disease has spread across most of Africa since it was first described about 70 years ago. Lumpy skin disease is on the Office Internationale des Epizooties (O.I.E) List A. animal diseases. and its presence in member countries should be reported immediately. In September 1989 the European Economic Community (EEC) added LSD to the Council Directive 82/894/EEC on the notification of animal diseases within the Community, and, as such, this disease must be reported to all other member states and the Commission within 24 hours of a primary outbreak.

The spread of the disease has been frequently associated with epizootics, although these have been interspersed with periods when the disease has been apparently absent. In some countries there appears to be a cycle of epizootics, and, although these have not been correlated quantitatively with meteorological data, there appears to be an association between the onset of the rainy season and of outbreaks of the disease (Carn, 1994). This correlation with climatic factors has not been carried out in most of the countries where LSD is endemic; for example in Southern Africa (Hunter and Wallace, 2001)

1.2.6 Transmission of Lumpy Skin Disease

The transmission of LSD seems to be more closely associated with insects, and the spread frequently follows high rainfall and moves along

water courses. Vector transmission would explain the difficulty in containing an outbreak, and the rapid spread across large distances (MacOwan, 1959). There is no documented evidence for the biological transmission of capripoxviruses.

When LSD first entered South Africa control measures were implemented such as restriction of animal movement with quarantine periods in low rainfall areas for some cattle, to minimise spread if the animals should become diseased. This measure indicated the great importance attached to rainfall in the spread of the disease. Despite these precautions disease spread continued, following main cattle routes that ran alongside water courses and it was assumed that insects were responsible. The application of insecticides was found to arrest spread of disease within the herd (Diesel, 1949).

Transmission of capripoxvirus by *Stomoxys calcitrans* between sheep has been demonstrated (Kitching and Mellor, 1986). From the large number of papules that developed on susceptible sheep following the feeding of infected *Stomoxys calcitrans* it was estimated that 10% of the flies transmitted virus.

In nearly all the areas where there have been outbreaks of LSD, the prevalence of biting flies and the favourable conditions for their breeding have supported the suggestion that the disease is spread by insects (Haig,

1957). Outbreaks follow roads, cattle routes and insects, especially mosquitoes, are believed to play an important role in the spread of the disease (Macowan, 1959; Burdin and Prydie, 1959). In Egypt, there is higher disease prevalence in certain localities; this is thought to be due to great numbers of insect vectors in these regions (Ali *et al.,* 1990). In the Sudan, epidemics of LSD have been associated with ticks, mainly *Amblyoma species* (Ali and Obeid, 1977). Nevertheless, the same authors found out that the prevailing conditions favoured the breeding of biting arthropods.

Insects often transmit viruses that cause disease in plants and vertebrates. These vectors may carry the virus mechanically or actually serve as a second host (Poinar and Thomas, 1978). However, most of the viruses which are vectored by insects are non pathogenic to their hosts. There are quite a number of biting flies that have been implicated as vectors of various types of viruses. These examples include *Phlebotomus papatasi*, which transmits the sandfly fever Naples virus (SFN) in humans (Bishop, 1986; Lewis, 1973); and *Aedes aegypti* Linnaeus that transmits dengue virus.

There are also biting flies which have been associated with transmission of the Capripoxviruses, and these include *Stomoxys calcitrans* (Kitching and Mellor, 1986), *Glossina morsitans morsitans* (Webb, 1990), *Biomyia fasciata* and *Tabanus spp.* (Macowan, 1959). Tabanids are recognized

mechanical vectors of more than thirty pathogens (Krinsky, 1976) that cause disease in animals, six of which are viral diseases. Mechanical transmission occurs when a fly feeding on a host with a patent infection is interrupted and the arthropod moves to a susceptible host. "The vector potential of each fly species for a given pathogen, is however, quite variable and is dependent on:

a) the stability of the agent in the fly mouthparts,
b) the quantity of infected material that can be transferred between hosts; and
c) the abundance and biting habits of individual fly species" (Foil, 1989).

Recovery of a pathogen from a particular vector and vertebrate host does not necessarily imply that vector and host are associated. Similarly the fact that a particular vector is incriminated within a given geographical area does not necessarily imply that it serves as a vector elsewhere. Assessment of vector status involves intensive long-term study. Too often vectors are listed as such on the basis of wholly inadequate evidence. Neither successful experimental transmission nor recovery of a pathogen in nature is sufficient by itself to incriminate a particular arthropod as an effective vector - yet vectors are often uncritically listed on such evidence (Mattingly *et al.*, 1973).

The concentration of a pathogen in the blood and local tissues of a host determine the number of infected cells that can partially be ingested or contaminate the mouthparts of a fly during feeding. Experimental results by Carn (1994) show that lower titres (10^1 Tissue culture infective dose ($TCID_{50}$) of virus are required to establish a generalized infection than are found in the skin lesions of LSD infected animals (10^4-$10^{8.2}$ $TCID_{50}$). Carn (1994) thus concluded that the important determinants for severe outbreaks of LSD are the presence of intravenous feeding arthropod vectors, which predispose to generalized infections as opposed to local lesions.

Davies (1976) speculated that aerosol spray was a possible means of transmission of the capripoxviruses as occurs with smallpox in humans and rabbit pox. However, this hypothesis was rejected by Carn (1994) who found out that close contact of infected and uninfected animals with shared feeding and watering utensils did not lead to transmission of LSDV. Thus, there is no spread between animals housed together in the absence of arthropods. Alexander (1949) also found that insect-proof stables prevented spread of the disease, therefore implicating biting insects as being necessary for transmission.

1.2.6.1 Arthropods as disease vectors

Most Dipterans shown to be involved in arbovirus transmission belong to the Suborder Nematocera (Mattingly, 1973), with the families Psychodidae, Ceratopogonidae and Culicidae having members that are known virus vectors. The Culicidae are capillary feeders having greatly elongated, flexible mouthparts, which allow penetration of individual blood vessels and direct imbibitions of circulating blood. The other families are pool feeders, as in the Tabanidae, where mouthparts are used to excavate a small depression in which blood collects and from which it is imbibed.

1.2.6.2 Evidence for arthropod transmission of poxviruses

Arthropods have been implicated in the mechanical transmission of a number of poxviruses, suggesting that they are a significant means of poxvirus transmission. Tripathy, *et al.* (1981) reported circumstantial or experimental evidence for arthropod transmission in Tanapox (Yaba-like disease), Shope fibromatosis, hare fibromatosis, and squirrel fibromatosis. Squirrel pox has been experimentally transmitted by mosquitoes, and the occurrence of Tanapox in children living along the Tana River in Kenya has also been linked to mosquitoes. Kilham and Dalmat (1955) showed that the Shope fibroma virus was localised in the head region of the mosquito, and that the mosquitoes remained infective for 5-6 weeks.

Swinepox, which may be spread through direct contact and skin abrasions, is mechanically transmitted by the louse *Haematopinus suis.* The louse greatly influences the severity and course of the disease with infestations resulting in generalised skin lesions. The louse carries infectious virus for weeks or months and biting insects are suspected of being responsible for spread between premises (Tripathy *et al.*, 1981).

Fowlpox has been shown to be mechanically transmitted by mosquitoes (Brody, 1936) and in order for them to transmit effectively, they must feed on lesions and not unaffected areas of skin.

The mosquito *Aedes aegypti* transmits myxomatosis quite effectively. Fenner *et al.* (1952), concluded that, with respect to its ability to transmit myxomatosis, the mosquito was essentially a flying pin. Mosquitoes were not infective unless they contaminated their proboscis by penetrating infected epithelial cells

1.2.7 Blood feeding by biting arthropods

In different haematophagous dipterans, blood may be temporarily stored in different parts of the digestive tract. In tsetse flies it passes through the midgut, then the next portion of the meal goes to the crop to be later transferred to the midgut (Harmsen, 1973), whereas in mosquitoes (*Aedes* species), the blood enters the midgut and only a small percentage (6%),

goes to the crop (Day, 1954). In the stable fly, it goes to the midgut immediately, and then to the crop depending on the age of the flies and the feeding temperature (Venkatesh and Morrison, 1979). The midgut of the adult stable fly Stomoxys calcitrans is functionally divided into an anterior non-digestive (storage) and a posterior digestive region, where blood protein undergoes proteolytic hydrolysis (Lehane, 1976).

Mosquitoes search for blood by repeatedly thrusting their mouthparts into the host's deep network of skin vessels. Saliva is ejected during this process and it aids in probing rather than in blood ingestion (Ribiero, 1987). The salivary glands of mosquitoes such as *Aedes aegypti* contain the enzyme apyrase, which has got antiplatelet activities thus reducing the period of blood finding which reduces host contact (Edman and Kale, 1971). Decreased host contact apparently enhances survival, since many hosts kill mosquitoes or prevent them from feeding on blood. Sandflies have very short mouthparts (less than 0.5 mm) that are unable to penetrate beyond the most superficial layers of skin (Lewis, 1975). The flies feed by lacerating the capillary loops and ingesting the blood pooling into the resultant haematomas. Saliva in sandflies is ejected during feeding and probing and it also has the enzyme apyrase (Ribiero, 1987).

Electron micrographs show that internal mouthparts of stable flies would retain 0.03 microlitres of blood (Weber *et al.*, 1988). Regurgitation occurring during a second feeding in Stomoxys has been described using

in vitro techniques by Butler *et al.* (1977). The biology of these insects enhances their transmission of disease agents. For example, tabanids exhibit various adaptations related to blood-feeding such as, taking a blood meal approximately equal to their unfed weight (Hollander and Wright, 1980), which in addition to increasing the chance of fly survival and reproduction, increase the probability of transmission of disease agents from one animal to another (Krinsky, 1976). *Stomoxys* flies take up blood meals about three times their own body weight while *Glossina* take up approximately twice their body weight of blood per meal (Parr, 1962).

1.2.8 The control of lumpy skin disease

1.2.8.1 Vaccines

The first work on a homologous vaccine against LSD was carried out in the Republic (then Union) of South Africa, following the severe epidemics of LSD in the 1940s and 1950s. Van Rooyen *et al.* (1969) attenuated the Neethling strain of LSDV In embryonated eggs. Another lyophilised vaccine, produced in Chad from a Madagascan strain of LSDV (Ramisse et al., 1969a), was passaged 101 times in rabbit kidney cells, and five times on foetal calf kidney cells (Ramisse et al., 1969 b).

The first use of heterologous virus for protection of cattle was described by Capstick and Coackley (1961), who used the Kedong and Isiolo strains of capripoxvirus isolated from sheep and passaged in lamb testis and kidney

cells (Capstick *et al.*, 1959). This vaccine was used extensively in Kenya between 1958 and 1959, with good results, although some *Bos taurus* breeds did develop mild generalised infection, and it was recommended not to use the vaccine near sheep, as it was still virulent for that species. Two other strains of heterologous virus have been used in cattle, for the control of the outbreaks in Egypt and Israel (Davies, 1991 b). The Egyptians used the Romanian strain of sheep poxvirus, and the Israelis the RM65 strain. There were no recorded complications with the use of these vaccines, and they were reported to be immunogenic in the field.

1.2.8.2 Other control measures

Capripox free countries maintain their disease-free status by restrictions on imports of livestock and animal products from affected areas. In countries remote from enzootic areas the swift implementation of a radical slaughter policy, strict movement control, and ring vaccination within a radius of 25-50km, has been used.

Countries in which capripox is enzootic control disease through vaccination, and occasionally, if the outbreak is well confined, by slaughter. Slaughter of clinical cases is limited in its application as animals are infectious for a number of days before clinical signs are seen. Isolated outbreaks may be controlled through slaughter of infected and in-contact animals and destruction of contaminated hides, coupled with local vaccination. Ring vaccination is frequently practised during outbreaks in enzootic areas, but

usually only the species that are clinically affected are vaccinated, despite the possibility that strains may cross infect between species. In areas at high risk of LSD outbreaks there may be strategic use of vaccines: generally young stock will be vaccinated before they enter the herd. Intensive dairy farmers, working with exotic, high-yielding breeds of cattle, most often adopt this regime. In Kenya vaccination of animals is usually carried out and quarantine is imposed if an outbreak occurs, though this is not always consistently carried out in all the Districts (Republic of Kenya, 2000). Prohibition of animal movements reduces long distance dissemination of the virus.

1.3 RATIONALE FOR THE STUDY

Kitching and Mellor (1986) illustrated that capripoxviruses can be experimentally transmitted between sheep using *Stomoxys calcitrans* as a vector. Now it is important to determine whether this fly and other biting flies are the natural capripoxvirus vectors in endemic areas. There is also need to isolate viruses from African Stomoxyinae, which are quite diverse and are also known to be mechanical vectors of other pathogens (Mihok *et al.*, 1995 a).

The ability to harbour a pathogen, adequate contact with the hosts and sufficient longevity for the parasite to mature are all equally essential and none of them alone is sufficient to define an effective vector: the study of

one aspect in isolation is never sufficient for this type of assessment (Mattingly *et al.*, 1973). In the case of LSD not all these aspects have been researched and thus it is important to do some exhaustive incrimination studies of potential vectors. Recognition of the principal vector for LSD is a prerequisite for the development of effective strategies to control the spread of the disease. So far no specific vector for lumpy skin disease virus (LSDV) has been conclusively identified. It is not known whether the vectors are biting flies or other arthropods such as ticks. Control would therefore be difficult from the vector management point of view because it would not be feasible to find a technique that would control such a wide range of arthropods.

Lumpy skin disease causes major economic loss through decreased milk yield, poor growth, hide damage and infertility (Diesel, 1949). Its spread outside Africa caused renewed concern and interest in the disease. Consequently in September 1989 the then EEC (now European Union); added LSD to its list of animal diseases which must be reported to all other member states and the commission within 24 hours of a primary outbreak. If LSD requires neither a wildlife reservoir (as in Egypt) nor environmental conditions peculiar to subtropical African countries as in Israel- (Abraham and Zissman, 1991), then its potential for spread is huge, and a distribution at least as extensive as that of sheep pox and goat pox (capripoxviruses) would be possible.

1.3.1 Statement of the problem

The rationale of the study is to clarify the method by which LSDV is transmitted under field conditions and to determine the specific vector(s) of the virus.

1.3.2 Research questions

In the light of the problem mentioned above it is important to address the following issues:

a) Which insects are capable of transmitting/harbouring the virus?

b) Which arthropods are the potential natural vectors of lumpy skin disease in endemic areas?

c) What other factors facilitate the transmission of LSDV?

1.3.3 Justification / significance of the study

Capripoxviruses have received considerable attention, with the focus being primarily on the small ruminant disease problem. With increasing rates of morbidity and mortality caused by LSD in Africa, the focus has changed and research is being directed towards the immunology and epidemiology of LSD (Kitching *et al.*, 1989). Better understanding of these areas will enable the formulation of more acceptable control measures

This study aimed at determining the potential vectors of LSDV especially in the African context where LSD continues to affect livestock. The study involved the use of indigenous cattle and correlation of meteorological variables with LSD outbreaks. This type of interrelation has not been done before. If the method by which LSD is spread is determined, then the appropriate control measures can be employed in reduction of the severity of the disease as well as its spread to neighbouring locations.

1.4 HYPOTHESES

The null hypotheses were:

a) Biting arthropods are not vectors of lumpy skin disease virus

b) LSD outbreaks are not influenced by climatic variables

c) Rainfall does not facilitate/determine the occurrence of Lumpy skin disease.

1.5 OBJECTIVES OF THE STUDY

1.5.1 General objective

The overall aim of the study was to identify and determine if biting insects and acarines were possible vectors of lumpy skin disease virus.

1.5.2 Specific objectives

a) To identify biting insects and acarines associated with livestock in areas where LSD had recently occurred and in which LSD occurred during the course of the study.

b) To establish the ecological factors, which facilitate arthropod transmission of LSDV.

c) To identify the probable vectors of LSDV in outbreak areas.

d) To determine the capacity of these vectors to transmit LSDV by conducting laboratory - based transmission studies.

CHAPTER 2: CLIMATIC VARIABLES AND ARTHROPODS ASSOCIATED WITH LSD OUTBREAKS IN SOME DISTRICTS IN KENYA

2.1 SUMMARY

There has been speculation about the occurrence of LSD being associated with the onset of the rainy season but there has been no recorded evidence correlating climatic variables to LSD outbreaks in different localities. In this study, arthropods were collected from different outbreak areas and the weather variables in those localities antable d other districts in Kenya, were recorded. A total of 30 species of arthropods were collected. In each of the LSD outbreak areas visited, the farmers reported the presence of insects, which were a nuisance to the animals just before the outbreaks, although they could not specify which particular insects were involved. The results of correlation between climatic variables and LSD outbreaks in Kenyan Districts during a twenty-year period revealed that wind, humidity and temperature play an important role in facilitating the occurrence of LSD. The effects of quarantine and vaccination of cattle against LSD were also considered. The results from this study indicate that integration of the knowledge of the biology of various biting flies as well as application of meteorological data, and the imposition of quarantine and vaccination, would play an important role in the control of LSD in Africa.

2.2 INTRODUCTION

There has been a recent emergence of LSD outbreaks in Kenya since 1990's. The countrywide distribution of outbreaks despite quarantines and movement controls suggested involvement of insects in some cases, (Ministry of Livestock Development, 1978-2000). During the course of this study, outbreaks of LSD occurred in several Districts in Kenya including Machakos, Kajiado, Thika and Kiambu Districts hence providing an opportunity to study insect populations during outbreaks.

Capripoxviruses have received considerable attention, with the focus being primarily on the small ruminant disease problem. With increasing rates of morbidity and mortality caused by LSD in Africa, the focus changed and research was being directed towards the immunology and epidemiology of LSD (Kitching *et al.*, 1989). Better understanding of these areas would enable the formulation of more acceptable control measures.

Insects have also been implicated as vectors of LSDV because of the fact that there is no transmission of LSDV when cattle are confined to insect-proof houses. Nevertheless, the method by which LSDV is transmitted under field conditions is not clear and no specific vector has been conclusively pinpointed. Further work was necessary to identify the method by which both mechanical and biological transmission occurs and to identify the specific potential vectors of LSDV in Africa.

2.3 MATERIALS AND METHODS

2.3.1 Sampling in outbreak areas

An observational study was conducted on each of the 16 farms, and records were made of anatomical sites on the animals preferred by the different biting flies in relation to lesions due to LSD virus infection. The LSD infected cattle were checked for infestation with ticks and other ectoparasites. Skin biopsies of the nodules and sera from convalescent cattle were collected from affected cattle for laboratory testing to confirm lumpy skin disease. The specimens were prepared as described by Ali and Obeid (1977) for virus and antibody detection.

2.3.2 Traps

A variety of sampling techniques were employed so as to avoid biases, which a particular trap might have towards a certain group of insects. It has been shown that different insects behave differently in terms of peak hours of activity, flying heights, and preferred odours. Therefore several approaches to collection of flies such as sweep nets, CDC light traps and sticky traps were used.

The following traps were used for collecting the different families of biting insects:

a) "Nzi" trap-for general survey use as the standard trap. The Nzi trap (Swahili for fly) is a cloth trap for biting flies (tsetse flies, horse flies, stable flies, etc.) evolved from the "Ngu" series of

traps (Brightwell *et al.*, 1991) designed for community- based tsetse control by Maasai pastoralists. It is triangular, roughly one metre to a side (Mihok, 2002). The body is made from squares and rectangles of blue and black cloth. Cutting out a triangle from a 1-m square piece of white mosquito netting forms the upper cone. Flies are collected into a plastic collecting bag at the top of the cone.

b) CDC (Centres of Disease control) light traps, Rentokil sticky traps, and suction traps were also used whenever possible. The CDC light traps are battery-powered traps with a small bulb and netting material at the base of the trap. They were switched on in the evenings between 6.45 p.m. and 7.00 p.m. The mouth suction traps were used to catch flies directly off animals on which they had landed.

Trapping was done both with and without odour attractants for sampling various communities of biting flies. These odours included acetone, urine (from cattle) and octenol (1-octen-3-ol). The concentration of the odours was acetone 880mg/h, and octenol 2.0mg/h (Mihok *et al.*, 1995a).

Mosquitoes and sandflies were collected for 4 -5 consecutive days per month from July to November 1995 in Machakos district. The sticky traps were placed in termite mounds and animal burrows close to the cattle pens at around 6.00 p.m. and CDC light traps were placed within

10 metres of the cattle pens and switched on at 7.00 p.m. The insects were collected the following morning between 6.00 a.m and 7.00 a.m. They were placed briefly in antibiotic and fungizone fortified media, placed in sterile water and transferred into 2 ml cryovials containing 10 % DMSO. The vials were then transferred to the vapour phase of liquid nitrogen for 30 minutes for transportation and storage at -196^0 C in the laboratory.

2.4 STUDY SITES

2.4.1. LSD outbreak areas – insect sampling sites

The outbreak areas visited were located in three districts in Kenya namely: Machakos, Kajiado and Kiambu (Figure 1). These districts represent three different geographic regions and livestock production systems in Kenya, namely the large-scale dairy cattle production, small-scale dairy cattle production and small-scale dairy-meat cattle production (Peeler and Omore, 1997). These geographic regions are located in different agro-climatic zones (Figure 2) in Kenya, (Jaetzold and Schmidt, 1982, 1983). Insect collections from the Nairobi National Park bordering Ngong in Kajiado, and from the National Veterinary Research Centre (NVRC) Muguga (bordering some parts of the Kiambu outbreak) acted as controls at the time when the LSD outbreaks occurred in Ngong and in the Kikuyu area.

Figure1. Map of Kenya Showing the study sites

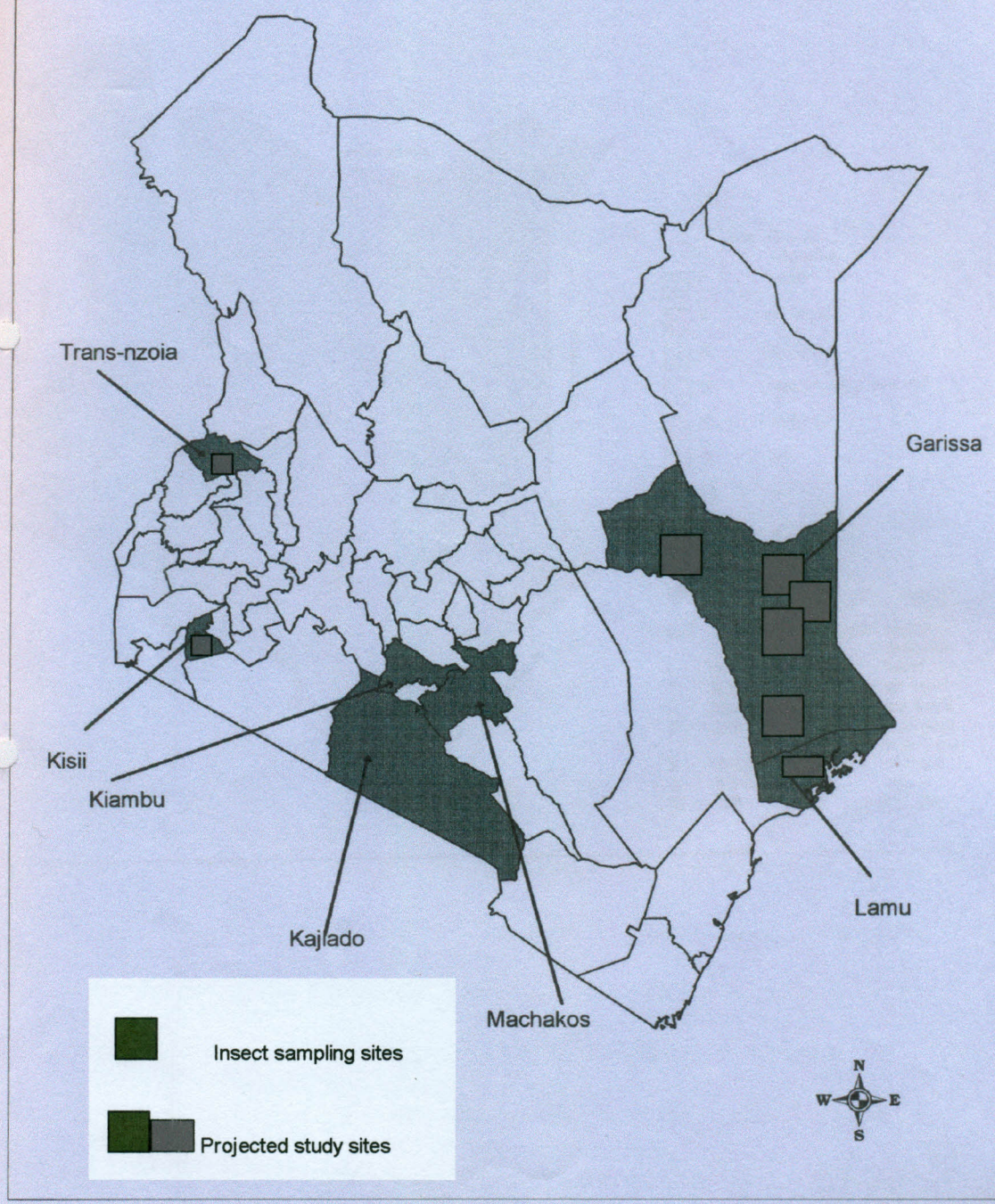
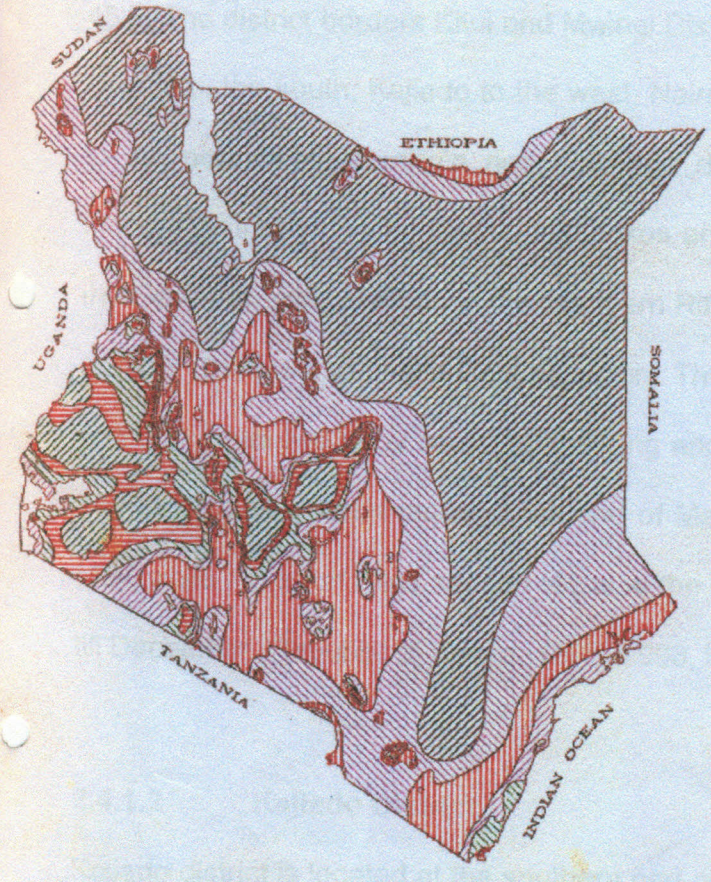


Figure 2. Agro-climatic zone map of Kenya



KEY

Zone	Climatic designation
I	humid
II	sub-humid
III	semi-humid
IV	semi-humid to semi-arid
V	semi-arid
VI	arid
VII	very arid
	Lakes

LEGEND EXPLANATION

Zone	r/EO ratio	r/EO ratio in %	climatic designation
I	>0.8	>80	humid
II	0.65-0.80	65-80	sub-humid
III	0.50-0.65	50-65	semi-humid
IV	0.40-0.50	40-50	semi-humid to semi-arid
V	0.25-0.40	25-40	semi-arid
VI	0.15-0.25	15-25	arid
VII	<0.15	<15	very arid

2.4.1.1 Machakos District

Machakos District is found in Eastern Province of Kenya. From north to south, the district stretches from latitude 0^0 45' S to Latitude 1^0 31' S. From east to west it is located between Longitudes 36^0 45' E and 37^0 45'E. The district borders Kitui and Mwingi Districts to the east, Makueni District to the south, Kajiado to the west, Nairobi City and Thika District to the northwest, Murang'a and Kirinyaga districts to the north and Mbeere district to the northeast. Machakos and parts of Thika being in the Eastern region, Kajiado in the Southern Rift region, and Kiambu and parts of Thika being in the Central region. The district is generally hot and dry. It has two rainy seasons, the long and the short rain seasons. The long rains season starts at the end of March and continues up to May, while the short rains season starts at the end of October and lasts till December (Republic of Kenya, 1997, 1998,1999, 2000, 2001).

