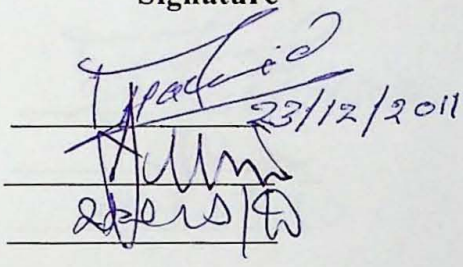
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BY

MOHAMMED ELSADIG AHMED

Academic Advisors **Signature**

1. Prof. Abdelhamid Elfadil (SUST, Sudan)
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DECEMBER, 2011

DEBRE ZEIT, ETHIOPIA

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1. Prof. Abdelhamid Elfadil (SUST, Sudan)	25/12/2011
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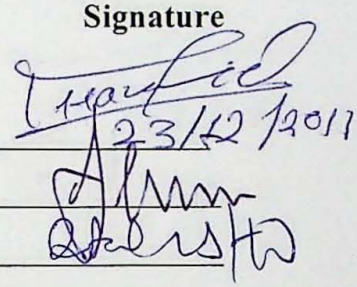
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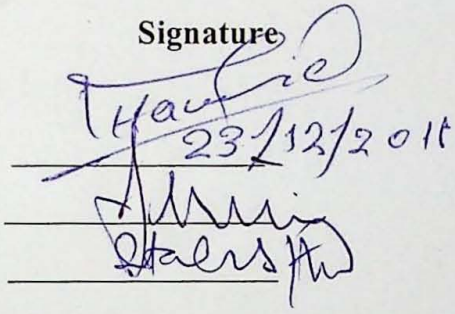
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MOHAMMED ELSADIG AHMED

A thesis submitted to the school of Graduate Studies of Addis Ababa University, Freie Universität-Berlin and Sudan University of Science and Technology in partial fulfillment of the requirements for the joint master in Transboundary Animal Disease Management program (MTADM)

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FREIE UNIVERSITÄT BERLIN

SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

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ACADEMIC ADVISORS

1. PROF. ABDELHAMID A.M. ELFADIL (SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY)

2. PROF. KARL-HANS ZESSIN (FREIE UNIVERSITÄT -BERLIN)

3. DR. YILKAL ASFAW (ADDIS ABABA UNIVERSITY)

APPROVAL BY THE BOARD OF EXAMINERS

This thesis is hereby approved by following members of board of examiners

Name	Title	Signature	Date
1. PROF. KARL-HANS ZESSIN	PROFESSOR	.....	.....
2. DR. KITMA ELMALIK	ASSOCIATE PROFESSOR	.....	.....
3. PROF. ABDELHAMID ELFADIL	PROFESSORS	.....	.....
4. DR. YILKAL ASFAW	ASSOCIATE PROFESSOR	.....	.....

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LIST OF ABBREVIATIONS

AAHL	Australian Animal Health Laboratory
AU	Administrative Unit
AUC	Area Under Curve
CFR	Case Fatality Rate
CPE	Cytopathic Effect
ELISA	Enzyme Linked Immunosorbent Assay
ETDA	Ethylene diamine-tetra acetic acid
GDP	Gross Domestic Product
GMEM	General Minimum Essential Medium
GTP	Goat pox
GTPV	Goat pox Virus
H	Hour
H& E	Hematoxlyin and Eosin stain
ICTV	International Committee on Taxonomy of Viruses
LK	Lamb kidney
LSD	Lumpy Skin Disease
LSDV	Lumpy Skin Disease Virus
LT	Lamb testis
N I	Neutralization Index
NKS	North Kordofan State
NM	Nanometer