2.4.1.2 Kajiado District

Kajiado district is located at the southern part of the Rift Valley province of Kenya. The Republic of Tanzania borders it to the southwest, Taita-Taveta District to the Southeast, Machakos and Makueni Districts to the east, Nairobi to the Northeast, Kiambu District to the north and Narok District to the west. The District is situated between longitudes 36^0 5' and 37^0 5' east and between latitude 1^0 0' and 3^0 0' south. The land rises to about 2500 metres above sea level at the Ngong hills area where the LSD outbreaks occurred. The District has a bimodal rainfall pattern. The short rains fall between October and December while the

long rains fall between March and May. (Republic of Kenya, 1997, 1998, 1999, 2000, 2001).

There was an outbreak in the Ngong area of Kajiado district from September to end of October 1996 and a survey was carried out from the end of October 1996 to February 1997 to determine the possible vectors of the disease during this outbreak. An initial survey was carried out in which eight farms in Kibiko and Kerarapon areas (in Ngong) were chosen randomly and Nzi traps and CDC light traps were set up in them. Serum samples were collected from the animals that had been involved in the outbreak. Following this survey, Nzi traps were set up in these farms and weekly collections of insects were carried out.

2.4.1.3. Kiambu District

Kiambu District is located in Central Province of Kenya. The district borders Nairobi City and Kajiado District to the south, Nakuru district to the west, Nyandarua District to the northwest and Thika District to the east. The District lies between latitudes 0^0 75" and 36^0 85" (Republic of Kenya, 2001)

In the Kikuyu outbreak, the traps were set up in various localities in Ondiri (5 sites), Kiambu (2 sites) and Karai (2 sites). Biopsies were obtained from the nodules on the legs and thighs of two of the affected

animals with the permission of the farmers who owned those particular animals.

2.4.1.4. Muguga

Nzi traps were set up in three localities at the National Veterinary Research Centre (NVRC) in Muguga. The traps were placed at the Gacuthi, Kenyambu and Kevevapi compounds. The Gacuthi site was in the middle of a large paddock and the trap was put next to a large tree. The Kevevapi site overlooked a dam and the trap was placed on a slope. In Kenyambu the trap was placed next to a tree near the cowsheds where cattle rest at night.

2.4.1.5 Nairobi National Park

Nzi-traps were set up at four different sites in the park. The first trap was placed about 2 metres from a dam, positioned two kilometres from the main gate. The next site was at the edge of the forest in the Park and the last two sites were situated right in the middle of a forest. The sites were in the western part of the Park that is, in the direction opposite to Ngong in Kajiado District where LSD outbreaks had occurred.

2.4.2 Other study sites

Data was collected from the Director of Veterinary Services annual reports and from the District Annual reports on LSD outbreaks in all Districts in Kenya (Appendix 7) from 1981 to 2001. In eight Districts (Figure 1), namely Transnzoia, Kisii, Kiambu, Kajiado, Thika, Machakos, Lamu and Garissa, various climatic variables were also collected for the same period of time. The climatic variables were correlated with the LSD outbreaks from 1981 to 2001.

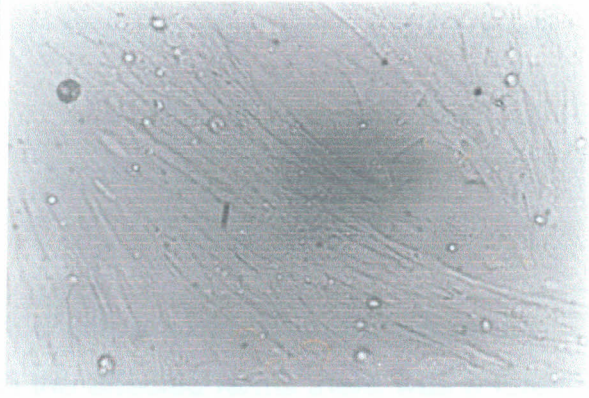
2.5 VIRUS ISOLATION

The flies were identified using various keys by Zumpt (1973), and sorted into various pools. The insect pools were ground up using a sterile mortar and pestle. The grinding was done in a solution of fortified medium (BAPBS and fortified with a penicillin, streptomycin and neomycin (PSN). The mixtures were then transferred to 1.5ml Eppendorf tubes and spun at 3000rpm for 10 minutes in a refrigerated centrifuge. The supernatant was harvested and stored at $^{-}70^{o}C$. These insect samples were later inoculated onto monolayers of prepubertal Lamb testes (LT) cell cultures in 25 cm^2 Falcon flasks. The cells were observed daily for cytopathic effect (CPE) (Plate 1) for up to twelve days.

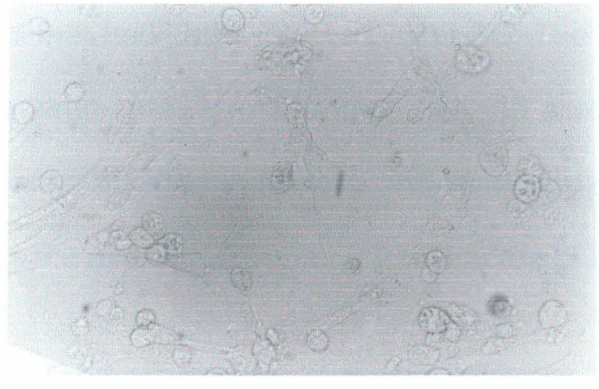
2.6 BLOOD MEAL ANALYSIS

Blood was obtained from the guts of engorged insects and tested using an Enzyme linked immunosorbent assay (ELISA) technique, to determine the animal species from which the blood meal was derived. Anti-human, anti-bovine, anti-goat, anti-sheep, anti-chicken, anti-dog, and anti-lizard horseradish peroxidase conjugates were used in the detection of insect host species. The reagents were highly specific for the individual animals tested. The tests were carried out at KEMRI and ICIPE, which serve as the Eastern Africa regional referral centres for such assays.

PLATE 1 Lamb testis cells uninfected cells (Top), LSD infected cells (bottom).



Non Infected Lamb testis cells with intact monolayer of spindle shaped cells



LSD infected cells with refractile-rounded cells due to cytopathic effect. Monolayer not intact.

2.7 WEATHER CONDITIONS

Data of rainfall, minimum and maximum temperatures, relative humidity (at 9.00 a.m. and 3.00 p.m.), sunshine hours, radiation and wind speed, were obtained from the daily records of nearby meteorological stations, namely Muguga, Machakos, and Dagoretti.

2.8 RESULTS

2.8.1 Features of LSD outbreak farms

In all the affected farms there was a forested area or bush land in the vicinity as well as a stream or dam nearby (Table 1). In nearly all the areas where there were outbreaks there were no continuous rows of farms that had cattle infected with LSD. The farms in Machakos district were large-scale farms ranging from 15 acres to 5000 acres; whereas the farms affected in Kajiado and Kiambu districts ranged from 1 to 40 acres the average size being 2 to 3 acres of land.

The arthropods collected from these outbreak areas included mosquitoes (*A. gambiae* Giles and *Culex antennatus* Becker) Tabanids, (*Haematopota* Meigen and *Atylotus* Wiedemann species), stable flies (*Stomoxys calcitrans* (L.), *S. niger niger* Marquart, *S. ochrosoma* Speiser, *S.* sitiens Rondani, *S. taeniatus* Bigot, and *S. niger bilineatus* Grunberg) and ticks (*Boophilus decoloratus* Koch).

TABLE 1. LSD outbreak areas and arthropods collected

Location and no. of farms	Habitat	Out break dates	% Of animals affected	Arthropods collected
Machakos	4 Farms in Kima and Veterinary farm Forested hills, streams bordered by thickets, active and inactive termite mounds, undergrowth present, water borehole	Feb., Mar. and July 1995	13% (91/698)	Mosquitoes, Tabanids, sandflies,
Kiambu	5 Farms in Ondiri, and Kaimba in Kikuyu location Most farms bordered forested areas, with streams of water or irrigation canals in the vicinity	Mar. and April 1998	20% (8/41)	Stomoxyinae, ticks, mosquitoes, tabanids
Kajiado	7 Farms in Kerarapon A and B, and Ngong. Three of the farms had forested areas and the other three consisted of bush land, Flowing streams present	Sept. to October 1996	49 % (25/51)	Stomoxyinae, Culicoides, mosquitoes

2.8.2 Machakos

The outbreaks in Machakos occurred during the months of March, April and July 1995 and the sampling was done for 4 - 5 consecutive days per month from July to November 1995. The farms in Machakos were large-scale farms ranging from 15 acres to 5000 acres.

2.8.3 Kajiado

The farms affected in the Ngong area of Kajiado District outbreak mostly had exotic (*Bos taurus*) breeds of cattle. In one of the farms, the

farmers claimed that there were buffaloes in a nearby forest and they described the existence of some "large biting insects" which would cause the animals to get very irritable and run after they had been bitten.

In the affected farms in Kajiado District, a total of 4,118 *Stomoxys* flies were trapped. Flies of the species *Stomoxys* were predominant, with *Stomoxys niger*, and *S. calcitrans* forming the largest proportion (41%), and (40.7 %), respectively. The other species that were trapped included, *S. bilineatus* (14.9 %), *S. sitiens* (2.1 %), *S. taeniatus* Bigot (0.07 %), *S. ochrosoma* (0.95%). Non- biting muscids and three flies of *Culicoides* species were also caught. The "domesticated" species of *Stomoxys* such as *S. calcitrans* and *S. niger* were also found in the National Park though not in large proportions as they occurred in the farms with LSD outbreaks.

When the Student Newman's Keul test was performed on these data, the results obtained showed that the months and species showed some significant differences (Table 2). There was no significant difference between the sexes of the insects, the means for the females was 11.93 and that of the males was 11.91 (Table 2, $P > 0.05$).

TABLE 2. *Stomoxys* population during LSD outbreak in Kajiado District (Student Newman's Keul (SNK) Test)

SOURCE	DF	ANOVA SS	Mean Square	F Value	Pr > F
MONTH	3	39119.94797	13039.98266	10.08	**0.0001**
SEX	1	0.02778	0.02778	0.00	0.9963
SPP	5	53242.08951	10648.41790	8.23	**0.0001**
SEX*SPP	5	1832.28704	366.45741	0.28	0.9221

The SNK grouping shows no significant difference between the mean numbers of *S. niger* and *S. calcitrans* but both of them were significantly different from the rest of the *Stomoxys* species (Table 3).

TABLE 3. Student Newman's Keul Grouping of various species of *Stomoxys* in Kajiado District

Species	SNK Grouping	Mean
S. niger	A	30.926
S. calcitrans	A	27.5
S. bilineatus	B	11.130
S. sitiens	B	1.463
S. ochrosoma	B	0.463
S. taeniatus	B	0.056

Species with the same grouping are not significantly different

The insect population increased after the short rains had started (Figure 3) and *Stomoxys calcitrans* and *S. niger* had higher means compared to the other flies.

2.8.4 Kiambu

In Kikuyu division of Kiambu district, an outbreak occurred from March to April 1998 in farms that were as close as 100 metres apart and in other farms that were as far as seven kilometres from the nearest

affected farm. The clinical signs that were observed in these animals included skin nodules.

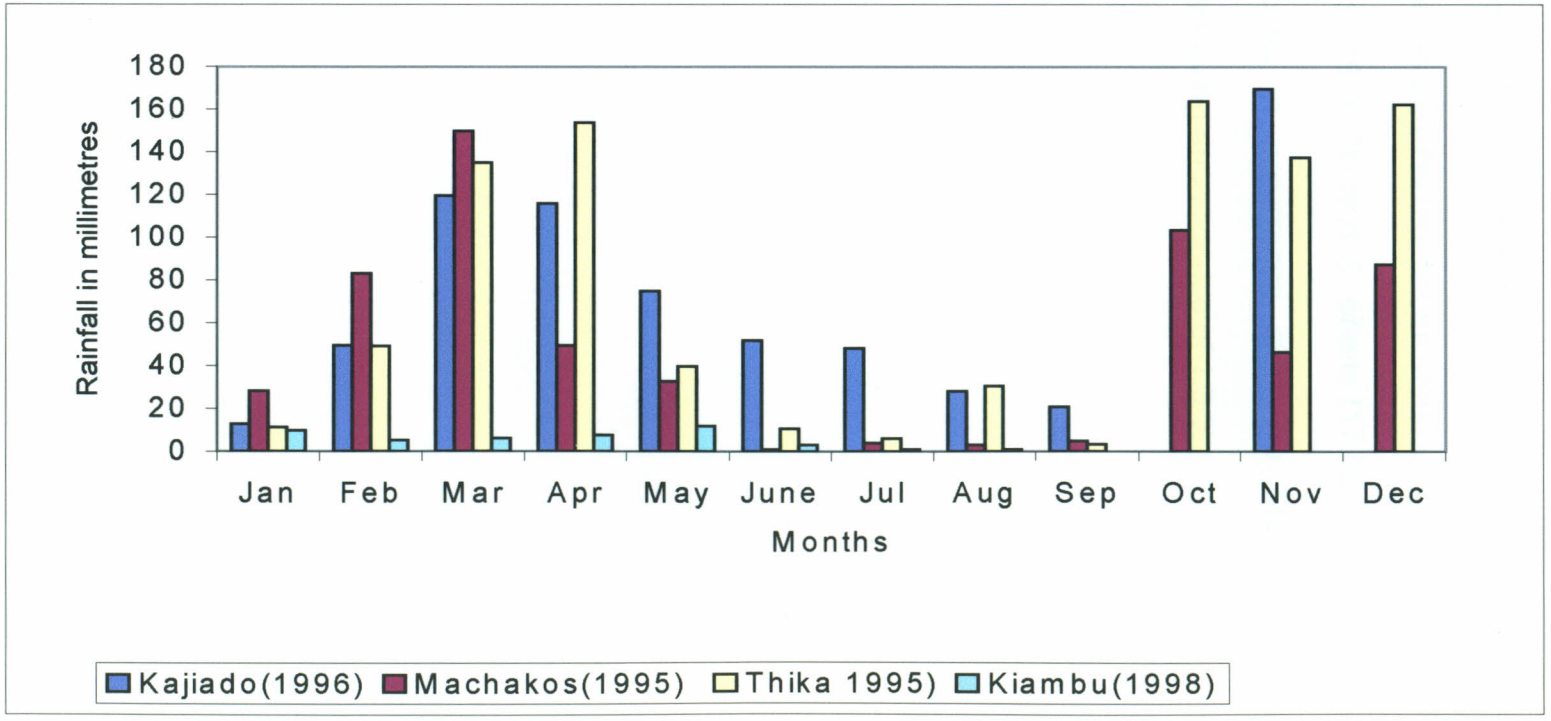
The LSD outbreaks were reported in Kahuho, Muguga and Kabete locations. The outbreak farms in Kiambu District (Kikuyu area) mostly had streams nearby and plenty of trees were present (Table 1). Ondiri swamp bordered several of the farms. In the first farm it was observed that quite a number of stable flies were feeding on the infected animal but the animal was also responding to the bites of the flies by whisking them off with its tail. Some of these flies were used in the blood meal analysis. In one of the farms studied, there was common grazing land shared with neighbours' animals. Mixed farming was practised in most of the farms- and sheep, donkeys, goats and cattle were kept.

In Ondiri location, vaccination had been carried out but the outbreak still affected some of the animals. The signs and symptoms noted in the animals that were infected with LSD in the farms included oedema of the legs, lumps and nodules on the skin, lesions and emaciation. The lesions were present especially on the head and legs. The animals affected ranged from five months old to three years old. Both cross-bred (*Bos taurus*) and zebu (*Bos indicus*) cattle were affected. The animals affected ranged from five months old to three years old.

A total of 4651 insects were collected from the Kikuyu area. Out of these insects, only one sample of *Prostomoxys* species yielded a positive result for the presence of LSDV. The arthropods collected from the Kikuyu outbreak included *Stomoxys* (forming 98.4% of the total catch), mosquitoes (2 %), tabanids (0.3%), and ticks (0.3%).

The specific species included *Stomoxys calcitrans* (27.5%), *S. bilineatus* (8.5%), *S. niger (*60.5%), *S. inornata and S. brunipes (0.8%)*, *S. sitiens (1.9%)*, *Prostomoxys* species (0.14%). *Anopheles gambiae*, *Culex* species, and *Boophilus* ticks. *Stomoxys* was again predominant. Here the females were apparently more abundant than the males. Even though both sexes feed on blood, the females need a blood meal for the purposes of formation of eggs and therefore will look for a host aggressively. Once again *S. niger* was the most abundant species followed by *S. calcitrans* and *S. bilineatus*. The abundance followed the same order as was observed in Kajiado District.

Figure 3. Rainfall in the outbreak areas



2.8.5 Incidence of arthropod species in the LSD outbreak areas

A total of more than twenty-nine different species of insects was collected from three outbreak areas at different times (Table 4). The *Stomoxys niger* species had the highest incidence 33.47 %, followed by *Stomoxys calcitrans* (18.04 %) and *S. sitiens* (17.03 %). The mosquito species were second in occurrence after the Stomoxyinae, with *Anopheles gambiae* having an incidence of 1.3 %.

TABLE 4. Frequency of occurrence of various insect species in the field during LSD outbreaks in Machakos, Kajiado and Kiambu Districts

SPECIES	FREQUENCY OF OCCURENCE	PERCENT	CUMULATIVE
Stomoxys calcitrans	90	18.04	18.04
S. sitiens	85	17.03	35.07
S. niger niger	90	18.04	53.11
S. niger bilineatus	77	15.43	68.54
S. ochrosoma	53	10.62	79.16
S. taeniatus	52	10.42	89.58
Sergentomyia bedfordi	2	0.40	89.98
Sergentomyia squamipleuris	2	0.40	90.38
Phlebotomus martini	2	0.40	90.78
Sergentomyia schwertzi	1	0.20	90.98
Sergentomyia antennatus	1	0.20	91.18
Sergentomyia africana	1	0.20	91.38
Culex antennata	1	0.20	91.58
Aedes ochraceous	1	0.20	91.78
Anopheles gambiae	6	1.2	92.99
Anopheles funestus	1	0.20	93.19
Boophilus decolaratus	1	0.20	93.39
Hematopota Spp.	1	0.20	93.59
Aedes spp.	2	0.40	93.99
S. brunipes	4	0.80	94.79
Prostomoxys large	3	0.60	95.39
Prostomoxys small	4	0.80	96.19
Atylotus spp.	2	0.40	96.59
Mosquitoes (unspeciated).	5	1.00	97.60
Mixed	2	0.40	98.00
Rhinomusca	1	0.20	98.20
Culicoides	3	0.60	98.80
Latifrons	1	0.20	99.00
S. hirtifrons	1	0.20	99.20
S. inornata	1	0.20	99.40
Stygeromyia	1	0.20	99.60
S. varipes	1	0.20	99.80
Tabanus spp.	1	0.20	**100.00**
Total	**499**	**100.00**	

When a one-way Anova was performed to determine whether there were any differences between the various groups of insects, it was found that the differences between the male and females insects were significantly different (Table 5).

TABLE 5 Comparison of the sex of the insects from Kajiado, Kiambu and Machakos LSD outbreak areas (One-way analysis of variance)

Insects	Degrees of Freedom	F-Factor	Prob. > F
Between species (males)	32	4.46	0.0000
Within species (males)	454		
Between species (females)	32	9.68	0.0000
Within species (females)	454		

Bartlett's test for equal variances (Males): chi^2 (14) = 795.4719
Prob>chi2 = 0.000
Bartlett's test for equal variances (Females): chi^2 (16) = 940.8115
Prob>chi2 = 0.000

The hypothesis that on average the number of females between the species-groups is statistically the same is not accepted (p-value = 0.0000). Therefore at least one pair of species is statistically different for example the mosquitoes, *A. gambiae* and *C. antennatus* (Appendix 2b). The average number of male flies collected from the field was 18 whereas that of the female flies was 27 for any given day. The Bonferroni test further showed the specific differences among the male and female species. These are highlighted in Appendix 2a and b. The

results from the Bonferroni test reflect differences between *Stomoxys* males and *Phlebotomus* and Mosquito males.

The hypothesis that on average the number of males between the species- (between groups) is statistically the same is not accepted (p-value = 0.0000). Therefore at least one pair of species is statistically different for example *S. bedfordi* and *S. squamipleuris* have a mean difference of 294 flies (Appendix 2a).

2.8.6 Probable vectors of lumpy skin disease

A total of 124 insects were analysed and the results revealed that 14.52% had fed on human blood, 22.58% bovine blood, 14.52% goat, 2.42% mixture of goat and bovine blood, 13.71% sheep and 32.26 % lizard blood (Figure 4). A two-sample t test was further performed on the data to compare the bloodmeals from Machakos and Kiambu (Table 6). The average number of insects found feeding on sheep, humans and goats, are statistically the same between Machakos and Kiambu Districts (p-value > 0.05) (Table 6).

Virus was isolated from *Prostomoxys sp.* collected form the Kiambu outbreak. The lamb testis cells infected with the homogenate exhibited cytopathic effect (Plate 1) and this was later confirmed using the polymerase chain reaction (Plate 4)

TABLE 6 Two-sample t test comparing insect bloodmeals in Machakos and Kiambu

(a) Sheep

Group	Insect species	Mean	Std. Err.	Std. Dev.	(95% conf. Interval)	
Machakos	8	1.5	1.5	4.2426	-2.0469	5.0469
Kiambu	5	1	0.5477	1.2247	-0.5207	2.5207
Combined	13	1.3	0.9225	3.3262	-0.7023	3.3177
Diff		0.5	1.9748		-3.8466	4.8465

Degrees of freedom: 11: Ho: mean (1) - mean (4) = diff = 0
P < t = 0.5976 P > |t| = 0.8048 P > t = 0.4024

(b) Human

Group	Insect species	Mean	Std. Err.	Std. Dev.	(95% conf. Interval)	
Machakos	8	1	0.4226	1.195229	0.0008	1.9992
Kiambu	5	2	1.7606	3.937004	-2.8884	6.8884
Combined	13	1.30	0.9225	3.326275	-0.1269	2.8961
Diff		-1	1.4586		-4.2101	2.2101

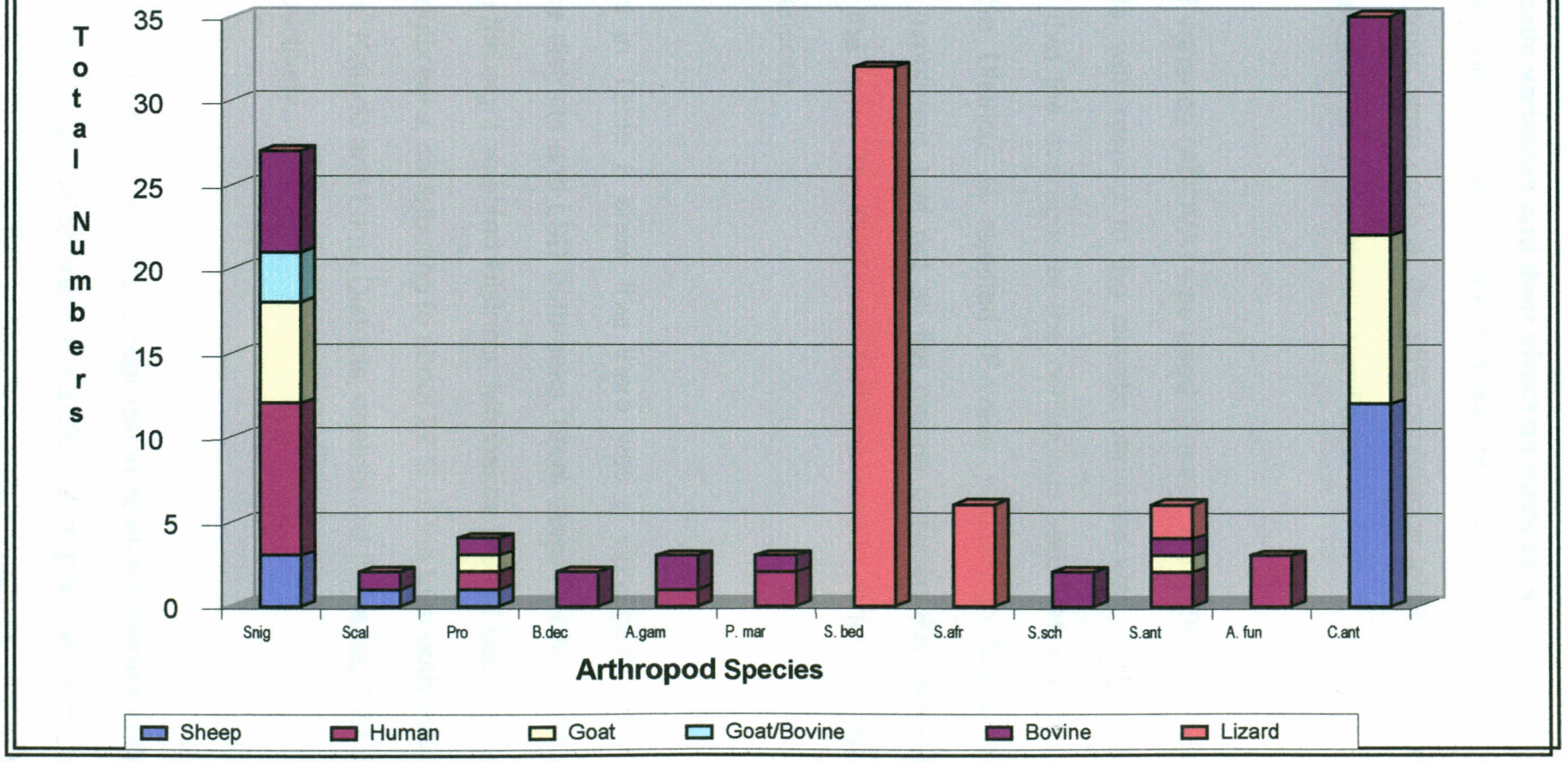
Degrees of freedom: 11: Ho: mean (1) - mean (4) = diff = 0
P < t = 0.2536 P > |t|= 0.5071 P > t = 0.7464

(c) Goat

Group	Insect species	Mean	Std. Err.	Std. Dev.	(95% conf. Interval)	
Machakos	8	1.38	1.2383	3.50255	-1.553205	4.303205
Kiambu	5	1.4	1.1662	2.607681	-1.837864	4.637864
Combined	13	1.38	0.8514	3.069703	-0.4703876	3.239618
Diff		-0.03	1.8278		-4.04796	3.99796

Degrees of freedom: 11
P < t = 0.4947 P > |t| = 0.9893 P > t = 0.5053

Figure 4. Hosts of Arthropods trapped during Kiambu and Machakos LSD Outbreaks



KEY

Sing- *Stomoxys niger niger* **Sca**- *Stomoxys calcitrans* **Pro**- *Prostomoxys s.p.p* **B.dec**- *Boophilus decolaratus* **A.gam**- *Anopheles gambiae* **P. mar**- *Phlebotomus martini*
S. bed- *Sergentomyia bedfordi* **S. afr**- *Sergentomyia africana* **S. sch**- *Sergentomyia schwertzi* **S. ant**- *Sergentomyia antennatus* **A.fun**- *Anopheles funestus* **C. ant**- Culex antennata

2.8.7 Climatic variables and their effect on outbreaks

The software that was used in the analyses was Strata version 7 (Texas, USA). The tests were done at the 95% confidence level. The p-value < 0.05 is considered to be statistically significant.

Analysis of variance (ANOVA) was used to test if the difference between the Districts, with respect to the climatic variables was significant. The hypothesis that the averages of the continuous variables are the same between the Districts is rejected (P-value < 0.05) in favour of the alternative hypothesis that not all the means between the Districts are statistically the same. The Bonferroni test was used in performing the pair-wise comparisons.

The results in Table 8 show that there was a significant association between the districts and LSD outbreaks. From these results it is evident that among the eight selected Districts, Machakos District had the highest number of outbreaks, contributing to about 22 % of the total outbreaks in all the Districts. Kajiado and Lamu Districts, contributed the least, 1.22 % and 5.28% respectively.

Analysis of data using the logistic regression analysis (Strata statistical package) showed that the different areas did not display uniformity as far the effects of the various variables were concerned. This was also reflected

in the species of insects that were present in the four districts. Different areas were affected by different variables even though relative humidity, maximum temperature and wind speed had significant effects on the occurrence of LSD in all the areas, in other words, the higher these variables were the higher the chances of outbreak. Rainfall on its own did not have a significant effect on the outbreak.

TABLE 7 Pearson chi- square distribution showing LSD outbreaks in eight Kenyan districts from 1981 to 2001

District	No of LSD Outbreaks	Total
Kiambu	41[a]	203[a]
	20.20[b]	100.00[b]
	16.67[c]	10.53[c]
Thika	36[a]	240[a]
	15.00[b]	100.00[b]
	14.63[c]	12.45[c]
Lamu	13[a]	252[a]
	5.16[b]	100.00[b]
	5.28[c]	13.08[c]
Machakos	54[a]	246[a]
	21.95[b]	100.00[b]
	21.95[c]	12.77[c]
Garissa	24[a]	252[a]
	9.52[b]	100.00[b]
	9.76[c]	13.08[c]
Kisii	24[a]	252[a]
	9.52[b]	100.00[b]
	9.76[c]	13.08[c]
Kajiado	3[a]	252[a]
	1.19[b]	100.00[b]
	1.22[c]	13.08[c]
Transnzoia	51[a]	230[a]
	22.17[b]	100.00[b]
	20.73[c]	11.94[c]
Total	246[a]	1927[a]
	12.77[b]	100.00[b]
	100.00[c]	100.00[c]

Pearson Chi2 (7) = 96.2322 Pr = 0.000

a =No. of observations ,**b** = proportion (%) , **c**= proportion of influence on outbreak

Relative humidity was significant in that the higher the relative humidity the greater the chances of occurrence of outbreaks. LSD outbreaks have previously been associated with rainfall but these results show that it is the factors that are affected by rainfall (for example relative humidity) that have a significant effect on the outbreak. These are further related to insect occurrence and conditions that are optimal for the hatching of the larvae and pupation. In the areas where relative humidity on its own was significant, the humidity was conducive to the development of the insect species that was found in large numbers in the particular area.

The results suggest that the effects of climatic variables are not constant over the three areas. In Ngong the log odds ratio for maximum temperature, relative humidity at 6 a.m., and wind are (1.277, P = 0.015), (0.992, P = 0.677), and (1.038, P = 0.000) respectively; whereas the same ratios for Kiambu are (1.6011, P = 0.000), (1.0684, P= 0.003), and (1.0367, P = 0.000). There was an indication that maximum temperature and wind had a significant effect on the outbreaks of LSD in both Kajiado and Kiambu districts although there was variation in the levels of their effects. In Ngong relative humidity at 6 a.m. had a significant effect while in Kiambu it had no effect.

The results in Table 8 show that in Machakos, wind was very important (P = 0.000) while the other variables tested (namely, maximum

temperature, relative humidity at 6.00 a.m., minimum temperature and rain), were not important. In (Ngong) Kajiado maximum temperature was very important (P = 0.000), (or else, relative humidity at 12 noon, as shown by Table 8 in which analysis is done by area but using maximum temperature, relative humidity at noon and rainfall). In Kiambu both minimum temperature and relative humidity at 12 noon were important. In Ngong relative humidity at 6 a.m. had a significant effect while in Kiambu it had no effect.

TABLE 8 Effects of climatic variables on LSD outbreaks in specific districts (Logistic Regression)

Machakos

Outbreak	Odds ratio	Standard error	Z	P>|z|	[95% Conf. Interval]	
Maxtemp	1.085623	.063218	1.411	0.158	.9685274	1.216876
Relhum6	1.022956	.013344	1.740	0.082	.9971332	1.049448
Wind	.9627914	.0051657	-7.067	**0.000**	09527199	0.9729693

Kajiado

Outbreak	Odds ratio	Standard error	Z	P>|z|	[95% Conf. Interval]	
Maxtemp	1.38726	.1141349	3.979	**0.000**	1.180664	1.630006
Relhum6	1.014922	.0139142	1.080	0.280	.988014	1.042563
Wind	1.00009	0.0036516	0.025	0.980	0.9929583	1.007273

Kiambu

Outbreak	Odds ratio	Std. Error	Z	P>|z|	[95% Conf. Interval]	
Mintemp	4.599683	0.904792	7.758	**0.000**	3.128169	6.763408
RH at 12	.9737454	.0122621	-2.113	**0.035**	.9500063	.9980778
Wind	.99825	.0033483	-0.522	0.602	.9917089	1.004834
Rainfall	.980026	.0185524	-1.066	0.287	.9443302	1.017071

Kajiado

Outbreak	Odds ratio	Std. error	Z	P>|z|	[95% Conf. Interval]	
Maxtemp	1.074471	.1016409	0.759	0.448	.892636	1.293348
RH at 12	.9621246	.0157922	-2.352	**0.019**	.9316649	.99358
Rainfall	.9730656	.03136	-0.847	0.397	.9135021	1.036513

Key:

Maxtemp- Maximum temperature; **Relhum6**- Relative humidity at 6a.m

Mintemp- Minimum temperature; **RH at 12**- Relative humidity at 12 noon;

Std error – Standard error; **Conf.** - Confidence

Table 9 shows that there was a significant difference in all the weather variables between the Districts, with all of them having a probability value that is less than 0.05. In other words, for the eight districts that were studied there was a significant difference in the weather variables in the districts, implying that there certain districts that displayed different degrees of the climatic variables studied. The Bonferroni test (Appendix 3) further specified the districts, which displayed the particular differences. In the case of minimum temperature, Kiambu differed significantly from Lamu with a mean difference of about 16^0 Centigrade, while Garissa and Lamu differed significantly by one degree centigrade (Figure 5). For maximum temperature, Kiambu differed significantly from Garissa by a mean of approximately 12^0 C, whereas Kajiado differed from Kiambu by a difference of about 2^0 C.

In terms of rainfall, only Machakos differed significantly from Kisii with a mean difference of about four millimetres. The statistical model shows significant differences between most of the districts with respect to relative humidity at 9.00 a.m. (0600 Z) (Appendix 3d, P = 0.000) except for Kiambu and Thika (P = 0.894), Kajiado and Thika (P = 0.287), Kajiado and Machakos (P= 0.135), and Transnzoia and Machakos (P = 1.000). Similarly relative humidity at 1200Z (3.00 p.m.) displayed differences between all the districts (Appendix1) except between Thika and Machakos (P = 0.571), Kisii and Kiambu, Kajiado and Thika, Thika and Transnzoia,

Machakos and Kajiado, Kajiado and Transnzoia, and Machakos and Transnzoia (P = 1.000). As far as mean radiation was concerned, the significant differences were observed between Kiambu and Machakos with a mean difference of about 4 Langleys while Kiambu and Kisii had a mean difference of approximately 8 Langleys. The mean sunshine hours showed significant differences between several districts, for example Lamu and Thika had a mean difference of about 2.2 hours of sunshine whereas Kiambu and Transnzoia had a mean difference of roughly 32 minutes.

TABLE 9 Summary data of various weather variables in the districts

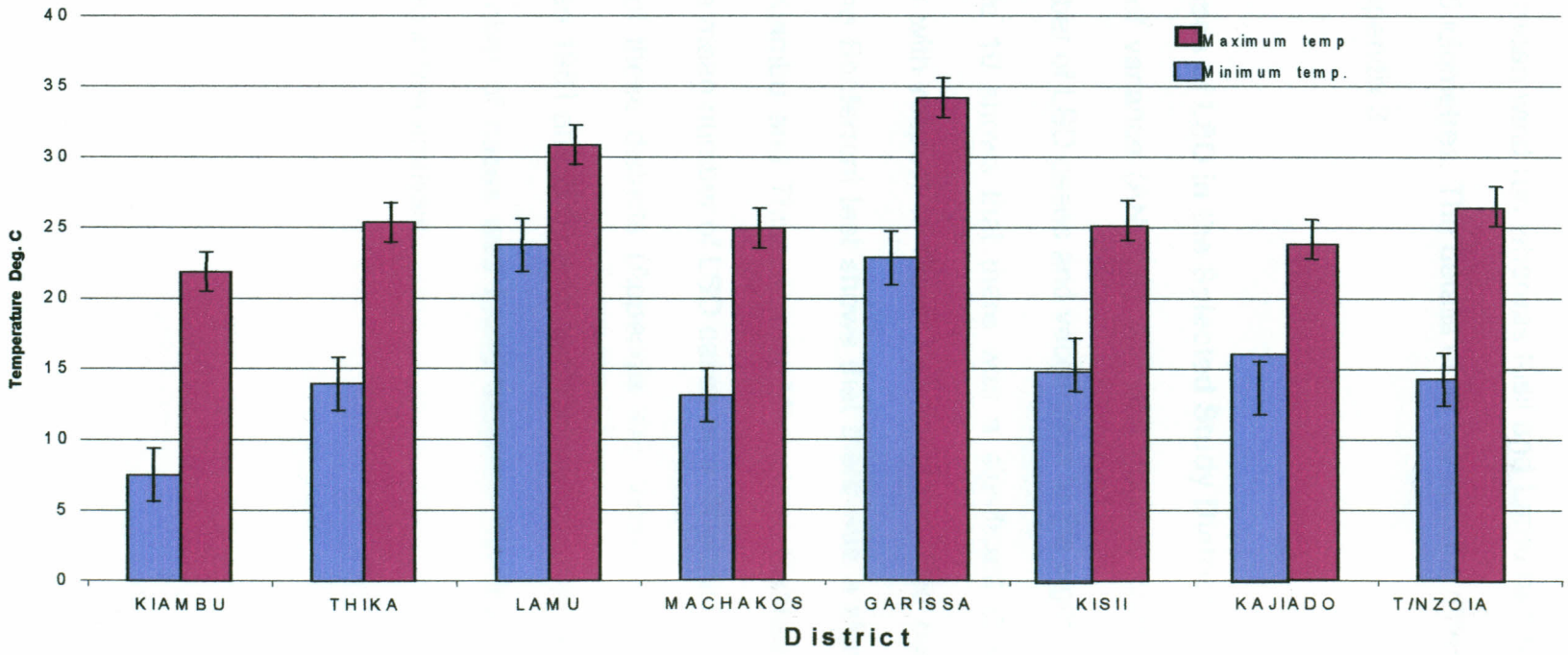
DISTRICT	KIAMBU	THIKA	LAMU	MACHAKOS	GARISSA	KISII	KAJIADO	TRANSNZOIA	ANOVA ANALYSIS	
									F-FACTOR	P>F
MINIMUM TEMP.	7.51	13.92	23.77	13.04	15.29	15.28	13.69	14.27	716.37	0.000
MAXIMUM TEMP.	21.86	25.35	30.85	24.97	25.51	25.51	24.19	26.51	109.78	0.000
MEAN RAIN	5.82	3.41	2.89	2.18	.	.	3.22	3.84	3.30	0.004
TOTAL RAIN	82.93	80.10	86.23	57.11	170.22	170.22	69.80	99.55	.	0.000
RH 600Z	92.21	81.01	75.41	78.21	67.31	67.31	79.57	77.84	166.23	0.000
RH 1200Z	56.72	51.69	67.18	50.36	57.31	57.31	50.33	49.68	107.85	0.000
MEAN RAD.	18.46	19.11	.	21.91	23.51	23.51	21.53	20.87	33.53	0.000
MEAN SUM	6.60	6.48	8.70	7.20	7.02	7.01	7.11	7.08	22.60	0.000
WIND RUN 6	.	.	.	175.47	118.57	118.57	44.36	157.66	30.00	0.000
WIND SPEED 6Z	.	.	127.47	3.70	8.10	8.10	79.97	3.96	ND	ND
WIND SPEED 12Z	3.30	.	11.23	7.19	4.43	4.43	3.39	6.786.78	ND	ND
WIND RUN 12	6.06	.	154.14	.	175.83	175.83	6.71	205.07	ND	ND

Temp. – Temperature
RH - Relative Humidity
P - Probability

ND - Not determined
Rad. – Radiation

Rain. – Rainfall
. - Missing data

FIGURE 5. Mean temperatures in the eight districts studied



Transnzoia and Garissa districts exhibited significant differences of about 57 kilometres for the mean wind run whereas Kisii and Lamu had a mean difference of about 15 kilometres. The details of all the specific differences are represented in Appendix 3.

2.8.8. Number of Cases of LSD in the Selected Study Districts

A one-way analysis of variance (ANOVA) test was done to check if the difference in the number of LSD cases and vaccination in the eight districts was significant. Table 10 shows that there was a significant difference between the districts with respect to these two variables. The pair-wise comparisons using the Bonferroni test shows that there was a significant difference between Kiambu and Thika, Lamu, Machakos, Garissa, Kisii, and Transnzoia in the mean number of LSD cases by a difference of about one case for each of these districts (Appendix 4a). When the 20-year period of time between 1981 and 2001 was considered, the only significant difference in the number of cases was found between Kiambu and the other six Districts used in this analysis.

TABLE 10 LSD cases and vaccination of cattle in the various districts (One-way ANOVA) (1981 – 2001)

Variable	Source	Sum of Squares	DF	Mean Sum of squares	F	P > F
Number of cases	Between districts	41.4343174	7	5.9191882	3.46	0.0011
	Within districts	2897.81554	1693	1.71164533		
	Total	2939.24985	1700	1.7289705		
Vaccination	Between districts	.181059929	7	.025865704	2.30	0.0246
	Within districts	20.5791358	1831	.011239288		
	Total	20.7601958	1838	.011294992		

When a logistic regression analysis was performed to find out the likelihood of the various climatic variables affecting the LSD outbreaks it was established that there was significant effect exerted by various variables (Table 11). The higher the relative humidity at 9.00 a.m. the higher the chances of an LSD outbreak and the same applied to the mean windrun.

There was a significant effect exerted by relative humidity at 9 a.m. (Kenyan time), and 3.00 p.m. Similarly the effect of wind was also significant.

TABLE 11. Interaction between the climatic variables and LSD outbreaks in the districts (Log odds regression)

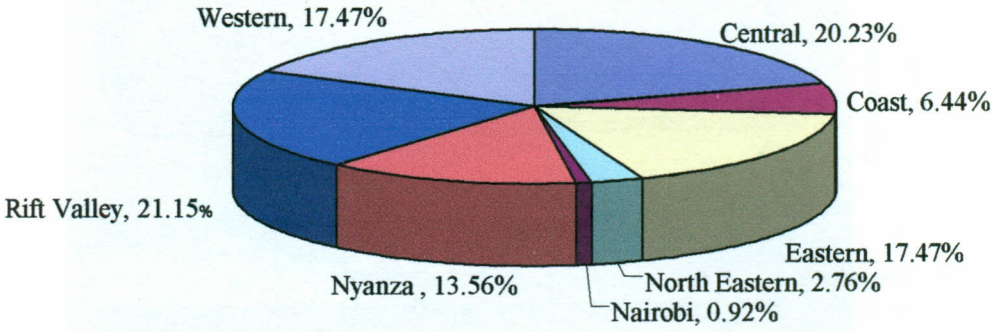
Varaiables	Odds Ratio	Std. Err.	Z	P>|z|	95%Conf. Interval]	
Machakos	2.389504	1.21523	1.71	0.087	.881885	6.474459
Kisii	1.755981	1.422901	0.69	0.487	.3587376	8.595332
Kajiado	.0418122	.0363064	-3.66	0.000**	.0076241	.2293081
Transnzoia	1.289861	.7233969	0.45	0.650	.4296953	3.871908
Minimum Temp.	1.018575	.0443305	0.42	0.672	.9352916	1.109275
Maximum temp.	.8730885	.0828272	-1.43	0.153	.7249489	1.0515
RH 0600 Z	1.109716	.0431388	2.68	0.007**	1.028306	1.19757
RH 1200 Z	.9408074	.0265955	-2.16	0.031*	.8900989	.9944048
Total Rainfall (mms)	1.001171	.0020259	0.58	0.563	.9972077	1.005149
MeanWindrun	1.010445	.0045614	2.30	0.021*	1.001544	1.019425
Wind speed12 Z	1.124796	.094964	1.39	0.164	.9532547	1.327208

* Significant at P < 5 % ** Significant at P < 1 %

2.8.9. LSD Outbreaks in Kenyan Districts in the period 1981-2001

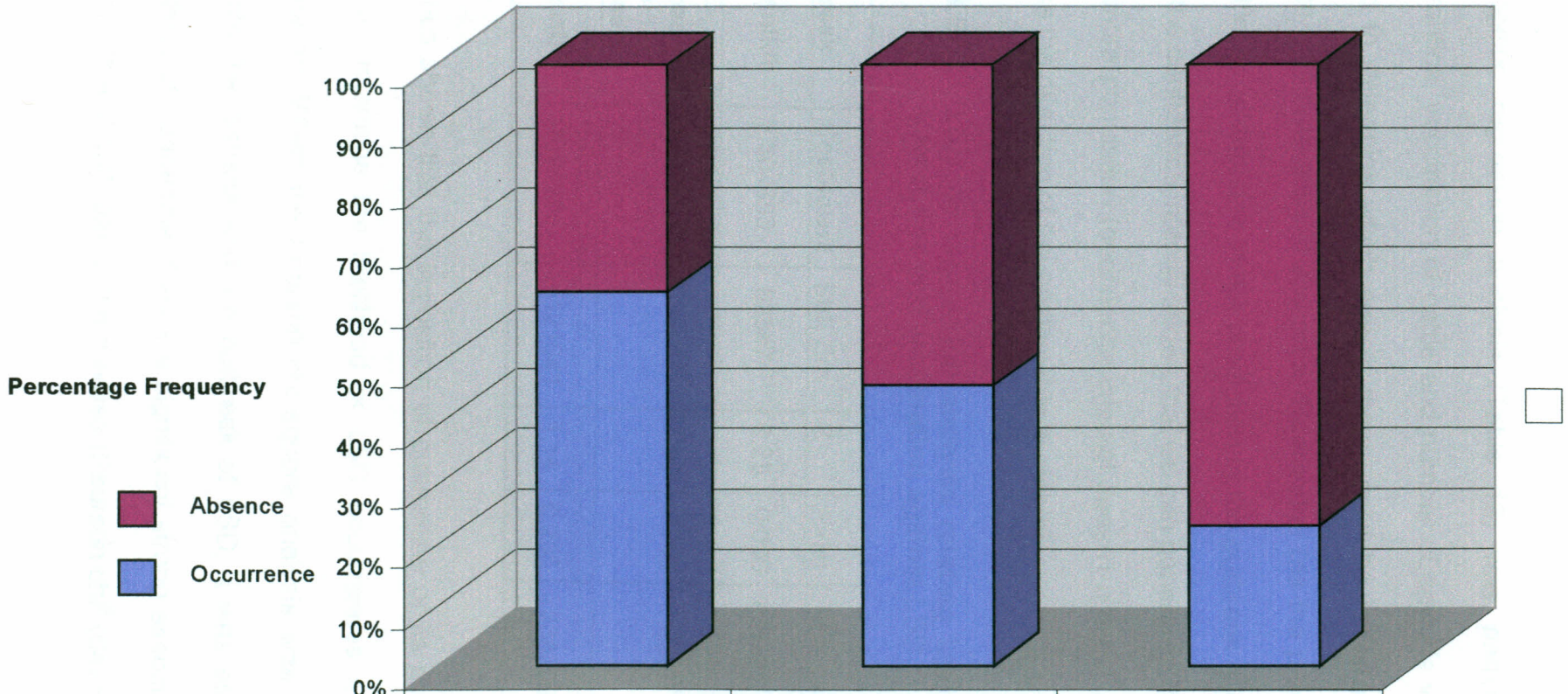
The pie chart (Figure 6.) shows that Rift Valley and Central Province had the largest proportion of outbreaks during this twenty-year period. Nairobi Province had the smallest number of outbreaks. The outbreaks appeared to be more frequent in certain agro-climatic zones than in others.

FIGURE 6. Lumpy skin disease outbreaks in Kenyan Provinces (1981-2001)



When the proportions of occurrence or absence of quarantine, vaccination and LSD outbreaks were compared it was noted that during this twenty-year period there were more times that there were no quarantines than the times that quarantine was imposed against LSD (about 77% and 23% respectively, Figure 7). The frequency of absence of vaccinations against LSD was also higher than that of vaccinations of cattle (54% and 47% in that order, Figure 7).

Figure 7 **Comparison of LSD outbreaks, vaccination of cattle and quarantine against LSD in Kenyan Districts between 1981 and 2001**



	Outbreak	Vaccination	Quarantine
Absence	38.00	53.53	76.67
Occurrence	62.00	46.47	23.33

A logistic regression analysis (Table 12) was performed in which quarantine, vaccination of cattle and number of animals vaccinated were used to determine which one of them would be important in predicting future LSD outbreaks in any area. Quarantine (as the predictor) makes a significant contribution in the prediction of the outbreak (p-value = 0.000), and the confidence interval shows that we can generalise these findings to the entire population (confidence interval doesn't cross 1 and is positive; hence the direction of the relationship is stable).

TABLE 12. Logistic regression analysis relating LSD outbreaks to quarantine and vaccination against LSD

Outbreak	Coefficient	Std. Err.	Z	P>|z|	95% Conf. Interval	
Quarantine	3.304653	.52847	6.25	0.000	2.26887	4.340435
Number of cattle vaccinated	$8.18e^{-06}$	$7.85e^{-06}$	1.04	0.298	$-7.22e^{-06}$	.0000236
Vaccination	.4694994	.4121041	1.14	0.255	-.3382099	1.277209

It is thus shown that the imposition of quarantine or its absence can be used to determine the likelihood of LSD occurrences as opposed to vaccination. When the Pearson chi square analysis was carried out to correlate the districts and the outbreak of LSD, it was established that regardless of quarantine, there's a significant strong association between the districts and outbreak of the disease (Pearson chi^2 (64) = 109.5995, p-

value = 0.000[measure of association], Cramer's V = 0.5102 [measure of strength]) (Appendix 3.)

2.9 DISCUSSION

The symptoms displayed by the animals infected in the field outbreaks resembled the ones described by Von Backstrom in 1945 when LSD was becoming established. It is interesting to note that these appeared when LSD was re-emerging in the cattle population in Kenya after a period of quiescence. The different climatic variables had different effects on LSD outbreaks in the areas studied. This could be related to the occurrence of different species of insects in these areas. In Machakos for example, wind plays an important part and sandflies were the predominant species of insects that were trapped in the outbreak areas. Sandflies are small in size and can easily be carried by wind. Their flight range is so limited (rarely over 50 metres) and usually their breeding habitats are within a few metres of their feeding places. The sandfly eggs hatch within 6-17 days but this period may be prolonged in cooler weather. This timing is similar for the other insect species that were encountered during these surveys and explains the increase in the numbers of different insects two weeks after the onset of rainfall in December in the Ngong area.

Quarantine may stop the animals from movement between the affected districts but it will not stop the disease agent, for example, the effect of

wind on the vectors. It is for this reason that there may still be outbreaks despite quarantines being imposed but quarantine helps as was observed in the significant relationship between quarantine and the districts with LSD outbreaks.