NVI	National Veterinary Institute, Ethiopia
OD	Optical density
OIE	Office Internationale des Epizooties
OK	Outbreaks of sheep pox in Kordofan region
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
ROC	Receiver Operation Characteristic
SGP	Sheep and goat pox
SKS	South Kordofan State
SPP	Sheep pox
SPPV	Sheep pox Virus
SPSS	Statistical Package for Social Sciences
$TCID_{50}$	Tissue Culture Infectious Dose
TMB	Tetramethylbenzidine dihydrochloride
VNT	Virus Neutralization Test
VRI	Veterinary Research Institute, Sudan

ABSTRACT

The current study is an attempt to better understand the epidemiology of sheep pox in the Kordofan region in the western part of the Sudan, which is one of the largest production sites for sheep. The specific objectives were the investigation of potential risk factors associated with sheep pox outbreaks, an assessment of the economic impact of sheep pox disease, the estimation of seroprevalence of sheep pox and to evaluate the test performance characteristics of the indirect ELISA test employed in this study. To practically achieve the objectives, the study was designed as a cross-sectional survey in the Kordofan region, with collection of specimen from the target population of sheep from March to September 2011; a concurrent questionnaire did address animal owners and veterinary professionals. The current study revealed that the estimated overall sero-prevalence of SPP in Kordofan region was 20.1% and that SPP prevails both in SKS and in NKS. Also, a number of risk factors like locality, herd size, insect bites and rain fall/year and traditional treatment were found to be significantly associated with SPP occurrence in the Kordofan region. The average cost of SPP was estimated at 20.5% of sheep flock production costs. The Indirect ELISA test to detect antibodies to capripox viruses was evaluated to have a diagnostic sensitivity of 33.33% and a diagnostic specificity of 60%. For VNT, 12 out of the 22 samples were positive, having Neutralization Indices (N.I $\geq$ 1.5) of 3.5, 3, 2.5, 2 and 1.5 recorded for 25%, 8.3%, 8.3%, 25% and 33.3 % of positive samples, respectively.

Key words: Sheep pox, prevalence, risk factors, economic effect, indirect ELISA, the Sudan

1 INTRODUCTION

The livestock sector in the Sudan is an important contributor to the national economy, accounting for 25% of the Gross Domestic Product (GDP) and employing 40% of the country's population. In 1999, livestock exports generated 20–25% of the national foreign exchange earnings (Central Bank of Sudan, 2004). Sudan has a large small ruminant population, estimated in 2006 at 50.1 million sheep and 42.1 million goats. Of the total livestock population of the Arab world, the main market for Sudanese livestock, Sudan accounts for about 70% of cattle, 31% of sheep, 49% of goats, and 25% of camels. Sudan also accounts for some 43% of the Arab world's red meat production (Dirani *et al.*, 2009). The main livestock production sites are located far from the major consumption centers and export outlets. Economically valuable livestock populations are concentrated in Northern and Southern Kordofan and Darfur States, accounting for 36% of cattle, 40% of sheep, 36% of goats, and 33% of camel populations. Blue Nile and Gedarif States are also important supply hinterlands for sheep export.

There are many constraints in livestock and livestock products confronting the developing countries. Diseases are considered one of the most important issues interfering with domestic and international livestock trade. One of these diseases is sheep pox (SPP) which causes considerable production losses in endemic areas as a result of reduced milk yields, decreased weight gain, abortion, poor quality wool and hides, increased susceptibility to pneumonia and fly strike, and mortality (Babiuk *et al.*, 2008; Yeruham *et al.*, 2007).

SPP is a viral disease of sheep characterized by fever, generalized papules or nodules, vesicles rarely, internal lesions particularly in the lungs, and death. Sheep and goat pox (SGP) are caused by viruses in the genus *Capripoxvirus*, which can infect sheep and goats. Although most of sheep pox virus (SPPV) strains examined cause more severe clinical disease in either sheep or goats, some strains are equally pathogenic in both species. It has been proposed that the malignant pox diseases of sheep and goats caused

by capripoxvirus as well as the Kenyan sheep and goat pox, the Indian goat dermatitis and the North African stone pox of sheep and goats should be referred to as capripox (OIE, 2008).