Biting insects appeared to be associated with LSD outbreaks although no virus was isolated from the field caught insects. *S. niger* and *S. calcitrans* are important pest species in Africa that plague livestock in many areas (Zumpt, 1973). In Zanzibar, *Stomoxys nigra* Marquart constitutes 90-98 % of the *Stomoxys* species. This species is found mostly in Africa and this may perhaps explain the higher incidence of LSD mostly in Africa compared to other continents. In Zanzibar these flies are found in high concentration in areas of the island that are heavily forested with poorly drained soils and in low concentrations in the sandy coastal areas" (Patterson, 1989). This can be compared to the findings of the spectra of insects/*Stomoxys spp.* collected during the LSD outbreaks in Kiambu and Machakos. Machakos has an abundance of termite mounds and sandy soils and thus sandflies were most prevalent during LSD outbreaks whereas in Kiambu and Kajiado, *Stomoxys nigra* was more prevalent. . *Stomoxys nigra* requires a high moisture level for the larval habitat (Kunz and Monty, 1976) and according to Patterson (1989), its' adult population normally increases two weeks after the heavy rains.

"*Stomoxys nigra* is one of the most underrated pests of cattle. By feeding on these animals, these flies weaken them, thereby predisposing them to other diseases (Patterson, 1989)". Ecological evidence suggests that stable flies are strongly agile dispersing far and wide (Eddy *et al.*, 1962). *Stomoxys* is an important animal pest because both the male and female flies feed on blood unlike sandflies and mosquitoes.

Biting flies are frequently disturbed and are likely to feed on a number of animals per day. The probability of contact with at least one infected animal is much greater for flies than for less mobile arthropods such as ticks. Therefore even if ticks were capable of harbouring the virus the chances that they would play a significant role in the transmission of LSD are hampered by their lack of mobility.

Vaccination of animals against Lumpy skin disease was among the main activities carried out by the Machakos District Veterinary officers just before and after the reported outbreaks in the district. In 1993, 528 animals were vaccinated; in 1994, 450 animals were vaccinated and in 1995, 90,900 animals were vaccinated. (Republic of Kenya, 1996). Apparently after the outbreak in 1995, many farmers opted to have their animals vaccinated and there was awareness of the effects of LSD on animals.

Some "wild" species of *Stomoxys* such as *S. ochrosoma* were present in the outbreak farms in Ngong and were also trapped in the control area (Nairobi National Park). This is an implication that they travelled some distance from the Park to these farms and therefore corroborates the finding that wind or the distance travelled by the insects plays an important role in outbreaks of LSD.

2.9.1 Climatic variables and the effect on outbreaks

Analysis of climatic data using the logistic regression analysis (Strata statistical package) showed that the different areas did not display uniformity as far the effects of the various variables were concerned. This was also reflected in the species of insects that were present in the four districts. Different areas were affected by different variables even though relative humidity, maximum temperature and wind-speed had significant effects on occurrence of LSD in all the areas, that is, the higher these variables were the higher the chances of outbreak.

The observation that rainfall alone (Tables 9 and 12) did not have a significant effect on the outbreaks could explain why the outbreaks of LSD are sometimes sporadic and unpredictable. The different species of insects associated with livestock are usually aided in their flight by wind movements and therefore the outbreaks can spread several kilometres

from the source of the original outbreak. The observation that in almost all the areas where there were outbreaks there was no continuous row of farms that had cattle infected with LSD, is further evidence that wind plays a significant role and thus some farms may be bypassed in the process of wind drifts carrying away the infected insects to different localities.

2.9.2 LSD outbreaks in Districts

The analysis of data showed that in all the areas rainfall was not a significant factor in the outbreaks of LSD but rather, the other variables that were affected directly or indirectly by rainfall, such as relative humidity and temperature.

Nairobi Province had the smallest proportion of outbreaks in the twenty-year period from 1981 to 2001 but it is also the smallest province with a small population of cattle compared to the other provinces. Even though Rift Valley Province had the largest proportion of outbreaks, it has more districts (total of 15 districts) and covers a wider area than Central Province, which came second. Therefore in terms of intensity of the outbreaks, Central Province had the largest number of outbreaks of Lumpy skin disease. The numbers of times that quarantine was imposed against LSD in the various districts was small in comparison to the occurrence of the disease (Figure 6). There should be consistency in government policy as far as dealing with LSD outbreaks is concerned. Although the results

from this study have shown that there is a strong association between LSD outbreaks and the districts regardless of quarantine, it is still important to prevent further spread of the disease from one district to the other. The association between the outbreaks and the various districts could be due to the differences in the location of the districts in different agro-climatic zones (this is also represented by the ANOVA results in Table 9), therefore quarantine should be imposed as soon as it has been established that there is danger of the disease occurring.

2.10 CONCLUSIONS

There seems to be different vectors of LSD in different areas and the arthropods that are most abundant in a given locality and can bite infected cattle and then susceptible animals are capable of mechanical transmission. This is evidenced by the occurrence of different species of insects in the different LSD outbreaks studied. In Machakos district for example, sandflies were most abundant (forming 85 % of the total insect catches in that district) but they were not caught in both Kajiado and Kiambu district LSD outbreak farms. A similar correlation has been observed with myxoma, (a rabbit poxvirus) whereby mosquitoes are the most important vectors of the disease in Australia while fleas are the most important in Great Britain (Foil, 1991). It was noted that there was a wide spectrum of insect species in the various localities where LSD occurred (Table 4). Comparable variation of species was also observed in Israel

when LSD outbreaks occurred there for the first time (Yeruham *et al*, 1995).

Besides insects, climatic variables play an important role in the transmission of LSD. Rainfall alone does have a significant effect on LSD outbreaks as has been previously speculated by earlier researchers such as Macowan (1959). Relative humidity, temperature and wind are important in the transmission of LSD outbreaks and can be used to predict the occurrence of LSD outbreaks in any given livestock population (Table 12). Relative humidity affects LSD transmission by having an effect on the development of the insects concerned in transmission in the given area.

The Central and Rift Valley Provinces in Kenya had the largest number of LSD outbreaks during the past twenty years (1981-2001) and Nairobi and North Eastern Provinces had the least number of outbreaks during the same period of time (Figure 6). There was a statistically significant association between LSD outbreaks and various districts in Kenya (Table 11), Kajiado in particular.

CHAPTER 3: EXPERIMENTAL INSECT TRANSMISSION OF LUMPY SKIN DISEASE VIRUS IN ZEBU CATTLE

3.1 SUMMARY

Studies on the transmission of lumpy skin disease virus (LSDV) were carried out using indigenous cattle (*Bos indicus*) and several insect species. Tsetse flies, sandflies, stable flies and mosquitoes were infected with the LSD Neethling virus and time series experiments were then carried out whereby the insects were fed on cattle on different days and dissections were carried out to isolate virus from the insects. Virus was isolated from all the insect species and these insects were able to cause seroconversion in 52% of the cattle upon which they had fed.

There was however, no animal that developed the typical clinical signs that are usually observed in some animals in the field situation. The virus was able to persist for up to 10 days in some of the insects, such as *Glossina morsitans centralis (G.m.c)*. It was concluded from these experiments that several insect species may serve as vectors of lumpy skin disease virus and that mechanical transmission of the virus is the most likely mode of transmission under natural conditions.

3.2 INTRODUCTION

There have been attempts to carry out transmission studies of the capripoxviruses using a few insects of the *Stomoxys spp.* (Kitching and Mellor, 1986) but the work was not done exhaustively and was carried out using the sheep poxvirus. Other studies (Webb 1990) demonstrated that LSD virus could be recovered from *Glossina morsitans morsitans* and *Stomoxys calcitrans* infected with the virus through intrathoracic inoculations. However the method by which both mechanical and biological transmission occurs and to identify the specific potential vectors of LSDV in Africa

In the current studies attempts were made to feed the insects on the virus through the natural route of feeding via the mouthparts and the fact that most viruses enter their hosts *per os* (Poinar and Thomas, 1978) was also taken into consideration. The findings of insects from the field studies gave an indication as to which species of insects was most predominant in the outbreak areas. An attempt was made to use insect species that were most abundant in the LSD outbreak areas, namely: *Stomoxys* and sandflies. *Glossina* species and *Aedes* that were most readily available in the laboratory were also used as models to study the transmission of LSD virus by insects.

3.3 MATERIALS AND METHODS

3.3.1 Experimental animals

Zebu cattle aged between nine months and two years old were obtained from Chuka in Meru district. This was an LSD free area and was chosen to minimise the risk of using animals that were previously exposed to LSD. The animals were been screened for antibodies to LSD virus using the virus neutralization test (Precausta *et al.*, 1979) and only those that were serologically negative were purchased. The animals were dipped and de-wormed as they acclimatized to conditions at NVRC, Muguga.

Prior to the experimental challenge with the virus, the average daily temperatures of the experimental animals were taken. This was carried out for three days to four weeks after the animals had been housed in the isolation unit.

3.3.2 Experimental infection of cattle with LSD virus

For each experiment two to three animals were inoculated both intradermally and intravenously at the neck region, with 4ml of tissue culture supernatant fluid containing 10^{-6} $TCID_{50}$ of LSD Neethling virus (0.2 ml intradermally and 3.8 ml intravenously). The animals were then observed daily for clinical signs of disease and their rectal temperatures recorded.

When the LSD lesions developed in these animals, flies were partially fed directly on the lesions and then transferred to complete their feeding on naive animals, at intervals of 24 hours, three days, seven days and ten days.

3.3.3 Collection of serum samples

Clotted blood samples were collected by jugular venipuncture from each animal immediately before exposure to the virus and at weekly intervals for 4 weeks. Serum was separated from each sample by centrifugation at 2500 X g for 15 minutes and then stored at - 20 ^{0} C until tested. All serum samples were examined for antibodies to LSD using virus neutralization tests (Precausta *et al.*, 1979)

3.3.4 Experimental insects

Preliminary studies were done to determine the time that is sufficient for the various biting flies to initiate feeding. The insects tested as vectors were newly emerged adults that had been fed on sugar water (10%) and then starved for 24 hours (in the case of *Stomoxys calcitrans*) and tenerals in the case of tsetse flies (*Glossina morsitans morsitans* (*G.m.m*) and *Glossina morsitans centralis* (*G.m.c.*). *Glossina m.m.* insects were

obtained from the Kenya Trypanosomiasis Research Institute (KETRI) whereas *Glosina m.c.* were supplied by the International Livestock Research Institute (ILRI). The stable flies, *Stomoxys* spp. were reared and maintained at the International Centre for Insect Physiology and Ecology (ICIPE) laboratory in Nairobi, Kenya. The sandflies (*Phlebotomus duboscqi)* and mosquitoes (*Aedes aegypti*) were obtained from the Kenya Medical Research Institute (KEMRI) insectaries.

3.3.4.1 *Glossina morsitans morsitans*

An experiment was carried out to determine the role of *Glossina morsitans morsitans* in the transmission of LSDV. Six Zebus and six cross-bred (*Bos taurus* X *Bos indicus*) cattle ranging in age from 9 months to 18 months were used for this experiment (LSD transmission by *G.m.m*). Two naïve cattle were challenged with the virulent LSD 2490 virus and then 200 *Glossina morsitans morsitans* were fed on them to engorgement.

Another set of flies was partially fed on LSD infected bovine blood through a silicone biological membrane. The feeding was interrupted and then completed on other naïve animals. The insects were fed on the animals (Plate 2) on days 0, 2, 4, 7, and 13 post-virus feeding. Feeding animals on day zero meant that the insects were feed on the infected blood or infected animal and then transferred to feed on naive animals on the same day. All

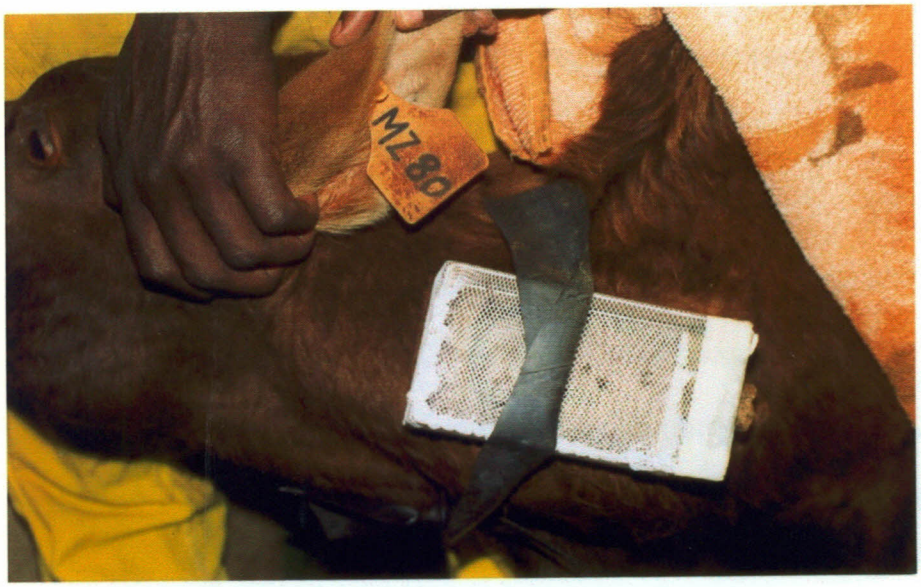
the animals were monitored for the development of clinical signs of LSD and their rectal temperatures were recorded daily throughout the experimental period. Serum samples were also collected from these cattle at weekly intervals for four weeks.

3.3.4.2 *Glossina morsitans centralis*

Two hundred *Glossina morsitans centralis* caged in groups of 20 insects were partially fed through a biological membrane for 45 seconds to one minute. These were then transferred to two naive animals to complete their feeding using a different side of the insect cage, that had no prior contact with the LSD infected blood.

Another group of two hundred tsetse flies (*G.m.c.*) were fed to engorgement and then dissected on days zero, day one, two, three, seven and ten following feeding on LSD virus infected blood. The feeding of the insects was done on both infected animals and through biological membranes as described for *Glossina morsitans morsitans.*

Plate 2. Artificial feeding of Insects on Zebu cattle



3.3.4.2.1. Experimental animals

Twenty Zebu cattle aged between one to two years were used for the *Glossina morsitans centralis* transmission experiment. Two of them were challenged with the LSD Neethling virus and laboratory reared *Glossina morsitans centralis* fed on them once LSD related nodules appeared on them. The insects were partially fed and completed their feeding on two naïve animals on day zero.

Two animals (MZ 01 and MZ 02) were each inoculated with 4 millilitres of the LSD Neethling virus. Once the skin nodules were formed, 150 *G.m.c.* insects were fed on the lesions to engorgement. Another 200 flies were partially fed and transferred to feed on three naive recipient cattle immediately and at intervals of 24 hours, 2, 7 and 10 days. The 200 insects were also fed to engorgement on three other naive animals after 24 hours. Groups of ten insects in both these groups of insects were dissected for virus isolation on different days (0, 2, 7 and 10). On days two, seven and ten post-feeding on the infected animals, the insects fed on two other naïve animals on each day.

Another set of 200 tsetse flies was membrane-fed on bovine blood mixed with the LSD virus. They completed their feeding on naïve animals immediately and also fed on other naïve animals on days two, seven and ten. Serum samples were collected from all the animals at weekly intervals for five weeks. Virus neutralization tests (VNTs) were performed on these

samples using the constant virus varying serum method (Precausta *et al*, 1979).

These insects were then dissected and grouped in batches of ten insects on days zero, one, three, eight and eleven post-feeding. The dissected insects were separated into heads, salivary glands, crops, midguts and hindguts, stored in 1 ml of fortified phosphate buffered saline containing 0.25 % bovine serum albumin (BAPBS). They were then inoculated in tissue culture for virus isolation and subjected to PCR tests after DNA extraction had been carried out on them.

3.3.4.3 *Stomoxys calcitrans*

The design of this experiment differed from that of the other insects because there was a limitation on the numbers of *Stomoxys* that could be obtained at the insectaries at any one time. The insects were reared at the International Centre for Insect Physiology and Ecology (ICIPE) in Nairobi. Different batches of insects were used instead of the same batch as for the other insects. Thirty insects were fed (individually) on the infected animals and then transferred to the naïve animals on different days. All the insects fed on any particular day were then dissected and a new batch would be fed.

Newly emerged insects that had been starved for 24 hours were used for these experiments. The insects were fed onto naïve animals on days zero, one, two three and seven-post virus feeding. For the *in vitro* feeding, the *Stomoxys* flies were fed on blood – virus mixtures that were poured onto Petri dishes and soaked on cotton wool. The cotton wool was then placed on top of the insect cage. Suid blood was used in this case.

A total of nineteen animals were used for this experiment. The animals that were inoculated with LSD Neethling virus for transmission studies involving *Stomoxys* were: MZ 52, MZ 58, and MZ 93. At the same time, animal numbers MZ 04, MZ 13, MZ 78 and MZ 34, which had been used in the previous transmission experiments (tsetse flies), were given a virulent challenge. The preliminary results of the virus neutralization test on their serum samples had showed that they had seroconverted; thus the challenge was to determine if they would be protected against LSD and to confirm the seroconversion.

3.3.4.4 Sandfly transmission studies

Some of the infected animals that were used in the *Stomoxys* studies, namely MZ52 and MZ 58 were used for feeding the sandflies. A total of seventeen animals were used for the sandfly transmission studies. Female sandflies of the species *Phlebotomus duboscqi* were used. Newly emerged flies that had not been fed on any blood were obtained from the Kenya Medical research Institute (KEMRI) in Nairobi. The insects that were partially fed on infected animals were fed alternately between naïve and infected animals. They were first fed for a few minutes (1 to 2 minutes) on an infected animal and then transferred to a naïve animal and then back to the infected animal if they were still hungry and then back to the naïve animal.

3.3.4.4.1 Membrane feeding by phlebotomine sandflies

For the membrane feeding, a biological membrane was used in the case of sandflies. Young Swiss albino mice (about one week old) were shaved after cervical dislocation. After that the mice were undressed and the skin cleaned in distilled water, then used to cover the jackets of the water-bath membrane feeder. The temperature was set at 37^0 C and the water movement through the tubes imitated the pulse of an animal. A blood-virus mixture was poured onto one feeder and the other feeder had only plain blood, which acted as the uninfected control. The blood used in this case

was rabbit blood. Two cages each containing thirty sandflies, were introduced to the two feeders and allowed to feed to engorgement. The insects were then fed on naïve animals on days one, three, five and nine post-virus feeding. The sandflies were maintained on apples or "Caro" sugar syrup (dark corn syrup with refiners' syrup) on the days when they were not feeding on the animals.

3.3.4.5 Mosquitoes

Mosquitoes of the species *Aedes aegypti* were used because they were readily available as compared to other species like *Culex,* which would have been preferred because of their abundance in the field during the surveys of LSD outbreaks. The feeding for the mosquitoes was done as described for the sandflies.

Following feeding of insects, all the animals were monitored daily for the development of fever and other clinical signs of LSD. The insects were maintained on naive rabbits or suid blood on the days when they were not fed onto cattle.

3.3.5 SAMPLE TESTING

3.3.5.1 Insect dissection

The flies were dissected on the different feeding days and divided into heads, crops and midguts. These were ground with sterile sand in sterile BAPBS using a mortar and pestle and stored at minus 70 ° C until tested using PCR and inoculation onto lamb testes (LT) cells.

3.3.5.2 Virus neutralization tests

These were carried out on the serum samples from the experimental cattle using the variable serum-constant virus method described by Precautsa *et al* (1979). Day zero (pre-insect feeding) and day twenty-eight (post - insect feeding) serum samples were used. Titrations were carried out using $\log_{10}$ dilutions of the cattle sera. A volume 50 µl of the test serum inactivated at 56^0 C for 30 minutes was added to an equal volume of virus dilution in each row. Each plate included a row of wells containing only LT cells to act as a control. The plates were covered and incubated for one hour at 37^0 C, and then LT cells added in 50 µl of growth medium. The plates were read by light microscopy on days 4 and 9 and the virus/antibody titre calculated using the method of Karber (1931) using the formula $TCID_{50}$ = M - D (S-0.5). (M=Maximum dilution affecting maximum proportion; D = Difference. between log. dilutions of virus; S = sum of all the proportions).In these

transmission experiments seroconversion was determined by a fourfold increase between pre-challenge antibody titre and week four-antibody titre, although in a few cases the fourfold change came about in the fifth week.

3.3 RESULTS

Most of the animals that were inoculated with virulent virus developed nodules at the inoculation sites (Plate 3, Tables 14, 15) Some of the infected animals exhibited lymph node enlargement (for example MZ 08 (Table 13) but NIL of the animals bitten by the experimentally infected insects displayed the typical clinical symptoms of LSD that are usually observed in naturally infected animals in the field (Tables 14, 15, 16, 17 and 18). Animal MZ 15 (Table 13) was a naturally infected animal that was used as a source of virus for feeding some of the *Glossina morsitans centralis* and *Stomoxys.* This animal had cutaneous nodules all over its body.

Most of the infected animals reached their peak temperatures during the second week after they had been fed upon by the insects or inoculated with the virus. One of the two-naïve cattle (Z011) that had been fed upon by the *G.m.m.* tsetse flies that had fed on LSDV infected blood 13 days post insect feeding, developed a large nodule (9 cms) at the site of feeding

(Table 16). However when the results of the virus neutralization test for LSDV antibodies showed that the animal not seroconverted.

The antibody titres in tables 13 to 17 are presented as the reciprocal of the highest dilution of serum that completely neutralized 100TCID^{50} of lumpy skin disease virus.

Plate 3. Infected Zebu animal with a single nodule

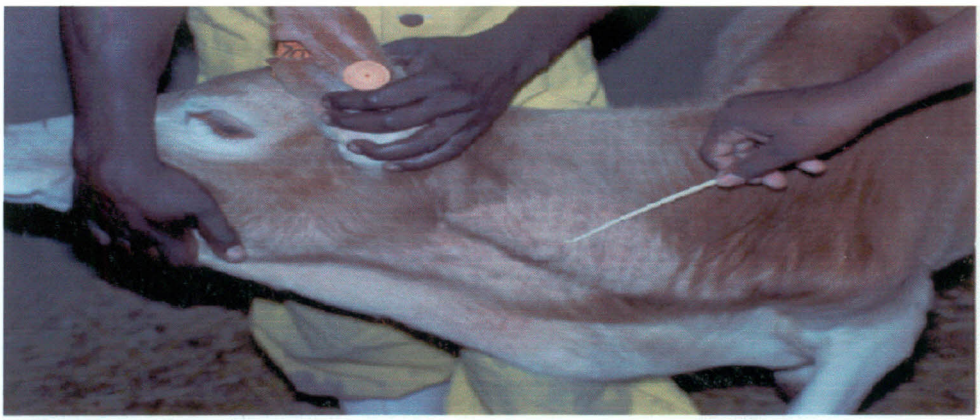


TABLE 13. Response of Zebu cattle to feeding by LSDV infected *Glossina morsitans centralis*

Feeding History of *G.m.c.*/ Animal ID	CLINICAL RESPONSE				SEROLOGY	
Day 0 PMF	Peak temp.	Lymph node enlarge-ment	Nodule swelling at local site	Satellite swellings	Pre-challenge antibody titre	Day28 antibody titre
MZ 04	38.1	++	NIL	On neck, 2cm wide	0	8
MZ 35	38.4	NIL	NIL	NIL	0	1
Day 0 Post Lesion Feeding (PLF)						
MZ34	38.4	NIL	NIL	NIL	0	12
MZ 08	38.1	++	NIL	Swelling on tail	2	8
MZ 26	38.1	++	NIL	NIL	2	12
Day 2 PLF						
MZ 11	38.4	+	NIL	Serous ocular discharge	2	8
MZ 45	38.2	++	NIL	NIL	0	2
Day 7 PLF						
MZ 22	38.1	++	NIL	NIL	0	2
MZ 12	38.5	++	NIL	NIL	2	8
MZ 27	38.2	+	NIL	NIL	0	4
MZ 32	38.2	+	NIL	NIL	0	12
Day 10 PLF						
MZ 13	36.5	+	NIL	Lesions on dewlap, probably not LSD related	1	12
MZ 18	38.2	+	NIL	NIL	2	6
MZ 03	38.7	++	NIL	NIL	1	2
MZ 36	38.3	++	NIL	NIL	0	1
Positive Control						
MZ 01	39.4	++	3.8 x 3cm	NIL	1	256
MZ 02	38.4	++	7.2 x7.3cm	Nodules on jugular	0	512
MZ15	39.6	++++	Naturally infected	All over the body	.	768

Key:

+- Denotes the degrees of enlargement 2-3cm
++-Denotes the degree of enlargement 3.1-4.0cm
+++- Denotes the degrees of enlargement 4.1-5.4cm
++++-Denotes the degrees of enlargement 5.5-7.6cm
PMF- Post membrane feeding
PLF – Post lesion feeding
NIL – No lymph node enlargement or nodules present

TABLE 14. Response of Zebu cattle to feeding by LSDV infected *Stomoxys species*

Feeding History of *Stomoxys*/ Animal ID	CLINICAL RESPONSE				SEROLOGY	
	Peak Temp	Lymph node enlarge-ment	Nodule swelling at local site	Satellite swellings	Pre-challenge antibody titre Day 0	Day 28 Antibody titre
DAY O PLF						
MZ 48	38.8	+	NIL	NIL	0	8
MZ 37	38.5	NIL	NIL	NIL	0	0
DAY 0 PCF						
MZ 85	38.4	+	NIL	NIL	2	3
DAY 1 PLF						
MZ 80	38.9	+	NIL	NIL	0	1
MZ 05	38.6	+	NIL	NIL	2	12
MZ 23	38.9	NIL	NIL	NIL	1	2
MZ 82	38.7	NIL	NIL	NIL	1	1
DAY 3 PLF						
MZ 17	39.1	+	NIL	NIL	0	4
MZ 33	39.0	+	0.5cm	NIL	1	1
MZ 28	38.9	+	1x1.3cm	NIL	0	2
DAY 3 PCF						
MZ 20	38.9	+	1.3x1.5cm	NIL	2	16
DAY 7 PLF						
MZ 10	38.5	+	1X1.3cm	Present on legs	1	4
MZ 63	39.0	+	NIL	NIL	0	4
MZ 94	38.4	+	NIL	NIL	0	0
Positive Controls						
MZ 54	38.9	+++	4X3.5cm	NIL	2	32
MZ 93	39.4	+++	7x7cm	Oedema of dewlap	0	6
Virulent Challenge						
MZ 04	39.5	+++	10X8cm	NIL	0	768
MZ 78	38.9	+	3x1.5cm	NIL	3	96
MZ 40	39.3	++	4.5x3.5cm	NIL	0	0
MZ 34	40.2	+++	21x4cm	NIL	0	2048

Key:

+- Denotes the degrees of enlargement 2-3cm
++-Denotes the degree of enlargement 3.1-4.0cm
+++- Denotes the degrees of enlargement 4.1-5.4cm
++++-Denotes the degrees of enlargement 5.5-7.6cm
PMF- Post membrane feeding
PLF – Post lesion feeding
NIL – No lymph node enlargement or nodules present