SPP and goat pox (GTP) are similar in terms of their geographical distribution. During the past 50 years, the geographical range of SGP has been restricted mainly to Asia and Africa, extending from North Africa to the Equator, from West Africa to East Asia, including Turkey, the Middle East and the Southern Regions of the former Soviet Union. Capripox has the potential to spread and become established in countries outside its normal distribution. In 1983 it spread into Italy, in 1985 and 1989 into Cyprus, and in 1988 and numerous subsequent occasions into Greece, but did not become established in these countries. In 1984, however, it spread into Bangladesh where since then it has persisted. In 2005, an outbreak in goats in Vietnam indicated that capripox has an even larger distribution than previously recognized.

In the Sudan, the first scientific investigation on SPP was evaluated by Bennet *et al.* (1944), who affirmed that the disease was endemic in the country as well as that the virus was host-specific. However, it has been early reported that no host specific SPPV in Sudan could infect goats (Hajer *et al.,* 1988). SPP in the Sudan is responsible for dramatic economic losses particularly in the animals prepared for export (Losos, 1986). Sheikh-Ali *et al.* (2004) reported alteration of some epidemiological patterns and virus heterogeneity recently observed in sheep pox outbreaks in the Sudan.

According to previously mentioned information no detailed epidemiological study about SPP though has been conducted in the west of the Sudan. Thus, the present study is an attempt to fill in gaps of the epidemiology of SPP in the Kordofan region of the western part of the Sudan.

Therefore the objectives of this study were:

- To estimate the sero-prevalence of SPP in sheep in the Kordofan region, Sudan.
- To investigate potential risk factors associated with SPP outbreaks.
- To estimate economic effects of SPP outbreaks.
- To evaluate the test performance characteristics of the new recombinant Capripox virus antigen indirect ELISA compared to the gold standard Virus Neutralization Test for the study area in the Sudan.

2 LITERATURE REVIEW

2.1 Aetiology

The virus causing SPP is one of the Capripoxviruses which are considered some of the largest viruses, measuring 170-260 nm by 300-450 nm (Matthews, 1982). It is closely related to the viruses that cause lumpy skin disease (LSD) and goat pox (GTP). SPPV (sheep pox virus) and LSD virus (LSDV) cannot be distinguished serologically. There exists only one serotype of SPPV. Various strains of SPPV cause disease only in sheep, and some in both sheep and goats (Davies, 1976, 1981; Kitching and Taylor 1985).

2.1.1 Taxonomy

SPPV belongs to the genus *Capripoxvirus*, classified in the subfamily *Chordopoxvirinae* of the family Poxviridae (Matthews, 1982). The virions are brick-shaped, enveloped with rounded ends and of complex symmetry (Dales and Pogo, 1981). The genome is a double stranded DNA of 73–91 MDa, with a central, less variable region of about 145 kilo base pairs and more variable termini with cross-links. Extensive DNA cross-hybridization occurs between species (Black *et al.,* 1986; Gershon *et al.,* 1989a; Murphy 1995). The virion is about 300 × 270 × 200 nm in size and resembles other viruses of the orthopox group. Two types of particles are observed in negatively stained preparations, namely the mulberry or 'm' form, which is electron-transparent, and the electron-dense capsule or 'e' form (Kitching and Smale, 1986).

The nomenclature of pox viruses is largely made up of the location of the country and the species from which it has been isolated, either from sheep or goats or from both sheep and goats. This issue of naming strains SPP, GTP or SGP remains a problem since it is based on field observation of the species affected, whether sheep or goats.

2.1.2 Morphology of SPPV

SPPV elementary bodies were among the first to be seen with the light microscope (Borrel, 1903a), but their morphology was not studied until 1957. The Pakistani strain showed an average length×mean and breadth×average axial ratio of 194±16.08µm ×15±10.73µm 1.76±10.176 (Abdussalam, 1957). Morphologically, SPPV resembles other animal orthopoxviruses in several aspects but is more elongated in shape. Electron microscopy can differentiate orthopoxvirus from parapoxvirus (Contagious pustular dermatitis) as orthopoxviruses have a distinct morphology of lateral bodies whereas parapoxviruses have an ovoid shape with a distinct pattern. The mature capripox virus particle is oval or rectangular in shape containing a dumbbell shaped core (Murray *et al.*, 1973). The size of the lateral bodies is larger than that of vaccinia virus (Cohen *et al.*, 1971).

2.1.3 Strains and isolates of SPPV

A number of SPP strains/isolates are documented in the literature. These strains basically have been named after their places of isolation. Some have been used as vaccines after attenuation in different cell culture systems at different passage levels and were found effective candidates for vaccines.

These strains include Ranipet, Jaipur, Hyderabad, Mysore, Karnal, Mathura, Indo-China (India), Roumanian, Roumanian-Fanar (RF), Cairo, Chinese, K Strain, Perego M, RM/65, Kazakhstan, Stavropol, Nairobi, Kenyan, SP8, Pakistan, Turkey, Russian, A1 strain, Oendic, Kedong, Soba, Persian, Bucharest, Bakirkoy, Algerian, Pendic, SPV/RH, Mauritanian, Chitinsk, Mongolian, Niski and SP6 (Bhanuprakash *et al,*. 2006).

2.1.4 Genomic structure of SPPV

SPP virions have a complex construction and can have different forms depending on the number of lipid envelopes surrounding the viral core. They contain a surface membrane,

a core, and lateral bodies. The SPP virion surface is characterized by tubules. The core is biconcave with two lateral bodies nested between the core or surface membranes. During their life cycle, virions produce extracellular and intracellular particles, but the infection is initiated by extracellular virions. Virus may be sequestered within inclusion bodies that are not occluded and typically contain one nucleocapsid. Virions mature naturally by budding through the membrane of the host cell. The viral genome encodes structural and non-structural proteins. Lipids are located in the envelope, constitute to the tune of 4% by weight and are composed of glycolipids similar to host cell membranes. The lipids are host-derived and synthesized de novo (ICTV, 2002).

Phylogenetic analysis of nucleotide sequence data of the capripoxvirus intracellular mature virion envelope protein P32 revealed that SPPV, GTPV and LSDV clustered into host species-specific groups (Hosamani *et al.,* 2004), but further investigations on different gene sequences from additional virus isolates are needed to confirm this observation.

2.1.5 Cross reactivity of SPPV

Capripoxviruses are antigenically related, and SPPV and GTPV share one or more precipitin band, depending upon the antiserum used (Subba Rao *et al.,* 1984). The viruses cross-react in the complement fixation test (Sharma and Dhanda, 1971) as well as in Virus Neutralization testing. Though the major portion of the antigens of GTPV and SPPV are similar, there may exist some differentiating antigenic components in each virus (Rao *et al.,* 1996). Overall, however, within isolates or strains of SPPV, and possibly GTPV, the antigenic make-up is largely similar (Bhat, 1993).

Furthermore, it seems that neither GTPV nor SPPV is serologically related to contagious pustular dermatitis virus, a parapoxvirus (Ngichabe *et al.,* 1999); however, the results of investigations on cross-reactions often vary, which is probably due to the different methods used by different workers, employing different isolates of capripoxvirus (Kitching *et al.,* 1986b).

Munz and Owen (1966) reported strains of capripox virus that cause LSD, such as Neethling found in cattle, are immunologically related to SPPV and protect cattle against LSD. The SPPV 'Kedong' strain did protect cattle against virulent LSD caused by the 'Neethling' type virus; in contrast, the Sudan SPPV isolate 'Soba' did not confer such protection (Muzichin and Ali Beih, 1979).