TABLE 15 Response of Zebu Cattle to feeding by LSDV Infected Phlebotomine sandflies

Feeding History of Sandflies/ Animal ID	CLINICAL RESPONSE				SEROLOGY	
	Peak temp.	Lymph node enlarge-ment	Nodule swelling at local site	Satellite swellings	Pre-challenge antibody titre	Day28 antibody titre
Day 0						
MZ 91	38.7	+	NIL	NIL	2	6
MZ 90	38.9	+	Reactions to insect bites; 2 nodular swellings	NIL	0	2
MZ 56	38.5	+	NIL	NIL	0	2
Day 1						
MZ 62	39.2	++	NIL	Present	0	4
MZ 57	38.5	+	NIL	Present	0	12
MZ 83	38.5	NIL	NIL	NIL	0	12
Day 3						
MZ 81	38.5	NIL	NIL	NIL	0	3
MZ 89	38.8	+	NIL	NIL	0	8
Day 5						
MZ 14	38.5	NIL	NIL	NIL	2	8
MZ 92	38.5	+	NIL	NIL	2	2
MZ 06	38.7	NIL	NIL	NIL	1	8
Day 7						
MZ 97	38.3	NIL	NIL	NIL	0	4
Day 9						
MZ 103	38.6	+	NIL	NIL	2	4
Positive controls						
MG 01	38.6	+	0 X 4cm	NIL	1	48
MG 02	38.4	+	NIL	NIL	0	6
AV Challenge						
MZ 32	38.9	++	4.6 x3cm swelling	NIL	1	96

Key:

+- Denotes the degrees of enlargement 2-3cm
++-Denotes the degree of enlargement 3.1-4.0cm
+++- Denotes the degrees of enlargement 4.1-5.4cm
++++-Denotes the degrees of enlargement 5.5-7.6cm
PMF- Post membrane feeding
PLF – Post lesion feeding
NIL – No lymph node enlargement or nodules present

TABLE 16. Response of Zebu cattle to feeding by LSDV Infected *Glossina morsitans morsitans*

Feeding History of Tsetse/ Animal ID	CLINICAL RESPONSE				SEROLOGY	
	Peak temp (^{0}C)	Lymph node enlarge-ment	Nodule swelling at local site	Satellite swellings	Pre-challenge antibody titre	Day28 antibody titre
Day 0						
GR 04	38.6	+	NIL	NIL	0	4
GR 02	39	NIL	NIL	NIL	0	0
GR 053	38.6	++++	Tenderness at site	NIL	0	6
Z 021	38.3	++	NIL	NIL	0	4
DAY 4						
GR 007	38.2	+	NIL	NIL	1	24
GR 075	38.4	NIL	NIL	NIL	0	0
GR 042	38.5	+	NIL	NIL	.	Dead
Day 8						
GR 039	38.4	NIL	NIL	NIL	0	24
GR 004	38.6	+	NIL	NIL	ND	ND
DAY 12						
Z 011	38.4	+++	Diffuse swelling at feeding site	NIL	0	0
Z 013	38.4	+++	NIL	NIL	0	3
Day 13						
Z 026	38.4	NIL	NIL	NIL	0	0
GR 057	38.7	NIL	NIL	NIL	0	2

Key:

+- Denotes the degrees of enlargement
+ 2-3cm
++- 3.1-4.0cm
+++- 4.1-5.4cM
++++- 5.5-7.6cm
PMF- Post membrane feeding
PLF – Post lesion feeding
NIL – No lymph node enlargement or nodules present
ND – Not determined

TABLE 17 Response of Zebu cattle to feeding by LSDV infected *Aedes aegypti*

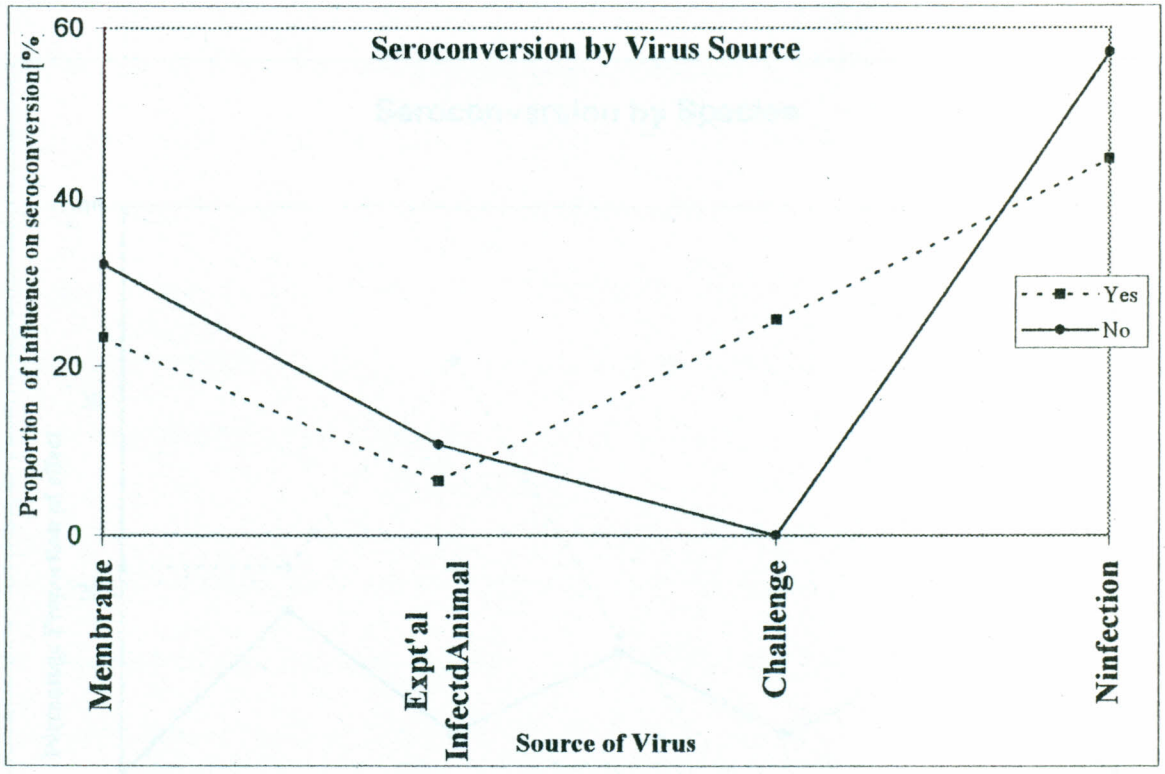
Feeding History of Mosquitoes/ Animal ID	CLINICAL RESPONSE				SEROLOGY	
	Peak temp.	Lymph node enlarge-ment	Nodule swelling at local site	Satellite swellings	Pre-challenge antibody titre	Day28 antibody titre
Day 0 PLF						
MZ 102	38.7	+	NIL	NIL	0	3
MZ 77	38.4	NIL	NIL	NIL	0	0
Day 0 PCF						
MZ 101	38.4	NIL	NIL	NIL	0	3
Day 3						
MZ 98	38.9	+	Reactions to insect bites	NIL	0	6
MZ 49	38.4	NIL	NIL	NIL	3	6
Day 5						
MZ 76	38.6	+	NIL	NIL	0	6
MZ 66	38.7	+	NIL	NIL	0	0
Day 9						
MZ 105	38.4	+	Reactions to insect bites	NIL	0	4
Controls						
MZ 52	38.1	++++	9x7 cm, 10X5.5 m	Present on legs	ND	ND
MZ 58	38.9	+++	8X5 cm 7.5 X 5cm	NIL	0	6

Key:

+- Denotes the degrees of enlargement
+ 2-3cm
++- 3.1-4.0cm
+++- 4.1-5.4cM
++++- 5.5-7.6cm
PMF- Post membrane feeding
PLF – Post lesion feeding
NIL – No lymph node enlargement or nodules present
ND Missing data

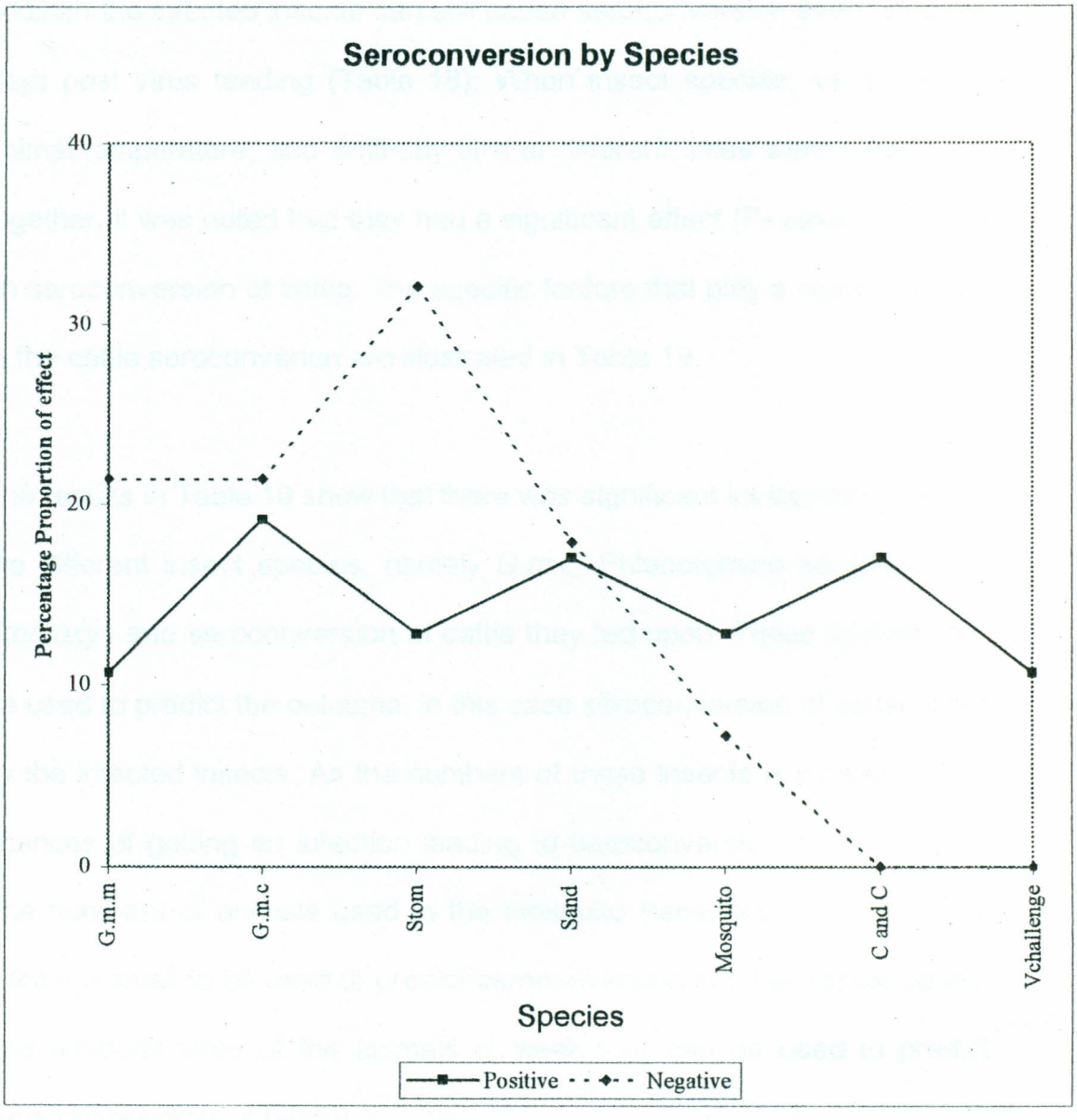
There was no significant association between animal sex and the source of virus (p-value = 0.698, Pearson chi^2 (4) = 2.2031). On the other hand the source of virus had an effect on seroconversion of the animals -Pearson chi-square = 8.6152, DF = 3, Pr < 0.035; Fisher's exact P < 0.015). The specific results are displayed in Figure 8. The Pearson chi squared test further revealed a significant difference between the different species of insects as far as seroconversion of the animals they fed upon was concerned-P < 0.043, DF = 6; Fisher's exact, P < 0.035. Figure 9 indicates that among the insects used in these experiments, *Glossina morsitans morsitans* and sandflies had the greatest effect in causing seroconversion in the cattle upon which they fed, followed by *Stomoxys* and mosquitoes.

Figure 8. Effect of virus source on seroconversion of cattle



Key: **Ninfection** – Naturally Infected animal **Exp'tal** - Experimentally

Figure 9. Effect of different insect species on seroconversion of cattle



Key
G.m.m- Glossina morsitans morsitans
Stom – *Stomoxys*
G.m.c. - Glossina morsitans centralis
C and C- Challenge and control
V challenge- Virus Challenge

The association between days post virus feeding by the insects, and seroconversion was statistically not significant (P- value = 0.321). In addition the infected insects can still cause seroconversion even up to ten days post virus feeding (Table 18). When insect species, virus source, animal temperature, and antibody titre at different times were considered together, it was noted that they had a significant effect (P–value = 0.0028) on seroconversion of cattle. The specific factors that play a significant role in the cattle seroconverion are illustrated in Table 19.

The results in Table 19 show that there was significant interaction between the different insect species, namely *G.m.c*, Phlebotomine sandflies, and *Stomoxys* and seroconversion of cattle they fed upon. These species can be used to predict the outcome, in this case seroconversion of cattle bitten by the infected insects. As the numbers of these insects is increased, the chances of getting an infection leading to seroconversion are increased. The numbers of animals used in the mosquito transmission experiments were too small to be used to predict seroconversion in other similar cases. The antibody titres of the animals at week four can be used to predict seroconversion ($P < 0.034$).

Table 18. Relationship between virus feeding days and seroconversion

Day post-virus feeding by insects	Seroconversion Negative	Positive	Total
0	9[a] 28.13[b] 32.14[c]	23[a] 71.88[b] 48.94[c]	32[a] 100.00[b] 42.67[c]
1	3[a] 42.86[b] 10.71[c]	4[a] 57.14[b] 8.51[c]	7[a] 100.00[b] 9.33[c]
2	0[a] 0.00[b] 0.00[c]	2[a] 100.00[b] 4.26[c]	2[a] 100.00[b] 2.67[c]
3	3[a] 33.33[b] 10.71[c]	6[a] 66.67[b] 12.77[c]	9[a] 100.00[b] 12.00[c]
4	3[a] 66.67[b] 7.14[c]	1[a] 33.33[b] 2.13[c]	3[a] 100.00[b] 4.00[c]
5	3[a] 50.00[b] 7.14[c]	2[a] 50.00[b] 4.26[c]	4[a] 100.00[b] 5.33[c]
7	2[a] 25.00[b] 7.14[c]	6[a] 75.00[b] 12.77[c]	8[a] 100.00[b] 10.67[c]
8	0[a] 0.00[b] 0.00[c]	1[a] 100.00[b] 2.13[c]	1[a] 100.00[b] 1.33[c]
9	0[a] 50.00[b] 3.57[c]	1[a] 50.00[b] 2.13[c]	2[a] 100.00[b] 2.67[c]
10	0[a] 75.00[b] 10.71[c]	1[a] 25.00[b] 2.13[c]	4[a] 100.00[b] 5.33[c]
12	1[a] 100.00[b] 3.57[c]	0[a] 0.00[b] 0.00[c]	1[a] 100.00[b] 1.33[c]
13	1[a] 100.00[b] 7.14[c]	0[a] 0.00[b] 0.00[c]	2[a] 100.00[b] 2.67[c]
Total	28[a] 37.33[b] 100.00[c]	47[a] 62.67[b] 100.00[c]	75[a] 100.00[b] 100.00[c]

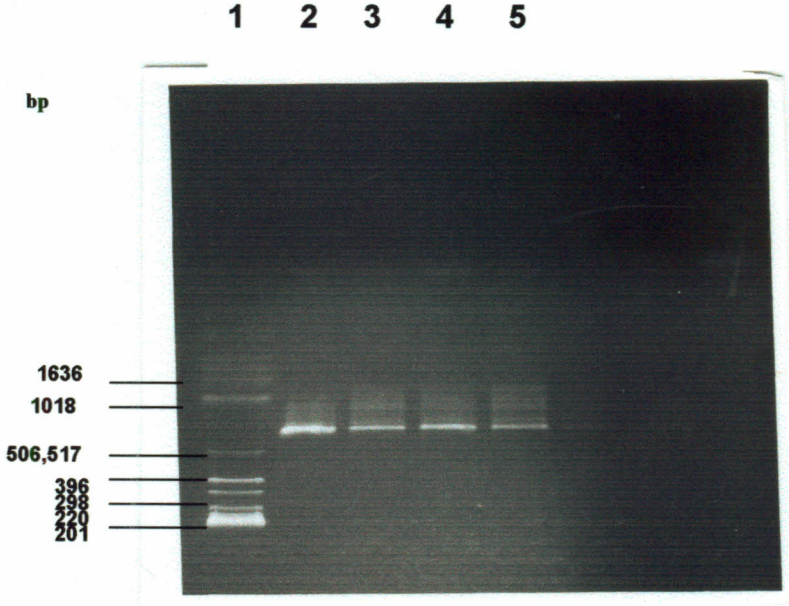
Pearson $chi^2(11) = 12.5963$ Pr = 0.321

a =No. of observations, b = proportion (%), c= proportion of effect on seroconversion

Plate 4. **Agarose gel electrophoresis of the polymerase chain reaction derived DNA products to amplify the gene encoding capripoxvirus protein P32 (Q_1 3L)**

Lane 1 DNA Kb marker

Lane 2 - 5 Positive PCR products - LSD Neethling ≈ 800bp



In some of the animals, MG 01and MG 02 (Table 15), there was a sixty-four fold and sixteen-fold increase in antibody titres respectively, without evidence of overt clinical signs of LSD. These particular animals were challenged directly with virus. They were inoculated both intravenously and intradermally with LSD virus of a titre of $10^{-5.7}$ $TCID_{50}$.

Table 19 Logistic regression analysis of various variables and seroconversion of cattle

Seroconv	Odds Ratio	Std. Err.	P>|z|	[95% Confidence Interval]	
G.m.c.	1.68 e-10	3.87 e-10	0.000**	1.83 e-12	1.54 e-08
Sandflies	1.71e-10	2.61 e-10	0.000**	8.59 e-12	3.41 e-09
Stomoxys	6.24e-11	9.64e-11	0.000**	3.03 e-12	1.29e-09
Mosquitoes	5.00e-10	-	-	-	-
Expt'al Inf.	.0071938	.0453202	0.433	3.12 e-08	1657.499
Natural inf.	.5788843	.6723133	0.638	.0594307	5.638621
Wk4temp	.0946444	.3651252	0.541	.0000492	181.9348
Abwk1	.9514196	.4777869	0.921	.3555601	2.54584
Abwk2	1.117662	.2448972	0.612	.7274431	1.717205
Abwk3	.852241	.142056	0.337	.6147233	1.181531
Abwk4	2.203366	.81925	0.034*	1.063152	4.566445

Key:
Seroconv – Seroconversion
Expt'al Inf. – Virus from experimentally infected animal
Natural Inf. – Virus from naturally infected animal
Wk4temp - Animal temperatures week 4
Abwk 1 - Antibody titres week 1
Abwk 2 - Antibody titres week 2
Abwk3 - Antibody titres week 3
Abwk4 - Antibody titres week 4
* - Statistically significant ($P < 0.05$)
** - Statistically significant ($P < 0.001$)
Std. Err. - Standard Error

Figure 10. Effect of various insect parts on LSD virus isolation in *Glossina morsitans morsitans*

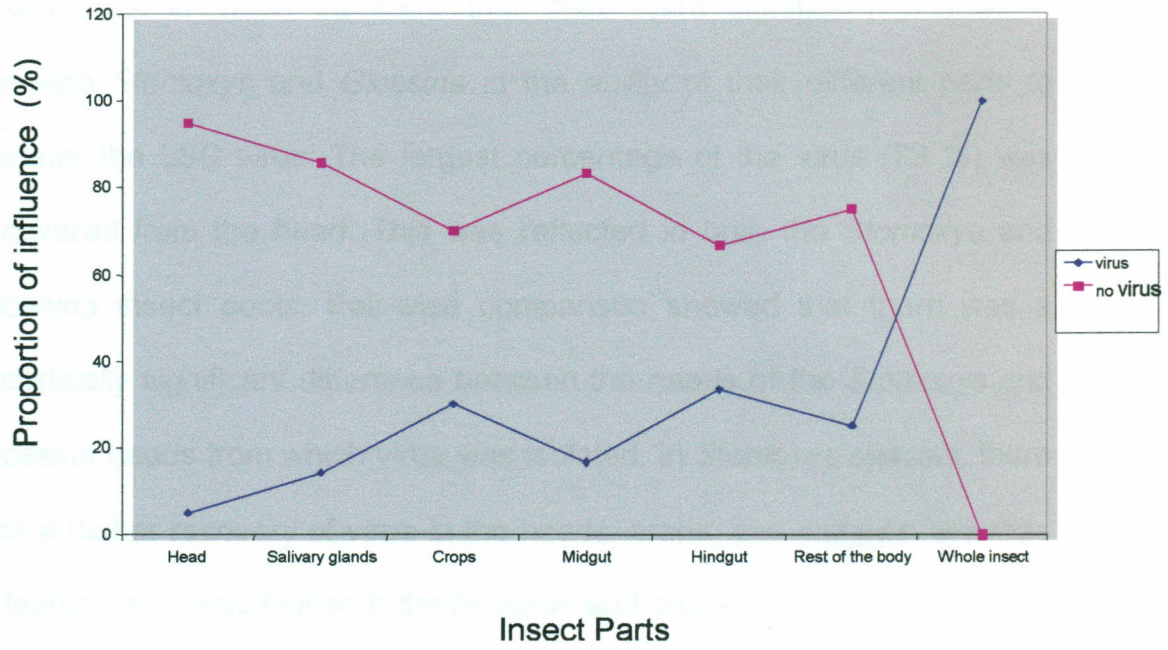


Table 20 LSD virus isolation from different insect parts

Insect part	*Glossina morsitans centralis*	*Stomoxys spp.*	Pearson $chi^2(1)$	P
	Proportion with virus			
Head	5.08% (3/59)	74%(37/50)	55.3312	0.000**
Crops	30% (3/7)	100 (4/4)	5.6000	0.018*
Hindgut	33.33 % (1/3)	100% (5/5)	4.4444	0.035*
Rest of the body	25% (1/4)	75% (9/12)	3.2000	0.074
Whole insect	ND	52% (13/25)	0.8914	0.345

Key:

* - Statistically significant ($P < 0.05$)

** - Statistically significant ($P < 0.001$)

ND - Not determined

In the tsetse flies (*G.m.c. and G.m.m.*), the crops and hindguts had the largest proportion of virus compared to the other body parts (Figure 10). The results in Table 20 show that there were significant differences between *Stomoxys* and *Glossina* in the ability of their different parts to harbour the LSD virus. The largest percentage of the virus (79 %) was recovered from the head. This was reflected in both the *Stomoxys* and *Glossina* insect pools. Pair-wise comparison showed that there was a statistically significant difference between the heads of the *Stomoxys* and *Glossina* heads from which virus was isolated. In *Stomoxys* species, there was a higher recovery of virus in the heads, crops, and midguts; whereas in tsetse flies it was higher in the hindguts and crops.

3.4 DISCUSSION

The logistic regression analysis results indicated that increasing the numbers of the insects used would result in higher chances of infection with LSDV in the animals. This finding is important and has implications for the field situation where there are large numbers of insects. In areas where there is an upsurge of biting flies during different seasons, the farmers should be advised to vaccinate their animals against LSD to reduce the chances of them being infected.

The results also indicated that the experimental animals did not develop the typical clinical signs of LSD. Apart from the clinical signs, the rectal

temperatures and the levels of antibody, the animals did not show any dramatic clinical signs as is usually observed in field conditions. For example, MZ 58 was directly challenged with the LSD virus and the increase in antibody titre was six-fold as compared to MZ 15, which was a naturally infected animal and displayed a dramatic increase and abrupt change in antibody titres (Tables 13 and 17).

Antibody titres in some of the challenged animals also showed a dramatic increase after challenge (MZ 02, Table 13). The level of antibodies in a given animal depends on the individual animals' immune status and the titre of the inoculum. The titre of infectious agent, the persistence of the agent and the infectiousness of the agent at the portal of entry are major factors in the ultimate probability of mechanical transmission. For blood-borne agents, the most frequent mechanism of transmission is considered to be on the mouthparts of the arthropod following an interrupted feed.

The more efficient mechanical vectors are telmophagous that is pool feeders (Foil, 1996). Examples of pool feeders include tabanids and stable flies. Stable fly mouthparts have been estimated to hold 0.03 nanolitres of blood following an interrupted feed, and as agents approach 10^8 infectious particles per ml of blood like murine leukaemia virus, single stable flies can transfer infection. Mosquitoes are vessel feeders (solenophagous).

The animal (Z011) that had been fed upon by the tsetse fly-*Glossina morsitans morsitans* (Table 16) and developed a huge swelling at the site of feeding and yet did not develop detectable antibodies to the disease could be a case of hypersensitivity to the insect bites. Tsetse flies not only inflict pain on their victims but may also cause large wheals in certain animals depending on the individual animals' sensitivity.

The fact that the analysis showed that large numbers of insects raise the chances of infection, explains the field situation where the animals are bitten at different times by many insects. Stable flies, for example, require blood meals about once every 24 hours and are found in close proximity to host aggregations (Allan, *et al*, 1987). There was evidence that there is a significant association between the source of virus and seroconversion of the animals (Figure 8).

The cattle that were fed upon by insects that had fed on a naturally infected donor animal contributed the largest proportion (44.68%) of seroconversion in the recipient cattle. On the other hand, animals whose source of virus were insects that had fed upon experimentally infected animals, showed the least proportion of seroconversion (6.38%, 3/47). The reason for this is that the latter group of donor animals were fed upon by insects which may not have picked up the virus or may have picked up virus whose titre was considerably low as compared to the insects that fed

on the naturally infected animal which had high viral loads from the nodules that were covering the whole body.

3.4.1. Virus isolation

The results indicated that there were certain insect parts, namely the heads, crops and hindguts (Table 20, Figure 8), that showed significant differences between the insect species. This could be due to the differences in the insect anatomy and physiology. It could also be attributed to the outcome of the digestion of tsetse flies and *Stomoxys* insects that may possibly have resulted in the LSD virus persisting for longer periods in one species and not the other.

It has previously been shown that *Stomoxys calcitrans* can regurgitate whole blood cells, and serum, from previous meals (Butler *et al.*, 1977) and this could partly explain the significant differences between the heads as far as isolation of virus from them was concerned. The rest of the insect bodies that remained after the guts were removed did not show any difference between the species and the same case applied when whole insect bodies were homogenized and virus isolated from them. This may have been so because all the organs were combined in this case and the differences in the insect anatomies would therefore not be reflected by the results that were obtained.