2.2 Epidemiology

Outbreaks of SPP have been reported from different parts of the world regularly (Bhanuprakash *et al.,* 2005). Like any other infectious disease, SPP outbreaks are accompanied with morbidity, mortality and case fatality rates (CFR).

Morbidity and mortality rates may be as low as 1% and go up to 100% (Woldemeskel and Ashenafi, 2003). Although morbidity is usually high in lambs (Yashpal *et al.,* 1997), such effects are also reported in adults, essentially depending on the location and the season. Breed wise, disease incidence is high in pure and crossbred lambs and hoggets (Belwal *et al.,* 1982). However, morbidity is generally high in lambs, yearlings and immunologically naïve sheep. Significant interspecies differences in morbidity and mortality in caprines have also been reported (Batta *et al.,* 1999). Mortality may shoot up to 100% (Daoud, 1997), which is normally seen among lambs and crossbred hoggets rather than adults (Belwal *et al.,* 1982).

CFR range from 15.8% to 57.9% (Mishra and Singh, 1984) and are highest in crossbred hoggets. Factors of the host (age, sex, breed, nutritional and immunological status), agent (strain, virulence, and pathogenicity), environment (micro and macro), poor management, feed scarcity and inadequate veterinary services have direct influence on the epizootiology of SPP (Woldemeskel and Ashenafi, 2003).

The disease has been reported in various countries of the world from time to time and outbreaks occur throughout the year depending upon season, climate, agent and the breeds of sheep native to that place (Gulbahar *et al.*, 2000). SPP outbreaks associated

with unusual winters (Hailat *et al.*, 1994) and drought (Chamoiseau, 1985) have also been reported. Peak outbreaks in India have been reported during winter and summer months and least during August in certain parts of the country. However, outbreaks in other parts of India have been recorded throughout the year, as reported also from other parts of the world. During seasons, sheep are exposed to various geoclimatic conditions, thereby causing the release of stressors, which, in turn, suppress the sheep immune system, in turn predisposing the animals to disease. Other probable causes include migration and ovination, which may aid disease spread (Bhanuprakash, 2001).

2.2.1 Host range

Capripoxviruses only infect some ruminant species and have a tropism for certain cell types (McFadden, 2005); they are not infectious to humans (Regnery, 2007). SPPV and GTPV cause clinical disease in sheep and goats, respectively; however, there is a wide range of clinical disease seen with different field isolates. There is little evidence to support the species strain designation theory, except when the viruses are used to experimentally infect both hosts under controlled conditions.

Some isolates are uniformly pathogenic in both sheep and goats such as some capripoxvirus strains from Kenya (Davies, 1976) and the Middle East (Kitching *et al.*, 1986). Most isolates, however, cause more severe disease in either sheep or goats and cause only mild or sub-clinical infection in the other species. It is unknown whether closely related North American animal species, such as mountain goats and mountain sheep, would be susceptible to SPPV or GTPV, but it is likely that they would exhibit at least some degree of susceptibility.

An extensive serological study involving a wide range of African wildlife demonstrated low capripoxvirus virus neutralization activity, between 1–10%, for giraffes, impalas, springboks, kudus, waterbucks and reedbucks, with African buffaloes and wildebeest being negative (Hedge and Hamblin, 1983). However, in 2008 Sudan reported 30 cases

of SGP in Bohor Reedbuck (*Redunca redunca*) to the OIE. This diagnosis though was based only on clinical findings and on post-mortem examination.

Capripox viruses are non-zoonotic unlike parapoxvirus causing orf which can lead to mild lesions of small red papules, followed by vesicles on the hands and arms, such as having been reported for humans working with parapoxviruses. With orf, no generalised infections occur.