3.4.2. Seroconversion

There could be cases in the field when an outbreak has already occurred and perhaps it is not noticed immediately due to lack of clinical symptoms which both the farmers and the field veterinary officers can easily recognise. In the experimental transmission, some animals such as MG 01 and MG 02 were challenged to be used as donor animals for the virus but they did not show any major clinical reactions and therefore externally, it could not be determined whether they had the infection or not. If this occurs in the field then perhaps one cannot tell the exact time that an outbreak actually starts. It is for this reason that it is important to consider climatic data covering several seasons and correlating it to outbreaks of LSD before coming up with conclusions as to what climatic factors are important in determining occurrence of this disease.

3.5 CONCLUSIONS

An important deduction from the findings of these experiments is that different species of insects play an important role in the transmission of LSD. This is derived from the observation that more than one species of biting insects is capable of harbouring the virus and transferring it to cattle (Table 13-17; Figure 9). It can therefore be concluded that the contact that different insect species make with LSD infected animals in the field and the insects' different modes of feeding, enhance the spread the LSD within a flock or a locality where there is an outbreak of the disease.

Among the insects that were used in the transmission studies and from which isolation of virus was carried out, *Stomoxys spp.* was found to be most significant in passing on virus to cattle thus causing seroconversion in most of the affected animals. The finding that *Glossina morsitans centralis,* Phlebotomine sandflies and *Stomoxys spp.* can be used to predict seroconversion of cattle bitten by insects that have fed upon LSDV infected blood, has further implications on the vectorial potential of these insects as well as other insects such as mosquitoes. Chihota *et al.* (2001) previously concluded that mosquitoes were possible competent vectors of LSDV.

Mechanical transmission is the most likely mode of transmission of LSDV though there was an indication that biological transmission of the virus may also be a possibility because of the insects' ability to hold the virus for more than seven days. The heads/mouthparts of the insects harboured more virus than the rest of their body parts (Table 20) and this would assist in mechanical transmission when the insects are interrupted during feeding and they find other animals to feed on.

Experimentally infected animals do not always display the typical symptoms of LSD that are usually observed in the field situation. This may be attributed to the abundance of different species of insects in the field that would bite the animal at the same time thereby producing lesions in different areas of the body.

CHAPTER 4: GENERAL DISCUSSION

4.1. ARTHROPODS ASSOCIATED WITH LUMPY SKIN DISEASE

In Zanzibar, *Stomoxys nigra* Marquart constitutes 90-98% of the *Stomoxys* species. "These flies are found in high concentration in areas of the island that are heavily forested with poorly drained soils and in low concentrations in the sandy coastal areas" (Patterson, 1989). This can be compared to the findings of the spectra of insects/*Stomoxys spp.* collected during the LSD outbreaks in Kiambu and Machakos. Machakos generally has plenty of termite mound and sandy soils and thus sandflies were most prevalent during LSD outbreaks whereas in Kiambu, *Stomoxys nigra* was more prevalent. *Stomoxys nigra* requires a high moisture level for the larval habitat (Kunz and Monty, 1976) and according to Patterson (1989), its' adult population normally increases two weeks after the heavy rains. "*Stomoxys nigra* is one of the most underrated pests of cattle. By feeding on these animals, these flies weaken them, thereby predisposing them to other diseases (Patterson, 1989)".

In East Africa, suitable breeding sites from *Stomoxys* are found within cattle enclosures which have rotted cattle dung, rotted straw or foliage and shade whereas during the wet season, suitable breeding conditions occur in open grassland and banana plantations, wherever cattle manure and vegetation occur together (Parr, 1962). This could partly explain the occurrence of LSD outbreaks even during dry seasons when generally speaking, the outbreaks would not be expected.

The habitats of the various insects trapped during the course of this work were varied. In Machakos for example, there were termite mounds from which sandflies were trapped and in the other areas such as Kajiado district, the outbreaks reportedly started in locations situated near water sources such as streams. These varied habitats are conducive breeding sites for different insects in different localities and potential vectors of LSD may thus be varied as has been noted with other diseases such as myxomatosis. Mosquitoes are the most important vectors of the myxoma (a rabbit poxvirus) in Australia while fleas are the most important in Great Britain (Foil and Issel, 1991). Perhaps there is also a similar correlation with regards to the LSD virus where there seems to be different vectors in different areas and the arthropods that are most abundant in a given locality and can bite infected cattle and then susceptible cattle, are capable of mechanical transmission.

Biting flies are frequently disturbed and are likely to feed on a number of animals per day. The probability of contact with at least one infected animal is much greater for flies than for less mobile arthropods such as ticks; thus even if ticks were capable of harbouring the virus the chances that they would play a significant role in the transmission of LSD is hampered by their lack of mobility. They would have to be biological vectors in order to play a significant role.

The observation that quite a number of stable flies were observed feeding on infected animals and the finding that *Stomoxys* species harboured virus which caused seroconversion in cattle, strongly implicates this species as having great potential in LSDV transmission. Warnes and Finlayson (1987) found that livestock that responded with tail, foot, and head manoeuvres to the feeding of stable flies had fewer flies than more placid contemporaries.

Extrapolations of these observations to disease situations suggest that, although an acutely ill host may be the potential source of an infectious agent, it may not expend energy for defensive movements. But in a disease such as LSD where the skin nodules are easily accessible, an insect that has fed would fly off and transmit the disease to another animal which would either be nearby or far away.

During the current investigations, sandflies, mosquitoes and Stomoxys were actually collected from the outbreak areas thus fulfilling three of the factors employed to incriminate vectors, as spelled out by DeFoliart *et al.* (1987) The presence of *Glossina* species has also been reported in some LSD endemic areas such as Busia in Kenya (Republic of Kenya 1997-2001), and there were some unconfirmed reports by farmers of their presence in some farms in the study area in Kajiado District. There seems to be different vectors of LSD in different areas and the arthropods that are most abundant in a given locality and can bite infected cattle and then susceptible cattle are capable of

mechanical transmission. The significant occurrence of biting insects in the field during LSD outbreaks (Tables 2 and 4) and observations of insects feeding on infected cattle in the field (Fig. 5) attest to this deduction. Insects can clearly be incriminated as having vectorial potential with regards to Lumpy skin disease. Recent work by Chihota *et al.,* (2001 and 2003), also reported the competence of *Aedes aegypti*, and *Culicoides spp.* as possible mechanical vectors of LSD. Insects are not only capable of harbouring the LSDV but they can also transfer infection from infected to naïve animals (Tables 13-16,20).

4.2 ECOLOGY OF LSD TRANSMISSION BY ARTHROPODS

Stomoxys nigra requires a high moisture level for the larval habitat (Kunz and Monty, 1976) and according to Patterson (1989), its' adult population normally increases two weeks after the heavy rains. "*Stomoxys nigra* is one of the most underrated pests of cattle. By feeding on these animals, these flies weaken them, thereby predisposing them to other diseases (Patterson, 1989)". The cyclical patterns of blood ingestion by the Stable flies could be potential times for uptake of viruses from infected animals and transmission as well. Venkatesh and Morrison (1980a and b) showed that the peak periods of blood ingestion was the fifth day and the ninth days for both the unmated and mated females although the mated insects ingested more blood per meal. Stable flies require about five blood meals to complete

development of the first batch of eggs. This type of feeding has also been reported in the mosquito, *Aedes aegypti* (Judson, 1967).

4.2.1 Locality of LSD occurrence

Apparently LSD does not occur as frequently in the coastal or Lakeland regions e.g. Lamu and Homabay as it does in the inland areas such as Nyeri, Kiambu or Machakos (Table 8, Appendix 4). This could be due to the differences in the combination of the weather variables such as relative humidity and mean wind run which play an important role in the LSD outbreaks. (Appendix 1) Temperature and humidity are among the important factors that affect mosquitoes in their selection of daily resting and hibernation sites. Overwintering adults generally choose dark, humid, sheltered areas such as caves, animal burrows, and man-made shelters. (Allan *et al.,* 1987).

On a more general note, it is these climatic variables that may have hindered the occurrence of LSD in other temperate countries previously. It is a well-known fact that there has been global warming in recent years and this may have an effect even on the disease patterns that emerge globally. Linthicum *et al.* (1990) and Rweyemamu *et al.* (2000) reported that the upsurge of Rift Valley fever in East and West Africa was associated with climatic changes. As far as biting arthropods are concerned, they are generally present in many countries of the world except for some species such as *Stomoxys niger niger*, which are African *Stomoxyinae*. The explanation for the occurrence of LSD

predominantly in African countries, involves several factors such as vaccination schedules of the animals against LSD, tropical climatic conditions, control of insects and strict enforcement of quarantine once there are outbreaks. In the 20-year period (1981-2001), that the different Kenyan districts were looked into as far LSD outbreaks are concerned, it was noted that there was regular vaccination carried out in certain Provinces and Districts more than in others. This does not help reducing LSD outbreaks in those particular provinces since the insect vectors would not know any boundaries. Furthermore if quarantine restrictions are not followed closely as is the case some of the times, then carrying out vaccinations in one district and not the neighbouring one would not be useful to nationwide control. These integrated control management measures would ensure that the outbreaks do not move from one district to the other as the outbreaks of 2002 in Kenya did (Personal communication from the Director of Veterinary Service's (DVS) office).

Relative humidity was significant in that the higher the relative humidity the greater the chances of occurrence of outbreaks. LSD outbreaks have previously been associated with rainfall but these results show that it is the factors that are affected by rainfall (for example relative humidity) that have a significant effect on the outbreak. These are further related to insect occurrence and conditions that are optimal for the hatching of the larvae and pupation. In the areas where relative humidity on its own was significant; the humidity was conducive to the

development of the insect species that was found in large numbers in the particular area.

4.2.2 Effect of wind

The effect of wind on the various insects can be two-fold, strong wind destroying the insects and thus slowing down the spread of the disease and wind can carry the infected insects so that they spread the disease further. The different species of insects associated with livestock are usually aided in their flight by wind movements and therefore the outbreaks can spread several kilometres from the source of the original outbreak.

Vaccination of animals against Lumpy skin disease was among the main activities carried out by the Machakos District Veterinary officers just before and after the reported outbreaks in the district. In 1993, 528 animals were vaccinated; in 1994, 450 animals were vaccinated and in 1995, 90,900 animals were vaccinated. (Republic of Kenya, 1997 b). Apparently after the outbreak in 1995, many farmers opted to have their animals vaccinated and there was awareness of the effects of LSD on animals.

4.3 PROBABLE VECTORS OF LSD IN OUTBREAK AREAS

Biting flies are frequently disturbed and are likely to feed on a number of animals per day. The probability of contact with at least one infected

animal is much greater for flies than for less mobile arthropods such as ticks and it is unlikely that ticks play a big role in the transmission of LSDV.

4.3.1 Feeding patterns versus infection of insects by viruses

Different species of insects seem to be able to hold the virus for different periods of time, e.g. in the *G.m.c* transmission experiments the tsetse could still infect other naïve cattle even 10 days post virus feeding. Insects such as *Glossina* that take up plenty of blood (up to double the flies' original pre-feeding weight (Parr, 1962) during feeding have a great chance for uptake of viruses.

4.3.2 Establishing the infection

The female mosquito draws up its food, such as blood, by means of a muscular pharyngeal pump, and, apparently, stimulation of special sensory organs in the buccal cavity (Day, 1954), determines the disposition of the meal in the digestive tract. The presence of intact blood cells stimulates a partial or complete contraction of sphincter muscle of the diverticula, causing the bulk of the meal to go to the diverticula receiving all or most of the food upon closure of the sphincter of the proventriculus. Since the hind part of the mid-gut receives most or all of the ingested infected blood, it is assumed that initial infection occurs in cells of that area, e.g. La Motte (1960) found that Japanese B

encephalitis virus occurred in higher titres in the rear part of the mid-gut of *Culex pipiens* Linnaeus before it could be detected in tissues farther forward. In the current LSD transmission studies it was noted that in insects such as *G.m.c.*, the proportion of virus present in the hindgut was relatively higher (33.33%) than that found in the other parts of the gut such as the crops (30%) and the difference between them were significant (Table 19).

Apparently infecting the animals with the LSD virus does not give them a sterile immunity. Some animals e.g. MZ 32 (Table 15) that had previously seroconverted were still able to transmit virus when challenged and insects fed on them still transmitted the virus to naive animals. They were immune to disease but may have showed anamnestic antibody responses to re-infection. The immunity is not a sterile immunity so the virus is able to replicate in recovered animals and can be picked up and transmitted by insects. This would make control by vaccination alone difficult. Integrated control measures including vaccination and vector/insect control are required.

4.3.3 Arthropod transmission of viruses

Williams *et al.* (1981) suggested that grooming by live mosquitoes as well as possible inhibitors of Equine Infectious Anaemia virus (EAIV) associated with mouthparts, was responsible for inability to find the virus on the mouthparts. Negative results in transmission trials of EAIV

and Bovine leukaemia virus (BLV) indicate that regurgitation that occurs in stable flies feeding on in vitro systems (Butler *et al.,* 1977) does not occur with high frequency under natural conditions or that viral inhibitors are present in the gut of stable flies. In the current study, the heads/mouthparts of the insects harboured more virus than the rest of their body parts and this would assist in mechanical transmission when the insects are interrupted during feeding and they find other animals to feed on. The *Stomoxys* mouthparts harboured a larger proportion of the virus compared to other *Stomoxys* body parts and to *Glossina* insects. Stable flies are pool feeders and their mouthparts retain blood (Weber *et al.,* 1988). These aspects of their biology enhance their transmission of diseases and would play an important role in the mechanical transmission of LSD when they move from one animal to the other and still retain virus-containing blood in their mouthparts. Yeruham *et al.* (1995) also implicated *Stomoxys spp.* as the most likely vector of lumpy skin disease in the first outbreak in Israel because of the large numbers of these insects in the Peduyim area where the outbreak occurred. It can therefore be concluded that *Stomoxys niger* which is closely related to *Stomoxys calcitrans,* is a major suspected vector in most African countries even though Chihota *et al.* (2003) failed to demonstrate the ability of *Stomoxys* to transmit LSD virus to Jersey cattle.

Mechanical transmission trials are often difficult to relate to what actually happens in nature even if live insects are given an interrupted

feeding on an appropriate donor and recipient. When insects are dissected, homogenized, or fed on artificially spiked donors (animals or membranes), the application of the results to real situations is reduced (Foil and Issel, 1991). Warnes (1992) also suggested that laboratory flies might show different patterns of behaviour to wild flies. It is theoretically possible for any blood-feeding arthropod to be a mechanical vector under favourable circumstances; namely a high concentration of virus in peripheral areas of the vertebrate host and close association of the arthropod with the host, for example *Stomoxys calcitrans* L. normally remains close to aggregates of host animals, such as around cattle enclosures, cowsheds and stables (Gatehouse and Lewis, 1973). Stable fly dispersion however, can also play an important role in the transmission of virus from one area to another. These flies display both short- and long-distance dispersal, the short-distance movement ranging from 90cm to 13km, while the long–distance dispersal goes up to 80 kilometres and wind plays an important role in this phenomenon (Hogsette *et al.*, 1989).

The critical virus level required would vary according to the susceptibility of the next host, the amount of virus carried by the vector, the stability of the virus, the degree of protection furnished the virus by the vector to prevent its inactivation, the time period between feedings, and the temperature and humidity at the time of virus transfer. Factors such as mouth-cleaning habits and probing activity of individual vector species

fluids, impermeability of the peritrophic membrane, variations in permeability of gut cell membranes, limited number of specific virus receptor sites on the gut cell, and a surface-type defence mechanism. (Chamberlain and Sudia 1961).

Davies *et al.* (1933), upon grinding and titrating *Aedes aegypti* days after infection with yellow fever virus, found an initial decrease over that ingested, followed by an increase, but never to a level surpassing that originally ingested. Sellards (1935) later interpreted this to mean initial virus decrease, infection of the insect and subsequent multiplication. In experimental transmission studies the insects may not replicate the field situation exactly and the insects' blood meal needs may not be the same as they are in the field. It is for this reason that the experimentally infected LSD animals do not replicate the infections as seen in the field situation; furthermore the viral intake of the insects may thus be limited resulting in lower titres as opposed to the viral intake from infected animals in the field.

The caged insects do not expend as much energy in movement as their wild counterparts thus their energy needs are not as high. In the field situation the animals are usually fed upon repeatedly by either the same insects or by new ones and there is no single group of insects feeding on an animal at a prescribed time. In the case of MZ 97 (Table 15) the feeding exercise was aborted and continued the following day when the insects now fed fully on the animal. It is possible that repeated feeding

is required for the animals to become infected, for example, partial feeding due to interruptions of the insects, necessitates the search for another animal host for completion of the feeding. This could range from a few minutes from the time of the previous feeding to several hours (24 hours in the case of MZ 97), thus the insect may transmit the virus (mechanically) even though its mouthparts or the insect itself may have been infected or contaminated much earlier. This phenomenon could also suggest that the repeated biting leads to the required threshold of virus within the insect that elicits the development of antibodies detected by the virus neutralisation tests.

The work of Whitman (1953) indicates that a shifting of virus infection sites occurred as incubation progresses, with an increase of concentration in the salivary glands and a decrease of amounts elsewhere in the mosquito body, perhaps caused by a depletion of susceptible cells in the sites first infected. He found that during early incubation more yellow fever virus was present in the mid-gut than in the salivary glands of *A. aegypti* but that later the reverse was true.

In the LSD transmission experiments it was found that those insects containing the most virus or that had fed on higher titres of virus, generally transmitted with greater efficiency. Chamberlain *et al.* (1959) also found similar results when working with St. Louis encephalitis virus in *Culex quinquefasciatus* and *C. pipiens*. Probably, high levels of virus in the mosquito tissues allowed more viruses to spill out into the

haemolymph, causing greater exposure of the salivary glands to infection, which in turn resulted in an increased transmission rate. There is evidence that salivary glands are infected by way of the haemolymph (Chamberlain and Sudia, 1961). Failure to transmit to a susceptible host is obviously a sign that an infectious quantity of virus was not injected into that host during feeding.

DeFoliart *et al.* (1987) were right when they commented, "the most important concept emerging from arbovirus research was that there are no simple stories". The same authors also noted that it is not easy to quantify the dynamics of vector-host interactions when nonhuman vertebrates are involved in transmission cycles and also the behavioural components of vector and vertebrate competence and local ecology are of critical importance as epidemiological determinants. In the study undertaken to determine the role of arthropod vectors of LSD, it is thus concluded that no single vector can be pinpointed so far to be the culprit but that LSDV transmission involves multiple factors, the major ones being the presence of biting arthropods in large enough numbers, the presence of infected cattle and the favourable climatic conditions which involve high temperatures and high humidities seen in tropical Africa where the disease is endemic.

CHAPTER 5: A SUMMARY OF CONCLUSIONS

1. Biting insects play an important role in the transmission of lumpy skin disease virus and therefore any conditions that would enhance the survival and burgeoning of these arthropods would greatly increase the chances of spread of the disease.

2. The animals used in the transmission experiments were indigenous Zebu cattle (*Bos indicus*) and for the first time it has been demonstrated that several species of insects are capable of transferring LSDV to these local cattle.

3. Mechanical transmission is the most likely mode of transmission of LSDV and there was an indication that biological transmission of the virus may also be a possibility because of the insects' ability to hold the virus for more than seven days.

4. Experimentally infected animals do not always display the typical symptoms of LSD that are usually observed in the field situation. The contact that different insect species make with LSD infected animals in the field and the insects' different modes of feeding enhance the spread the LSD within a flock or a locality where there is an outbreak of the disease.

5. There are different vectors of LSD in different areas and the arthropods that are most abundant in a given locality and can bite infected cattle and then susceptible animals are capable of mechanical transmission.

6. Climatic variables play an important role in the transmission of LSD. Rainfall alone does have a significant effect on LSD outbreaks. Relative humidity, temperature and wind are important in the transmission of LSD outbreaks and can be used to predict the occurrence of LSD outbreaks in any given livestock population.

7. The Central and Rift Valley Provinces in Kenya had the largest number of LSD outbreaks during the past twenty years (1981-2001). On the other hand, Nairobi and North Eastern Provinces had the least number of outbreaks during the same period of time.

8. LSD control strategies must involve decreasing the abundance of insects in the LSD endemic regions.

CHAPTER 6: RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

1. One of the major achievements of this work was the demonstration of the role of the interaction between various weather variables and insects in the outbreaks of lumpy skin disease in cattle. There is need to find out the specific conditions that are conducive to LSD outbreaks in the different countries and whether the predictors of LSD occurrence that were found in the Kenyan situation are actually similar to the other areas.

2. Since LSD is a disease that has been spreading to various countries within the past 70 years, its pattern of occurrence should be studied in various countries and prediction models between climatic variables and LSD occurrence made as has been done with Rift Valley fever.

3. Secondly it would be important to do localised area studies to find out the distribution of insects before, during and after an LSD outbreak. It is similarly important to find out the microclimatic conditions prevailing before and after an outbreak.

4. The department of veterinary services should closely monitor climatic conditions and prepare for LSD outbreaks in the endemic areas by carrying out vaccination in all the potential areas where

outbreaks would occur, without any disparities. Since the same herd of animals can be infected every year from recovered animals, which may become re-infected, regular vaccination is of utmost importance if LSD is to be brought under control in any given locality.

4. Kenya has the potential of having LSD outbreaks throughout the country at any given time. Since LSD is a notifiable (O.I.E List A) disease, there should be no complacency in dealing with it and enforcement of the existing regulations must be carried out efficiently and regularly.

REFERENCES

Abraham, A. and Zissman, A. (1991). Isolation of LSDV from cattle in Israel, *Israel Journal of Vet. Medicine*, **46:**20-23.

Adler, S. (1964). Leishmania, In: Advances in Parasitology, Vol.II (Ben Daves Ed.) pp.35-96. Academy Press, London.

Agag, B.I., Mousa, S., Hassan, H.B., Saber, M.S., El Dehidy, N.S., and El Aziz, A.M.A. (1992) Clinical, serological and biochemical studies on lumpy skin disease. *Journal of Applied Animal Research* **1**:13-23.

Alexander, R.A., Plowright, W. and Haig, D.A. (1957). Cytopathic agents associated with lumpy skin disease of cattle. *Bulletin of Epizootic Diseases in Africa* **5**:489-492.

Ali, A. A., Esmat, M., Attia, H., Selim, A., and Abdel-Hamind, Y. M. (1990). Clinical and pathological studies on lumpy skin disease in Egypt. *Veterinary Record* **127**:549-550.

Ali, B.H. and Obeid, H.M. (1977). Investigation of the first outbreaks of lumpy skin disease in the Sudan. *British Veterinary Journal*, **133**:184-189.

Allan, S.A., Day, J.F. and Edman, J.D. (1987). Visual ecology of biting flies. *Annual review of Entomology* **32**:297-316.

Ashford, R.W. (1974). Sandflies (Diptera: Phlebotominae) from Ethiopia: Taxonomic and biological notes. *J. Med. Entomol.* **II**: 605-616.

Appleyard, G., Westwood, J.C.N. and Zwartouw, H.T. (1962). The toxic effect of rabbitpox virus in tissue culture, *Virology* **18:**159.

Bech- Nielsen, S., Pier, C.E. and Ferrer, J.F. (1978). Natural mode of transmission of the bovine leukemia virus: role of blood sucking insects. Am.J.Vet. Res., 39:1089-1092.6. Bishop, D.H.L. (1986). Replication of Arenaviruses and Bunyaviruses, In: Fundamental Virology (Fields.B.N. and Knipe, D.M. Chief Editors) pp. 449- 476. © 1986 by Raven Press Books, LTD. New York

Bellett, A.D.J., Fenner, F. and Gibbs, A.J. (1973). The viruses in "Frontiers of Biology Volume 31- Viruses and Invertebrates" edited by A.J. Gibbs. Pps 43-87.North Holland Publishing Company. Amsterdam.

Bishop, D.H.L. (1986). Replication of Arenaviruses and Bunyaviruses, In: Fundamental Virology (Fields, B.N. and Knipe, D.M. Chief Editors) pp 449-476. © 1986 by Raven Press Books, Ltd. New York.

Brigel, H. and Lea, A.O. (1975). Relationship between protein and proteolytic activity in the midgut of mosquitoes. *Journal of insect Physiology* **21**:1597-1604.

Brightwell, R., Dransfield, R.D. and Kyorku, C. (1991). Development of a low-cost trap and odour baits for *Glossina pallidipes* and *G. longipennis* in Kenya. *Medical Veterinary and Entomology* **5**:153-164.

Brody, A.L. (1936). The transmission of fowl-pox. Cornell University agricultural experimental station memorandum 195.

Boulter, E.A. and Appleyard, G. (1973). Differences between extracellular and intracellular forms of poxvirus and their implications. *Progress in Medical Virology* **16**:86-108.

Burdin, M.L. and Prydie, J. (1959). Observations on the first outbreak of LSD in Kenya. *Bulletin of Epizootiological Disease of Africa* **7**:21-26.

Burg, J.G., Neely, M.B., Williams, N.M. and Knapp, F.W. (1994). Retention and attempted mechanical transmission of *Ehrlichia risticii* by *Stomoxys calcitrans Medical and Veterinary Entomology* **8**:43-46.

Butler, J.F., Kloft, W.J., Dubose, L.A. and Kloft, E.S. (1977). Recontamination of food after feeding a ^{32}P food source to biting Muscidae. *Journal of Medical entomology,* **13**: 567-571.

Capstick, P.B. and Coackley, W. (1961). Protection of cattle against lumpy skin disease. I. trials with a vaccine against Neethling type infection. *Research in Veterinary Science* **3**:287-291.

Capstick, P.B., Pyrdie, J., Coackley, W. and Burdin, M.L. (1959). Protection of cattle against the Neethling type virus of lumpy skin disease. *Veterinary Record* **71**: 422-423.

Carn, V.M. (1994). The experimental transmission and diagnosis of LSD (Neethling) Ph.D. Thesis University of Reading, England.

Chamberlain, R.W. and Sudia, W.D. (1961). Mechanisms of transmission of viruses by mosquitoes. *Ann. Rev. Entomol.* **6**:371-90.

Chamberlain, R.W., Sudia, W.D. and Gillett, J.D., (1959). *Am. J. Hyg.* **70**:221-236.

Chapple,P.J. and Lewis, N.D. (1965). Myxomatosis and the rabbit flea. *Nature* **207**:388-389

Chihota, C.M., Rennie, L.F., Kitching, R.P. and Mellor, P.S. (2001). Mechanical transmission of lumpy skin disease virus by *Aedes aegypti* (Diptera: Culicidae). *Epidemiol. Infect.* **126**:317-321.