2.2.2 Distribution and occurrence

Hansen first reported goat pox in 1879 from Norway (Rafyi and Ramyar, 1959). Later, it was observed during the period of the First World War in Macedonia, where it became enzootic during the year 1926, with a mortality rate of 15% (Blanc *et al.*, 1928). The disease today is present mainly in Asia, Africa, the Middle East and parts of Europe, but it has also been reported from many other parts of the world.

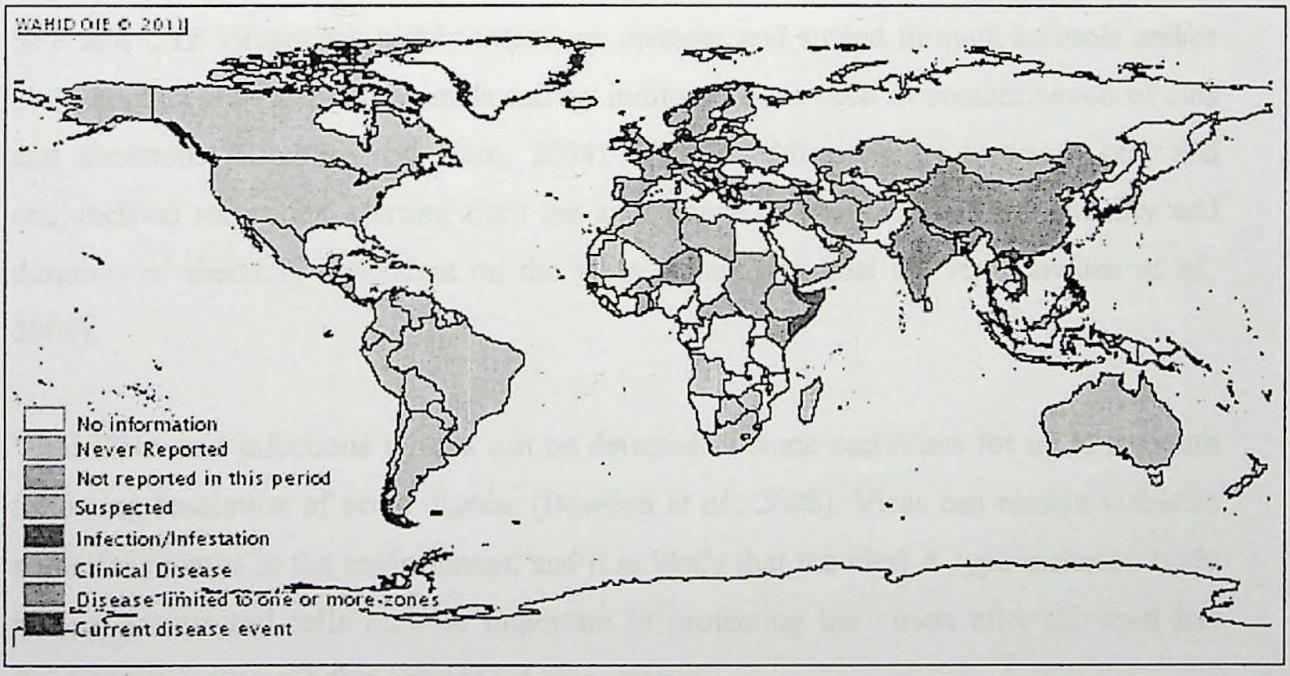


Figure 1: Distribution and occurrence of SPP (OIE, 2011)

There are distinct differences between the geographic distribution of SPP, GTP and LSD viruses. The geographic range of SPP had been restricted in the last 50 years mainly to Asia and Africa, extending from Africa north of the Equator (Kitching *et al.*, 1989; Mariner *et al.*, 1991; Achour and Bouguedour, 1999), into the Middle East (Daoud, 1997), Turkey (Oğuzoğlu *et al.*, 2006) and Asia, including regions of the former Soviet Union (Orlova *et al.*, 2006), India (Mondal *et al.*, 2004; Bhanuprakash *et al.*, 2005) and China (Zheng *et al.*, 2007). SPP or GTP did extend their range into Bangladesh in 1984 (Kitching *et al.*, 1987b) and more recently into Vietnam (2005 and 2008) and Mongolia (2006 and 2007) in the east, and repeated incursions have been reported in Greece in southern Europe (2007) (OIE, 2009).