Chihota, C.M., Rennie, L.F., Kitching, R.P. and Mellor, P.S. (2003). Attempted mechanical transmission of lumpy skin disease by biting insects. *Med. and Vet. Entomol.* **17**:294-300.

Davies, F.G. (1991). Lumpy skin disease of cattle: a growing problem in Africa and the Near East. *World Animal Review* **68**:(3) 37:42.

Davies, F.G. (1991b). Lumpy skin disease. A capripox virus infection of cattle in Africa. FAO. Rome 1991

Davies, C.R. (1990). Interrupted feeding: causes and effects. *Parasit. Today* **6**:19-22

Davies, F.G. (1982). Observations on the epidemiology of LSD in Kenya. *Journal of Hygiene, Cambridge* **88**:95-102.

Davies, F.G. (1976). Characteristics of a virus causing a pox disease in sheep and goats in Kenya with observations of the epidemiology and control. *Journal of Hygiene*, Cambridge **76**:163-171.

Davies, N.G., Frobischer, M. Jr. and Lloyd, W. (1933). *J. Experimental Med.*, **58**:211-226.

Day, M.F. (1954). The mechanism of food distribution to midgut or diverticula in the mosquito. *Aust. J. Biol. Sci.* **7**:515-524.

Day, M.R., Fenner, F.and Woodroofe, G.M. (1956). Further studies on the mechanical transmission of mosquito transmission of myxomatosis in the European rabbit. *J. Hyg.* **54**:258-83.

De Boom, H.P.A. (1947). Knopvelsiekie:'n Kort Oorsig *South African Science* **1**:44-46.

DeFoliart, G.R., Grimstad, P.R. and Watts, D.M. (1987). Advances in mosquito-borne arbovirus/vector research. *Annual review of Entomology* **32**:479-505.

Diesel, A.M. (1949). The epizootiology of lumpy skin disease in South Africa. Report of the 14th International Veterinary Congress, London, **2**: 492-500.

Eddy, G.W., Roth, A.R., and Plapp, F.W. (1962). Studies on the flight habits of some marked insects.

Edman, J., and Kale, H.W. (1971). Host behaviour: Its influence on the feeding success of mosquitoes. *Ann. Entomol. Soc. Am.* **64**:513-516.

Fenner, F., Day, M.F. and Woodroofe, G.M. (1952). The mechanism of the transmission of Myxomatosis in the European rabbit (*Oryctolagus cuniculus*) by the mosquito *Aedes aegypti. Australian Journal of Experimental Biology and Medical Science* **30**:139-152.

Foil, L.D. (1989). Tabanids as vectors of disease agents. *Parasit. Today* **5**:88 -96.

Foil. L.D. and Issel, C.J. (1991). Transmission of retroviruses by arthropods. *Annual Review of Entomology*, **36**:355-381.

Foil, L.D., French, D.D., Hoyt, P.G., Issel, C.J., Leprince, D.J., McManus, J.M. and Seger C.L. (1989). Transmission of bovine leukaemia virus by *Tabanus fuscicostatus*. *Am. J. Vet. Res.*, **50**:1771-1773

Foil, L.D., Adams, W. V. Jr., McManus, J.M., Issel, C. J. (1988). Quantifying the role of horse flies as vectors of equine infectious anaemia. *Equine Infectious Diseases. Proc. 5th Int Conf.* Ed. D.G. Powell. Pp. 185-95. Lexington, Ky

Francki, B.I.R., Fauquet, C.M., Knudson, D.L. and Brown, F. (1991). Classification and Nomenclature of Viruses: fifth report of the International Committee on Taxonomy of Viruses. New York, Spring-Verlag.

Fukuda, T., Goto, T., Kitaoka, S., Fujisaki, K. and Takamatsu, H. (1979). Experimental transmission of fowl pox by *Culicoides arakawae*. *Nat Inst Anim Hlth Quart* **19**:104-105

Gatehouse A.G. and Lewis, C. T. (1973). Host location behaviour of *Stomoxys calcitrans*. *Ent. Exp. & appl.* **16**:275 -290.

Gerhardt, R.R., Allen, J.W., Greene, W.H. and Smith, P.C. (1982). The role of face flies in an episode of infectious bovine keratoconjuctivitis. *Journal of the American Veterinary Medical Association* **180**:156-159.

Glasgow, J.P. and Phelps, R. J. (1970) Methods for the collecting and sampling of *Glossina* In: *The African trypanosomes.* (H.W. Mullighan, ed.), pp 395-415. George Allen and Unwin, London.

Glass, H.W.Jr., Gerhardt, R.R. and Greene, W.H. (1982). Survival of *Moraxella bovis* in the alimentary tract of the face fly. J.*Econ. Entomol.* **75**:545-546

Green, H.F. (1959). Lumpy skin disease- its effect on hides and leather and a comparison in this respect with some other skin diseases. *Bulletin Epizootic Diseases of Africa* **7**:63-74.

Gooding, R.H. (1974). Digestive processes of haematophagous insects: control of trypsin secretion in *Glossina morsitans* morsitans Westw. (Diptera: Glossinidae). *Bulletin of Entomological Research* **64**: 175-181.

Gooding, R.H. (1975). Digestive enzymes and their control in haematophagous arthropods. *Acta Tropica* **32**:2.

Ichihashi, Y., Matsumoto, S. and Dales, S. (1971). Biogenesis of poxviruses: role of A-type inclusions and host cell membranes in virus dissemination. *Virology* **46**:507.

Haig, D.A. (1957). Lumpy skin disease. *Bulletin of Epizootic Diseases of Africa* **5:** 421- 430.

Henning, M.W. (1956). Animal diseases in South Africa. Third edition, Cape Town, Central News Agency.

Harmsen, R. (1973). The nature of the establishment barrier for *Trypanosoma brucei* in the gut of *Glossina pallidipes. Trans. R. Soc. Trop. Med. Hyg.* **67**: 364-373.

Hogsette. J.A., Ruff, J.P. and Jones, C.J. (1989). Dispersal Behaviour of Stable flies (Diptera: Muscidae). United States Department of Agriculture (USDA), Miscellaneous Publications. **74**: 23-32).

Hoy, Marjorie, A. (1994). Insect Molecular Genetics: An introduction to Principles and Applications. Academic Press Inc. © 1994, California.

Hunter, P. and Wallace, D. (2000). Lumpy skin disease in Southern Africa: a review of the disease and aspects of control. *J.S. Afr. Vet. Assoc.* **72** (2): 68-71.

Jaetzold, R. and Schmidt, H. (1982/1983). Farm Management handbook of Kenya, Vol. II. Pub. Ministry of Agriculture, Kenya in o-operation with the German Agricultural Team for the German Agency for Technical Co-operation (GTZ).

Jones, C. J., Hogsette J. A., Patterson R. S. and Milne D. E. (1985). Effects of natural saccharide and pollen extract feeding on stable fly (Diptera: Muscidae) longevity. *Environ. Entomol.* **14**:223-227

Judson, C.L. (1967). Feeding and oviposition behaviour in the mosquito *Aedes aegypti* L. I. Preliminary studies of physiological control mechanisms. *Biol. Bulletin*. **133**:369-377.

Karber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Archiv fur Experimentelle Pathologie und Pharmakologie **162**:480-483.

Kilham, L. and Dalmat H.T. (1955). Host –virus- mosquito relations to Shope fibromous in cottontail rabbits. *American Journal of Hygiene* **61**:45-54

Kitching, R.P. and Mellor, P.S. (1986). Insect transmission of *capripoxvirus Research in Vet. Science* **40**:255-258.

Kitching, R.P., Bhat, P.P. and Black, D.N. (1989). The characterisation of African strains of capripoxvirus. *Epidemiology and Infection* **102**: 335-343.

Kitching, R.P., McGrane, J.J., Hammond, J.M., Miah, A.H., Mustafa, A.H. and Majumder, J.R. (1987) Capripox in Bangladesh. *Trop Anim Health Prod.* **19**(4): 203-8.

Kligler, I.J., Muckenfuss, R.S., and Rivers, T.M. (1929). Transmission of Fowl-Pox by Mosquitoes. *J.Exp.Med.* **49**:649-660

Kline, D.L. (1994) Olfactory attractants for mosquito surveillance and control: 1-octen-3-ol. *Journal of American Mosquito Control Association*, **10**(2): 280-287.

Kline, D.L., Dame, D.A. and Meisch, M.V., (1991) Evaluation of 1-octen-3-ol and carbon dioxide as attractants for mosquitoes associated with irrigated rice fields in Arkansas, *Journal of American Mosquito Control Association* **7**:165-169.

Krinsky, W.L. (1976). Animal disease agents transmitted by horse flies and deer flies (Diptera: Tabanidae). *Journal of Medical Entomology* **13**:225-275.

Kunz, S.E. and Monty, J. (1976). Biology and ecology of *Stomoxys nigra* Marquart and *Stomoxys calcitrans* (L.) (Diptera: Muscidae) in Mauritius. *Bull. Entomol Res.* 66:745-755.

La Motte, L.C. Jr. (1960). The infection of mosquitoes and bats with Japanese B Encephalitis virus with reference to survival of virus during simulated hibernation. Doctoral Thesis, The John Hopkins University, School of Hygiene and Public Health, Baltimore, Md.,)

Lee, R.M.K. and Davies, D.M. (1979). Feeding in the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae). I. Destination of blood, sucrose solution and water in the alimentary canal, the effects of age on feeding, and blood digestion. *J. Med. Entomol.* **15**:541-554.

Lee, D.J., Fenner, F., and Lawrence, J.J. (1958). Mosquitoes and fowl pox in the Sydney area. *Aust. Vet. J.* **34**: 230-237

Lehane, M.J. (1976). Digestive enzyme secretion in *Stomoxys calcitrans (Diptera: Muscidae). Cell Tissue Research* ***170****: 275 – 287.*

Lewis, D.J. (1982) A taxonomical review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bulletin of the British Museum (Natural History) (Entomology)*, 45:121-209.

Lewis, D.J. (1975). Functional morphology of the mouthparts in New World phlebotomine sandflies (Diptera: Psychodidae). *Trans. R. Entomol. Soc. London.* **126**:497-532.

Lewis, D.J. (1973) Phlebotomidae and Psychodidae In Insects and other Arthropods of Medical importance (Smith, K.G.V. ed) pp 155. Bri. Mus. Nat. Hist. London.

Linthicum KJ, Bailey CL, Tucker CJ, Mitchell KD, Logan TM, Davies FG, Kamau CW, Thande PC, Wagateh JN. (1990) Application of polar-orbiting, meteorological satellite data to detect flooding of Rift Valley Fever virus vector mosquito habitats in Kenya. *Medical and Veterinary Entomology*. 4(4): 433-8

Macdonald, R.A.S. (1931). Pseudo-urticaria of cattle in: Government of Northern *Rhodesia Department of Animal Health Annual Report for the year 1930.* Pp.20-21.

Macowan, K.D.S. (1959) Observations on the epizootiology of lumpy skin disease during the first year of its occurrence in Kenya. *Bulletin of Epizootic Diseases of Africa* **7**:7-20.

Manson-Bahr, P.E.C. and Downie, A.W. (1973). Persistence of Tanapox in Tana River Valley. *British Med J.* **2**:151-153

Mathews, R.E.F. (1982). Classification and nomenclature of viruses. *Intervirology* **17**: 41-46.

Mattingly, P.F. (1973). Diptera" pp 193-211. In Viruses and Invertebrates (A.J. Gibbs (Ed.), Frontiers of Biology (Eds. A. Neuberger and E.L. Tatum) **Vol.31**. North Holland Publishing Company, Amsterdam.

Mattingly, P.F., Crosskey, R.W. and Smith, K.G.V. (1973). Summary of Arthropod Vectors pp. 497-523. In: Smith, K.G.V., (ed.) *Insects and other arthropods of medical importance.* Trustees British Museum (National History) London.

Mihok, S. (2002). The development of a multipurpose trap (the Nzi) for tsetse and other biting flies. *Bulletin of Entomological Research* **92**:385-403.

Mihok, S., Maramba, O., Munyoki, E. and Kagoiya, J. (1995a). Mechanical transmission of *Trypanosoma spp.* By African Stomoxyinae (Diptera: Muscidae). *Trop. Med. Parasitol.* **46**:103-105.

Mihok, S., Kang'ethe, E.K. and Kamau, G.K. (1995b). Trials of traps and attractants for *Stomoxys spp. J. Med. Ent.* **32**: 283-289.

Minter, D.M. (1964) The Distribution of Sandflies (Diptera: Psychodidae) in Kenya. *Bull. Ent. Res.*, **55**:205-217.

Mitzmain, M.B. (1914). Experimental insect transmission of anthrax. *Public Health Reports*, **29**:75-77

Morris, H. (1918) Blood-sucking insects as transmitters of anthrax or charbon. *La Ag Exp Sta Bull.* **163**:3-15

Moss, B. (1974) "Reproduction of Poxviruses" pp 405- 474 in Comprehensive virology volume 3 (Fraenkel-Contrat and Heinz (Editors)). Plenum Press, New York.

Moss, B. (1987). The Molecular Biology of Poxviruses. In: *The Molecular Basis of viral replication. Bercoff NATO series A: Life sciences. 136.* Proceedings

Office International des Epizooties (OIE) 2004 Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 5th Edition Vols. 1 & 2. 1178pp © 2003 OIE, World Organisation for Animal Health.

Office International des Epizooties (OIE) 2000. Manual of Diagnostic tests and vaccines. © 1999. World Organisation for Animal Health

Ogston, C. W. (1981). Transfer of radioactive tracer by the bedbug *Cimex hemipterus* (Hemiptera: cimicidae): a model for mechanical transmission of Hepatitis B virus. *J. Med. Entomol.* **18**: 107-11

Packer, M.J. and Brady, J., (1990). Efficiency of electric nets as sampling devices for tsetse flies (Diptera: Glossinidae) Bulletin *of Entomological Research* **80**:43-47.

Parr, H.C.M. (1962). Studies on *Stomoxys calcitrans* (L.) in Uganda, East Africa. II. - Notes on Life-History and Behaviour. *Bulletin of Entomological Research* **53**:437-443.

Patterson, R.S. (1989) Biology and ecology of *Stomoxys nigra* and *S. calcitrans* in Zanzibar. *Misc. Pub. Ent. Soc. America.* 74:2-11.

Peeler, E.J. and Omore, A.O. (1997). Manual of Livestock Production Systems in Kenya. -cattle, sheep and goat systems (2nd Edition). KARI/DFID National Agricultural Research Project II, Kikuyu, Kenya.

Precausta, P., Kato, F. and Vellut, G. (1979). A new freeze-dried living virus vaccine against sheep-pox. *Compendium of Immunology, Microbiology and Infectious Diseases* **1**:305-319.

Poinar, G.O. Jr. and Thomas, G.M. (1978). Diagnostic manual for the identification of insect pathogens © 1978 Plenum Press, New York.

Pratt, D.J., Greenway, P. J. and Gwynne, M. D. (1966). A Classification of East African Rangeland, (with an Appendix on Terminology). *J. Applied Ecology* Vol 3 and 4, 369-382.

Prozesky, L., and Barnard, B.J.H. (1982). A study of the pathology of lumpy skin disease in cattle. Onderstepoort *Journal of Veterinary Research* **49**: 167-175.

Prullage, J. B., Williams, R.E. and Gaafar, S.M. (1993). On the transmissibility of *Eperythrozoon suis* by *Stomoxys calcitrans* and *Aedes aegypti*. *Veterinary Parasitology* **50**:125-135.

Ramisse, J, Serres, H. and Rakotondramary, E (1969a) Isolement à Madagascar de virus associes à la dermatose nodulaire. *Revue d'Elevage et de Médecine Véterinaire des Pays tropicaux* **22:** 357-362

Ramisse, J, Serres, H. and Rakotondramary, E (1969b) Adaptation aux cellules renales de lapin de virus associes à la dermatose nodulaire. *Revue d'Elevage et de Médecine Véterinaire des Pays tropicaux* **22:** 363-371.

Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E. Estes, M., Lemon, S., Maniloff, J., Mayo, M.A., McGeoch, D. Pringle, C.R. and Wickner, R.B. (eds) (2000). Virus Taxonomy. 7th Report of the International Committee on Taxonomy of Viruses. Academy Press. New York, San Diego.

Republic of Kenya (2000) Department of Veterinary Services, Annual Report, Ministry of Agriculture and Rural Development.

Republic of Kenya (1999) Department of Veterinary Services, Annual Report, Ministry of Agriculture and Rural Development.

Republic of Kenya (1998) Department of Veterinary Services, Annual Report, Ministry of Agriculture and Rural Development.

Republic of Kenya (1997) Department of Veterinary Services, Annual Report, Ministry of Agriculture and Rural Development.

Republic of Kenya (1996) Department of Veterinary Services, Annual Report, Ministry of Agriculture and Rural Development.

Republic of Kenya (1997a) Machakos District Development Plans (1997-2001). Rural Planning Department, office of the President and Ministry of Planning and National Development; Printed by the Government Printer, Nairobi, Kenya.

Republic of Kenya (1997b) Kajiado District Development Plans (1997-2001). Rural Planning Department, office of the President and Ministry of Planning and National Development. Printed by the Government Printer, Nairobi, Kenya.

Republic of Kenya (1997c) Kiambu District Development Plan (1997-2001): Rural Planning Department, office of the President and Ministry of Planning and National Development; Printed by the Government Printer, Nairobi, Kenya

Ribiero, J.M.C. (1987). Role of saliva in blood-feeding by arthropods. *Ann. Rev. Entomol.***32**: 463-78.

Schneider, F., Houseman, J.G. and Morrison, P.E. (1987). Activity cycles and the regulation of digestive proteases in the posterior midgut of *Stomoxys calcitrans* (L.) (Diptera: Muscidae). Insect *Biochem.* Vol 17. No. 6:859-862.

Sellards, A.W. (1935). *Ann. Trop. Med. Parasitol.*, **29**:49-35

Spates, G. E. and Mayer, R.T. (1984). Mid-gut haemolytic activity of the horn fly, *Haematobia irritans* (Diptera: Muscidae). *J. Med. Entomol.* **21**: 58-62

Tarry, D.W., Bernal, L. and Edwards, S., (1991). Transmission of Bovine Virus Diarrhoea Virus by blood feeding flies. *Vet. Record.* **128**:82-84

Taylor, H.R. (1988). A simple method for assessment of association between synanthropic flies and trachoma. *Am. J. Trop. Med. Hyg.* **38** (3): 623-627

Tidwell, M.A., Dean, W.D., Tidwell, M., Coombs, G.P., Anderson, D.W., Cowart, W.O. and Axtell, R.C. (1972). Transmission of hog cholera virus by horseflies (Tabanidae: Diptera). *Am. J. Vet. Res.* **33**:615-622

Thomas, L.A. (1955) Development of the virus of Western Equine Encephalomyelitis in the mosquito vector, *Culex tarsalis* (Diptera: Culicidae). Doctoral thesis, Tulane University, New Orleans, La.

Tripathy, D.W., Hanson, L.E., and Crandell, R.A. (1981). Comparative diagnosis of virus diseases. Vol. 3:267-346.Eds. E. Kurstak and C. Kurstak, London Academic Press

Turrell, M.J., and Knudson, G.B. (1987). Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). *Inf Imm*, **55**: 1859-1861

Vale, G.A. (1974). New field methods for studying the responses of tsetse flies (Diptera: Glossinidae) to hosts. *Bulletin of Entomological Research* **64**:199-208.

Vale, G. A., (1969). Mobile attractants for tsetse flies. *Arnoldia* **4**:1-7.

Van Rooyen, P.J., Munz, E.K. and Weiss, K. E. (1969) The optimal conditions for the multiplication of Neethling type lumpy skin disease virus in embryonated eggs. *Ondestepoort Journal of Research* **36**: 165-174.

Venkatesh K. and Morrison, P.E. (1980 a). Crop filling and emptying by the stable fly *Stomoxys calcitrans* L. *Can. J. Zool.* **58**: 57-63.

Venkatesh K. and Morrison, P.E. (1980 b). Some aspects of oögenesis in the stable fly *Stomoxys calcitrans* (Diptera: Muscidae). *J. Insect Physiology,* **26**: 711-715.

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

infectious nature. *Journal of South African Veterinary Medicine Association* **16**:29-35

Warnes, M. L. (1992). Activation of three species of tsetse (*Glossina* spp.) in response to host derived stimuli. *Medical and Veterinary Entomology* **9**:349-354.

Warnes, M.L. and Finlayson, L.H. (1987) Effect of host behaviour on host preference in *Stomoxys calcitrans*. *Med. Vet. Entomology*.**1**: 53-57.

Webb, G. (1990). Studies on the mechanical transmission of animal viruses by biting flies. Ph.D. Council of National Academic Awards, UK.

Weber. A.F., Moon, R.D., Sorensen, D.K., Bates, D.W., Meiske, J.C., Brown, C.A., Rohland, N.L., Hooker, E.C. and Strand, W.O., (1988). Evaluation of the stable fly (*Stomoxys calcitrans*) as a vector of enzootic bovine leukosis. *Am. J. Vet.* Res., **49**:1543-1549.

Webster, K.A., Rankin, M., Goddard, N., Tarry, D.W. and Coles, G.C. (1992). Immunological and feeding studies on antigens derived from the biting fly, *Stomoxys calcitrans*. *Veterinary Parasitology*, **44**: 143-150.

Willemse, L. P. M. and Takken, W. (1994) Odor-induced host location in tsetse flies (Diptera: Glossinidae), *Journal of Medical Entomology* **31**(6): 775-794.

Williams, D.L., Issel C.J., Steelman, C.D., Adams, W.V. Jr. and Benton, C.V. (1981). Studies with equine infectious anemia virus: transmission attempts by mosquitoes and survival of virus on

vector mouthparts and hypodermic needles, and in mosquito tissue culture. (1981). *Am. J. Vet. Res.* **42**:1469-73.

Whitman, L. (1953). Annual Report of the Rockefeller Foundation 1952 (Rusk, D., Ed., Rockefeller Foundation, New York, N.Y

Woods, J.A. (1988). Lumpy skin disease- a review. *Trop. Anim. Hlth Prod.* **20**:11-17.

Yeruham, I., Nir, O., Braverman, Y., Davidson, M., Grinstein, H., Haymovitch, M. and Zamir, O. (1995). Spread of lumpy skin disease in Israeli dairy herds. *Vet. Record* **137**:91-93.

Zumpt, F (1973). The stomoxyinae biting flies of the world. Diptera: Muscidae. Taxonomy, biology, economic importance and control measures. Gustav Fischer, Stuttgart, Federal Republic of Germany

APPENDIX I REAGENTS USED AND GENERAL MATERIALS AND METHODS

1.1 BUFFERS AND SOLUTIONS

All chemicals were purchased from BDH or Sigma.

Phosphate buffered saline (PBS) (10 litres)

- Sodium chloride 80g
- Potassium chloride 2g
- Magnesium chloride 6 H_20 1g
- Potassium di-hydrogen orthophosphate 2g
- Sodium di-hydrogen orthophosphate 114g
- Calcium chloride 2 H_20 132g
- Double distilled water to a final volume of 10 litres
    - Magnesium chloride was dissolved in warm distilled water
    - Calcium chloride was added last, very slowly,
    - Sterilisation was done by filtration

Glasgow's Modified Essential Medium (GMEM)

- GMEM 1 sachet
- Double distilled water 10 litres
- Penicillin / streptomycin solution
- Fungizone (Amphotericin B)
- Sodium bicarbonate solution 7.5 % would vary every time

1 % Trypsin (Stock solution)

- Trypsin 1g
- Double distilled water 100ml
    - Sterilisation done by filtration through a 0.45µ syringe filter
    - Stored at -20 ^{o} C

Antibiotic/fungicide stock:

- 10^3 iu/l sodium penicillin
- 5mg/ml streptomycin sulphate
- $10^{2.7}$ iu/ml mycostatin
- $10^{2.9}$ iu/ml neomycin

Conjugate Diluent (BMA):

- Stock Phosphate Buffer (0.5 M) pH 8.0
- Solution A: 0.5M, $NaH_2PO_4.2H_2O$ (M.W. 156.01) – 78.0g/l
- Solution B: 0.5M $Na_2HPO_4.2H_2O$ (M.W. 178.0) - 89.0 g/l

  500 ml solution B was titrated with 40.00 ml of solution A to give pH 8.0
- To 1000ml of 0.005M Phosphate buffer pH 8.00 the following were added:
    - Potassium chloride - 75g
    - EDTA - 1g
    - Benzoic acid - 2.5g

- Tween-80 - 1ml

After allowing ample time to dissolve, pH was adjusted to 7.5 using 4 M NaOH.

All the buffers and stock solutions used for blood meal analysis (BMA) were stored at 4^0 C.

Substrate Diluent (BMA):

- Citric acid (F.W. 210.14) -4.8g in 500ml ddH_2O, pH adjusted to 4.0
- ABTS Chromogen - 0.27g in 12.5 ml ddH_2O
- H_2O_2 substrate - 500 µl of 30 % H_2O_2 in 7.5 ml ddH_2O

Hepes buffered saline X2, per litre:

- 16g Nacl
- 0.74g Kcl
- 0.25g Na_2HPO_4
- 2g glucose
- 5g hepes

TBE X 10:

- 89mM tris base
- 89mM boric acid
- 2mM EDTA

TE:

- 10mM tris-HCl, ph 8
- 1mM EDTA

1.2 GENERAL METHODS

1.2.1 Virus strains

A South African Neethling strain of capripoxvirus, originally recovered from an LSD infected cow and then passaged at the Institute of Animal Health (IAH), Pirbright, UK (Kitching et al., 1989) was used as the challenge virus for all the experimental cattle except during the *G.m.m.* experimental transmission studies. In the *G.m.m.* studies, the LSD 2490 strain of virus was used.

1.2.2 Lamb testis cells

The cells used for virus isolation were obtained from prepurbertal lamb testes that were obtained from Kakuzi farm in Thika, Kenya.

APPENDIX 2a COMPARISON OF NUMBER OF FIELD COLLECTED MALE INSECTS BY SPECIES BONFERRONI TEST)

Row mean-Col mean	S. cal	S. Sit	Sniger	Sbil	Sochr	Staen	Sbed	SSq	P.mart	Sschw	Sant	Safr	Cant	Aochr	Agamb	Anfun	Hema
Sbed	273.5 0.000	292.47 0.000	260.773 0.000	277.98 0.000	293.078 0.000	273.2 0.000		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SSq	NS	NS	NS	NS	NS	NS	-294 0.000	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P.mart	NS	NS	NS	NS	-289 0.003	5 1.000	*-289* *0.003*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cant	535.5 0.000	554.47 0.000	522.773 0.000	539.98 0.000	555.078 0.000	535.2 0.000	262 0.422	556 0.000	551 0.000	555 0.000	556 0.000	556 0.000	NS	NS	NS	NS	NS
Aochr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	--556 0.000	NS	NS	NS	NS
Anga	NS	NS	NS	NS	NS	NS	290.5 0.000	3.5 1.000	1.5 1.000	2.5 1.000	3.5 1.000	3.5 1.000	-552.5 0.000	3.5 1.000			
Anfun	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-556 0.000	0 1.000	-3.5 1.000		
Mixed	174 0.074	192.97 0.013	161.273 0.217	178.48 7 0.052	193.578 0.014	173.7 0.086	NS	NS	NS	NS	NS	NS	-361.5 0.002	NS	NS	NS	NS
Cul.							-293.6 0.000	NS	NS	NS	NS	NS	-555.67 0.000	NS	NS	NS	NS

APPENDIX 2B COMPARISON OF NUMBER OF FIELD COLLECTED MALE INSECTS BY SPECIES (BONFERRONI TEST)

Row mean-Col mean	S. cal	S. sit	Sniger	Sbil	Sochr	Staen	Sbed	SSq	P.mart	Sschw	Sant	Safr	Cant	Aochr	Angamb
Sbed	1016.0 0.000	1043.5 0.000	995.49 0.000	1023.56 0.000	1044.5 0.000	1039.0 0.000									
SSq	NS	NS	NS	NS	NS	NS	-1030.5 0.000								
P.mart	NS	NS	NS	NS	NS	NS	*-1044* *0.000*	NS							
Sschw	NS	NS	NS	NS	NS	NS	-1046 0.000	NS	NS						
Sant	NS	NS	NS	NS	NS	NS	-1033 0.000	NS	NS	NS					
Safr	NS	NS	NS	NS	NS	NS	-1033 0.000	NS	NS	NS	NS				
Cant	667.08 0.000	694.58 0.000	646.49 0.000	674.56 0.000	695.51 0.000	690.06 0.000	-349 1.000(NS)	681.5 0.000	695 0.000	697 0.000	684 0.000	681 0.000			
Aochr	NS	NS	NS	NS	NS	NS	-1045 0.000	NS	NS	NS	NS	NS	-696 0.000		
Anga	NS	NS	NS	NS	NSN	NS	-1041.5 0.000	-11 0.000	NS	NS	NS	NS	-695 0.000	NS	**NS**
Anfun	NS	NS	NS	NS	NS	NS	-1044 0.000	NS	NS	NS	NS	NS	-556 0.000	0 1.000	-3.5 1.000
Aedes	NS	NS	NS	NS	NS	NS	-1045.5 0.000	NS	NS	NS	NS	NS	-695.5 0.000		
Mixed	174 0.074	192.97 0.013	161.273 0.217	178.487 0.052	193.57 0.014	173.7 0.086	NS	NS	NS	NS	NS	NS	-361.5 0.002	NS	NS
Cul.	NS	NS	NS	NS	NS	NS	-1044.6 0.000	NS	NS	NS	NS	NS	--695.67 0.000	NS	NS

Appendix 2a and b (continued)

Key:

S.cal	*Stomoxys calcitrans*
S.sit	*S. sitiens*
Sniger	*S.niger niger*
Sbil	*S.niger bilineatus*
Staen	*S. taeniatus*
Sbed	*Sergentomyia bedfordi*
Ssq	*Sergentomyia squamipleuris*
Pmart	*Phlebotomus martini*
Sschw	*Sergentomyia schwertzi*
Sant	*Sergentomyia antennatus*
Safr	*Sergentomyia africanus*
Cant	*Culex antennatus*
Aochr	*Aedes ochrosoma*
Angamb	*Anopheles gambiae*
Anfu	*A. funestus*
Hema	*Hematopota spp.*
Mixed	Mixed species
Cul	*Culicoides*
NS	Not statistically significant

APPENDIX 3. COMPARISION OF CLIMATIC VARIABLES BY DISTRICTS

3a Comparison of minimum temperature by district (Bonferroni)

Row mean – Col Mean	Kiambu	Thika	Lamu	Machakos	Garissa	Kisii	Kajiado
Thika	6.4086 0.000						
Lamu	16.2528 0.000	9.844412 0.000					
Machakos	5.6262 0.000	-.7824 0.087	-10.6266 0.000				
Garissa	15.3651 0.000	8.95648 0.000	-.887684 0.020				
Kisii	7.77056 0.000	1.36195 0.000	-8.48221 0.000				
Kajiado	6.1584 0.000	-.2503 1.000	-10.0944 0.000	.5322 1.000	-9.2067 0.000	-1.6122 0.000	
Transnzoia	6.7587 0.000	.3501 1.000	-9.4941 0.000	1.1325 0.001	-8.6064 0.000	0.6003 0.663	-1.0119 0.003

3b Comparison of maximum temperature by district (Bonferroni)

Row Mean-Col Mean	Kiambu	Thika	Lamu	Machakos	Garissa	Kisii	Kajiado
Thika |	3.49141 0.000						
Lamu	8.99333 0.000	5.50192 0.000					
Machakos	3.11565 0.000	-.375759 1.000	-5.87768 0.000				
Garissa	12.3545 0.000	8.86311 0.000	3.36119 0.000	9.23887 0.000			
Kisii	3.64838 0.000	.156975 1.000	-5.34495 0.000	.532734 1.000	-8.70614 0.000		
Kajiado	2.33459 0.000	-.1.15682 0.899	-6.65874 0.000	-.781057 1.000	-10.0199 0.000	-1.31379 0.375	
Transnzoia	4.64958 0.000	1.15817 0.830	-4.34375 0.000	1.53393 0.107	-2.31499 0.000	1.0012 1.000	2.31499 0.000

3c Comparison of mean rain by district (Bonferroni)

Row Mean-Col Mean	Kiambu	Thika	Lamu	Machakos	Kisii	Kajiado
Thika	-2.40656 1.000					
Lamu	-2.9349 1.000	-.528336 1.000				
Machakos	-3.64065 0.107	-1.23408 1.000	-.705748 1.000			
Kisii	.345492 1.000	2.75206 0.251	3.28039 0.412	3.98614 0.006		
Kajiado	-2.59867 0.936	-.192103 1.000	.336233 1.000	1.04198 1.000	-2.94416 0.146	
Transnzoia	-1.9823 1.000	.4242 1.000	.9526 1.000	1.6583 1.000	-2.3278 1.000	.6164 1.000

3d Comparison of relative humidity of 00600 z by district (Bonferroni)

Row Mean-Col Mean	Kiambu	Thika	Lamu	Machakos	Garissa	Kisii	Kajiado
Thika |	-1.20372 0.894						
Lamu	-6.94438 0.000	-5.74067 0.000					
Machakos	-4.23818 0.000	-3.03446 0.000	2.7062 0.002				
Garissa	-11.4797 0.000	-10.276 0.000	-4.53531 0.000	-7.24151 0.000			
Kisii	-15.2347 0.000	-14.0309 0.000	-8.29028 0.000	-10.9965 0.000	-3.75497 0.000		
Kajiado |	-2.6401 0.000	-1.4364 0.287	4.3043 0.000	1.5981 0.135	8.8396 0.000	12.5946 0.000	
Transnzoia	-4.35575 0.000	-3.15203 0.000	2.58864 0.019	-.117568 1.000	-	-1.7165 0.299	-

3e Comparison of RH_1200z by district (Bonferroni)

Row Mean-Col Mean	Kiambu	Thika	Lamu	Machakos	Garissa	Kisii
Thika|	-5.03045 0.000					
Lamu	9.34051 0.000	14.371 0.000				
Machakos	-6.92045 0.000	-1.89 0.571	-16.261 0.000			
Garissa	-14.5601 0.000	-9.52965 0.000	-23.9006 0.000	-7.63965 0.000		
Kisii	-.264779 1.000	4.76567 0.000	-9.60529 0.000	6.65567 0.000	14.2953 0.000	
Kajiado	-6.3912 0.000	-1.3607 1.000	-15.7317 0.000	.5293 1.000	8.1689 0.000	-6.1264 0.000
Transnzoia	-6.89888 0.000	-1.86843 1.000	-16.2394 0.000	-.021573 1.000	-0.50768 1.000	-6.6341 0.000

3f Comparison of mean radiation by district (Bonferroni)

Row Mean-Col Mean	Kiambu	Thika	Machakos	Kisii	Kajiado
Thika	.652529 1.000				
Machakos	3.45106 0.000	2.79853 0.044			
Kisii	8.33098 0.000	7.67845 0.000	4.87993 0.000		
Kajiado	3.07162 0.000	2.41909 0.147	-.379433 1.000	-5.25936 0.000	
Transnzoia	2.87895 0.000	2.22642 0.209	-.572104 1.000	-5.45203 0.000	-.192671 1.000

3g Comparison of mean sunshine hours by district (Bonferroni)

Row Mean-Col Mean	Kiambu	Thika	Lamu	Machakos	Garissa	Kisii
Thika	-.119262 1.000					
Lamu	2.10321 0.000	2.22248 0.000				
Machakos	.66656 0.010	.785822 0.290	-1.43665 0.000			
Garissa	2.05594 0.001	2.1752 0.002	-.047274 1.000	1.38938 0.116		
Kisii	.26767 1.000	.386931 1.000	-1.83554 0.000	-.398891 0.759	-1.78827 0.006	
Kajiado	.5078 0.170	-6271 1.000	-1.5954 0.000	-.1587 1.000	-1.5481 0.039	.2402 1.000
Transnzoia	.535173 0.067	.654435 0.821	-1.56804 0.000	-.131387 1.000	-0.27351 1.000	0.2675 1.000

3h Comparison of mean windrun by district (Bonferroni)

Row Mean-Col Mean	Kiambu	Thika	Lamu	Machakos	Garissa	Kisii	Kajiado
Thika	4.71864 1.000						
Lamu	26.523 0.000	21.8043 0.071					
Machakos	30.6962 0.000	25.9776 0.006	4.17324 1.000				
Garissa	49.4714 0.093	44.7527 0.308	22.9498 1.000	18.7752 1.000			
Kisii	41.7849 0.000	37.0662 0.000	15.2619 0.042	11.0887 0.346	-7.6865 1.000		
Kajiado	27.3242 0.000	22.6056 0.021	.8013 1.000	-3.3719 1.000	-22.1472 1.000	-14.4607 0.009	
Transnzoia	-7.370 1.000	-12.088 1.000	-33.892 0.000	-38.065 0.000	-56.841 0.020	-34.693 0.000	-49.154 0.000

APPENDIX 4a. COMPARISON OF NUMBER OF LSD CASES IN DIFFERENT KENYAN DISTRICTS (1981-2001)

Row mean - Col Mean	Kiambu	Thika	Lamu	Machakos	Garissa	Kisii	Kajiado
Thika	-.52095 0.003						
Lamu	-.52095 0.002	0 1.000					
Machakos	-.52095 0.005	0 1.000	0 1.000				
Garissa	-.52095 0.003	0 1.000	0 1.000	0 1.000			
Kisii	-.52095 0.003	0 1.000	0 1.000	0 1.000	0 1.000		
Kajiado	-.38206 0.097	.138889 1.000	.138889 1.000	.138889 1.000	.138889 1.000	.138889 1.000	
Transnzoia	-.52095 0.006	0 1.000	0 1.000	0 1.000	0 1.000	0 1.000	-.138889 1.000

APPENDIX 4b COMPARISON OF NUMBER OF ANIMALS VACCINATED AGAINST LSD IN DIFFERENT KENYAN DISTRICTS (1981-2001)

Row Mean-Col Mean	Thika	Lamu	Machakos	Garissa	Kisii	Kajiado
Lamu	-008811 1.000					
Machakos	.024843 0.412	.033654 0.020				
Garissa	.008811 1.000	0 1.000	.033654 0.027			
Kisii	.003689 1.000	.0125 1.000	-.021154 0.989	.0125 1.000		
Kajiado	.000874 1.000	.007937 1.000	.025717 0.271	.007937 1.000	.004563 1.000	
Transnzoia	.005895 1.000	.014706 1.000	-.018948 1.000	.014706 1.000	.002206 1.000	.006769 1.000

APPENDIX 5. COMPARISON OF LSD OUTBREAKS IN KENYAN DISTRICTS BETWEEN 1981-2001 (PEARSONS CHI-SQUARE)

District	No Outbreak	Outbreak present	Total
Kiambu	4	23	27
	14.81	85.19	100.00
Kirinyaga	1	7	8
	12.50	87.50	100.00
Maragua	3	2	5
	60.00	40.00	100.00
Muranga	1	12	13
	7.69	92.31	100.00
Nyandarua	5	8	13
	38.46	61.54	100.00
Nyeri	2	17	19
	10.53	89.47	100.00
Thika	2	3	5
	40.00	60.00	100.00
Kilifi	2	2	4
	50.00	50.00	100.00
Kwale	4	0	4
	100.00	0.00	100.00
Lamu	1	2	3
	33.33	66.67	100
Malindi	1	2	3
	33.33	66.67	100.00
Mombasa	4	1	5
	80.00	20.00	100.00
Taita Taveta	2	4	6
	33.33	66.67	100.00
Tana River	1	1	2
	50.00	50.00	100.00
Embu	0	18	18
	0.00	100.00	100
Isiolo	1	1	2
	50.00	50.00	100.00
Kitui	2	5	7
	28.57	71.43	100.00
Machakos	5	9	14
	35.71	64.29	100.00
Makueni	1	5	6
	16.67	83.33	100.00
Marsabit	1	0	1
	100.00	0.00	100.00
Meru	2	7	9
	22.22	77.78	100.00
Moyale	1	1	2
	50.00	50.00	100.00
Mwingi	1	1	2
	50.00	50.00	100.00

APPENDIX 5 (Continued)

District	No Outbreak	Outbreak present	Total
Tharaka-Nithi	8	5	13
	61.54	38.46	100.00
Garissa	1	3	4
	25.00	75.00	100.00
Ijara	1	2	3
	33.33	66.67	100.00
Mandera	1	2	3
	33.33	66.67	100.00
Wajir	4	2	6
	66.67	33.33	100.00
Nairobi	0	1	1
	0.00	100.00	100.00
Gucha	3	2	5
	60.00	40.00	100.00
Homa-Bay	3	1	4
	75.00	25.00	100.00
Kisii	3	1	4
	75.00	25.00	100.00
Kisumu	3	2	5
	60.00	40.00	100.00
Kuria	3	1	4
	75.00	25.00	100.00
Migori	3	1	4
	75.00	25.00	100.00
Nyamira	3	1	4
	75.00	25.00	100.00
Nyando	3	2	5
	60.00	40.00	100.00
Rachuonyo	3	1	4
	75.00	25.00	100.00
Siaya	3	1	4
	75.00	25.00	100.00
Suba	4	1	5
	80.00	20.00	100.00
Baringo	1	5	6
	16.67	83.33	100.00
Bomet	0	4	4
	0.00	100.00	100.00
Elgeyo Marakwet	2	2	4
	50.00	50.00	100.00
Kajiado	4	5	9
	44.44	55.56	100.00
Kericho	3	5	8
	37.50	62.50	100.00
Laikipia	3	4	7
	42.86	57.14	100.00
Naivasha	0	1	1
	0.00	100.00	100.00

APPENDIX 5(Continued)

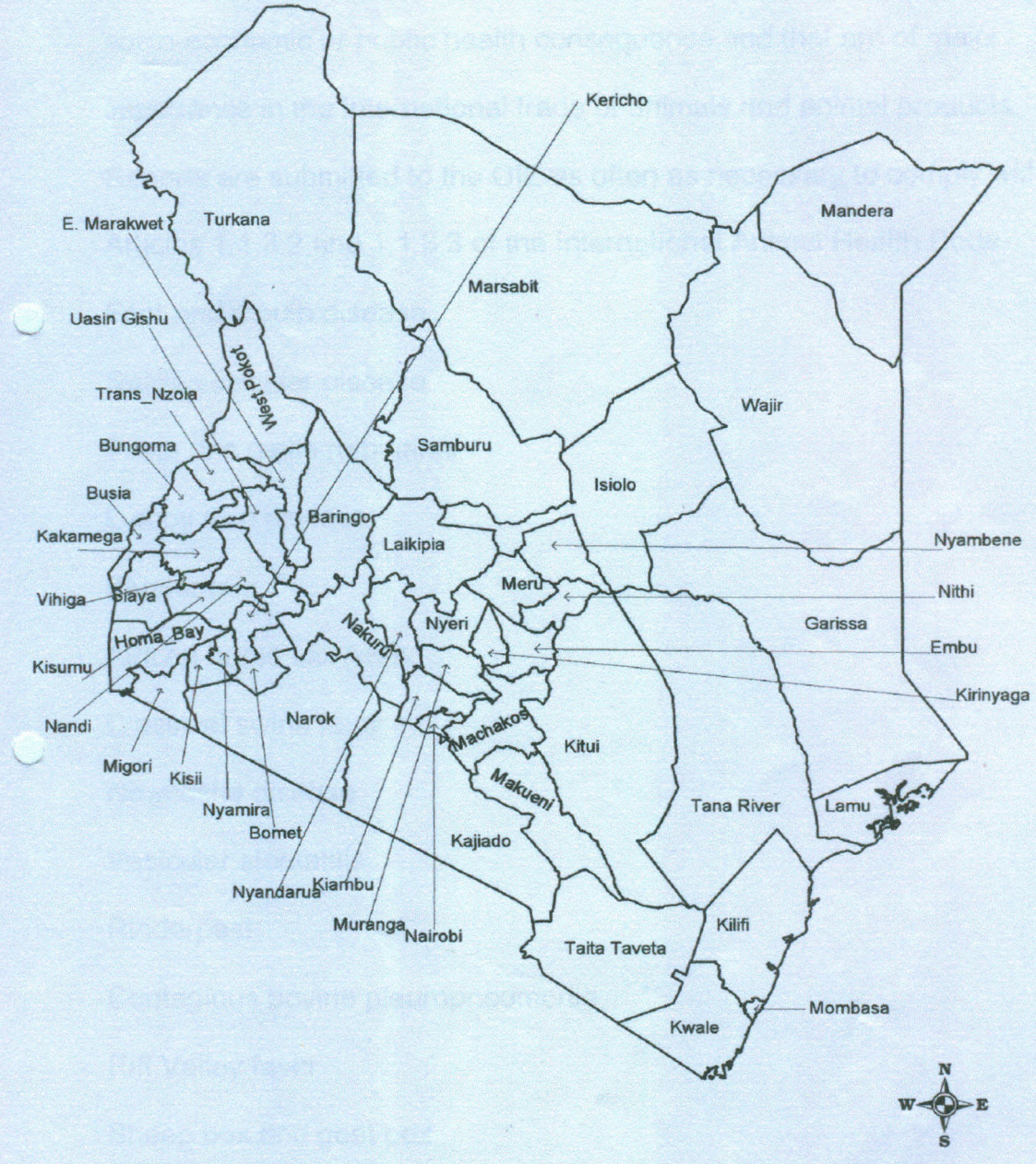
District	No Outbreak	Outbreak present	Total
Nakuru	4	9	13
	30.77	69.23	100.00
Nandi	2	7	9
	22.22	77.78	100.00
Narok	0	4	4
	0.00	100.00	100.00
Samburu	3	0	3
	100.00	0.00	100.00
TransNzoia	0	7	7
	0	100.00	100.00
Turkana	1	1	2
	50.00	50.00	100.00
Uasin Gishu	1	5	6
	16.67	83.33	100.00
West Pokot	0	4	4
	0.00	100.00	100.00
Butere-Mumias	3	4	7
	42.86	57.14	100.00
Bungoma	5	4	9
	55.56	44.44	100.00
Busia	4	8	12
	33.33	66.67	100.00
Kakamega	5	6	11
	45.45	54.55	100.00
Lugari	6	3	9
	66.67	33.33	100.00
Mount Elgon	5	4	9
	55.56	44.44	100.00
Teso	6	3	9
	66.67	33.33	100.00
Vihiga	6	4	10
	60.00	40.00	100.00
Buret	0	1	1
	0.00	100.00	100.00
Transmara	0	1	1
	0.00	100.00	100.000
Total	160	261	421
	38.00	62.00	100.00

Pearson chi2(64) = 109.5995 Pr = 0.000 Cramer's V = 0.5102

APPENDIX 6 FREQUENCY DISTRIBUTION OF CATTLE VERSUS INSECTS SPECIES USED IN THE TRANSMISSION EXPERIMENTS

Species	Frequency	Percentage	Cumulative
Glossina morsitans morsitans.	11	13.10	13.10
Glossina morsitans centralis	15	17.86	30.95
Stomoxys	17	20.24	51.19
Mosquitoes	8	16.67	67.86
Challenge/ Control	8	9.52	86.90
Virus Challenge	5	5.95	92.86
Negative Control	6	7.14	100.00
Total	**84**	**100.00**	

APPENDIX 7 MAP OF KENYAN DISTRICTS



APPENDIX 8 DISEASES NOTIFIABLE TO THE OIE

List A

Transmissible diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products.

Reports are submitted to the OIE as often as necessary to comply with Articles 1.1.3.2 and 1.1.3.3 of the International Animal Health Code.

Foot and mouth disease

Swine vesicular disease

Peste des petits ruminants

Lumpy skin disease

Bluetongue

African horse sickness

Classical swine fever

Newcastle disease

Vesicular stomatitis

Rinderpest

Contagious bovine pleuropneumonia

Rift Valley fever

Sheep pox and goat pox

African swine fever

Highly pathogenic avian influenza

List B

Transmissible diseases that are considered to be of socio-economic and/or public health importance within countries and that are significant in the international trade of animals and animal products. Reports are normally submitted once a year, although more frequent reporting may in some cases be necessary to comply with Articles 1.1.3.2 and 1.1.3.3 of the International Animal Health Code.

Multiple species diseases

Anthrax

Aujeszky's disease

Echinococcosis/hydatidosis

Heartwater

Leptospirosis

New world screwworm (Cochliomyia hominivorax)

Old world screwworm (Chrysomya bezziana)

Paratuberculosis

Q fever

Rabies

Trichinellosis

Cattle diseases

Bovine anaplasmosis

Bovine babesiosis

Bovine brucellosis

Bovine cysticercosis

Bovine genital campylobacteriosis

Bovine spongiform encephalopathy

Bovine tuberculosis

Dermatophilosis

Enzootic bovine leukosis

Haemorrhagic septicaemia

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis

Malignant catarrhal fever

Theileriosis

Trichomonosis

Trypanosomosis (tsetse-transmitted)

Sheep and goat diseases

Caprine and ovine brucellosis (excluding B. ovis)

Caprine arthritis/encephalitis

Contagious agalactia

Contagious caprine pleuropneumonia

Enzootic abortion of ewes (ovine chlamydiosis)

Maedi-visna

Nairobi sheep disease

Ovine epididymitis (Brucella ovis)

Ovine pulmonary adenomatosis

Salmonellosis (S. abortusovis)

Scrapie

Equine diseases

Contagious equine metritis

Dourine

Epizootic lymphangitis

Equine encephalomyelitis (Eastern and Western)

Equine infectious anaemia

Equine influenza

Equine piroplasmosis

Equine rhinopneumonitis

Equine viral arteritis

Glanders

Horse mange

Horse pox

Japanese encephalitis

Surra (Trypanosoma evansi)

Venezuelan equine encephalomyelitis

Swine diseases

Atrophic rhinitis of swine

Enterovirus encephalomyelitis

Porcine brucellosis

Porcine cysticercosis

Porcine reproductive and respiratory syndrome

Transmissible gastroenteritis

Avian diseases

Avian chlamydiosis

Avian infectious bronchitis

Avian infectious laryngotracheitis

Avian mycoplasmosis (M. gallisepticum)

Avian tuberculosis

Duck virus enteritis

Duck virus hepatitis

Fowl cholera

Fowl pox

Fowl typhoid

Infectious bursal disease (Gumboro disease)

Marek's disease

Pullorum disease

Lagomorph diseases

Myxomatosis

Rabbit haemorrhagic disease

Tularemia

Bee diseases

Acariosis of bees

American foulbrood

European foulbrood

Nosemosis of bees

Varroosis

Fish diseases

Epizootic haematopoietic necrosis

Infectious haematopoietic necrosis

Oncorhynchus masou virus disease

Spring viraemia of carp

Viral haemorrhagic septicaemia

Mollusc diseases

Bonamiosis (Bonamia exitiosus, B. ostreae, Mikrocytos roughleyi)

Marteiliosis (Marteilia refringens, M. sydneyi)

Mikrocytosis (Mikrocytos mackini)

MSX disease (Haplosporidium nelsoni)

Perkinsosis (Perkinsus marinus, P. olseni/atlanticus)

Crustacean diseases

Taura syndrome

White spot disease

Yellowhead disease

Other List B diseases

Leishmaniosis

APPENDIX 9: ABSTRACTS FOR CONFRENCES/SEMINARS/ WORKSHOPS

Abstract for KARI/DFID End of Project Conference held at KARI Headquarters, Nairobi on 23rd -26th March, 1999.

(Abstract also available on website: Http//karidfid.africaonline.co.ke**)**

ECOLOGICAL FACTORS IN THE TRANSMISSION OF LUMPY SKIN DISEASE OF CATTLE

Misiani, E A, Ngichabe, C K, Anjili, C, Mihok, S.

Lumpy skin disease is an economically crippling disease of cattle with epizootic occurrence particularly after the onset of the rainy season. In nearly all the areas where there have been outbreaks of LSD the prevalence of biting flies and the conditions favourable to their breeding support the suggestion that the disease is spread by insects (Haig, 1957). Biting arthropods have been implicated as vectors of Lumpy skin disease virus (LSDV) due to observations that there is no transmission of LSD when cattle are confined to insect-proof houses Carn (1994).

The clinical signs that were observed in these animals included skin nodules and lesions especially on the head and legs and oedema of the legs. The animals affected ranged from five months to three years old. Both hybrid and zebu cattle were affected. In all the affected farms there was a forested area or bushland in the vicinity as well as a stream or dam nearby.

The arthropods collected from these outbreak areas included sandflies (*Phlebotomus* martini Parrot, *Sergentomyia bedfordi* Newstead, *S.africanus* Newstead, *S.schwetzi* Adler, Theodor and Parrot, and *S.antennatus*) mosquitoes (*Anopheles funestus* Giles, *A. gambiae* Giles and *Culex antennatus*.Becker) tabanids, (*Haematopota* and *Atylotus* species), stable flies (*Stomoxys calcitrans* (L.), *S. niger niger* Marquart, *S. ochrosoma* Speiser, *S.* sitiens Rondani, *S. taeniatus* Bigot, and *S. niger bilineatus* Grunberg) and ticks (*Boophilus decoloratus* Koch). The results that have been analysed show that sandflies and Stomoxyinae formed the largest percentage of the total insects trapped.