LSD is endemic in Africa, including South Africa, whereas SGP are not present, and causes outbreaks in the Middle East in Israel.

2.2.3 Modes of transmission

SPP and GTP viruses are highly infectious diseases and spread through aerosols and/or close contact with infected animals and by indirect means such as contamination of cuts and abrasions (Kitching and Carn, 2004). Viral shedding occurs in nasal, oral and conjunctival secretions, starting from the appearance of papules, with the quantity and duration of shedding dependent on the virus isolate and host species (Bowden *et al.*, 2008).

Viral DNA and infectious virions can be detected in some secretions for up to a month following resolution of acute disease (Bowden *et al.*, 2008). Virus can remain viable in scabs for months in the environment, and it is likely that the viral A type inclusion body protein in infected cells may be important in protecting the virion after the scab has disintegrated, although this has not yet been proven.

The amount of viral shedding correlates with the severity of clinical disease, with sheep and goats displaying mild clinical signs, with shedding being lower in sheep, and goats

having more severe clinical disease. The high concentrations of virus in the skin may also contribute to the spread of SPP and GTP via insect vectors (Bowden *et al.,* 2008). For myxoma virus sufficient precedents exist for this proposition (Fenner *et al.,* 1952) as well as for fowlpox virus (Damon, 2007) and LSDV (Chihota *et al.,* 2001, 2003), all being transmitted mechanically by biting insects.

In contrast to SPPV and GTPV, LSDV appears mainly to be spread mechanically by biting insects (Mellor *et al.,* 1987; Chihota *et al.,* 2001, 2003), with transmission by direct contact between animals being insignificant. Under experimental conditions, it was demonstrated that no disease transmission nor immunity was generated in naïve animals housed with infected animals in the absence of suitable insect vectors (Carn and Kitching, 1995b). Animals that did not show clinical signs did not shed virus in oral, nasal or conjunctival swabs (Carn and Kitching, 1995a). LSDV can be isolated in the semen of infected animals for extended periods of time (Irons *et al.,* 2005; Osuagwuh *et al.,* 2007) and transmission may occur by this route.

2.2.4 Factors influencing transmission of SPPV

SPPV and GTPV are highly infectious, and intimate contact does assist transmission. This can occur during night herding or stabling in endemic areas (Davies, 1981). The movement of infected animals is the main means of spread over a large area. In endemic areas, spread occurs mainly in summer.

Capripoxviruses are very stable in the environment and can remain viable for long periods, on or off animal hosts. They are susceptible to sunlight, but may persist for up to 6 months in a cool, dark environment, such as in shaded animal pens (Davies, 1981). The SGPV may remain viable for at least 3 months in the exudate of skin lesions that has accumulated in wool and hair (Davies, 1981). No carrier state has been demonstrated in recovered animals. Also, the SGPV persists up to 6 months in the environment, including on fomites, clothing and equipment.

Capripoxvirus is listed by Hare (1985) as a virus known to be excreted in semen and being able to be transmitted by semen. No information is available on the transmission of the virus in embryos. It should be assumed that virus would be found in semen and embryos during the viraemic period. The closely related LSDV of cattle was cited by Weiss (Coetzer, 2004) to be shed in the semen of clinically affected bulls for up to 22 days and for at least 12 days in subclinically affected bulls. The extremely resistant nature of the virus in the environment would make transmission very likely (NRC, 1993).

2.2.5 Molecular epidemiology

Few studies have been carried out on the molecular epidemiology of SPPV and GTPV. Restriction analysis of genomes of the viruses isolated from different countries did reveal that disease in sheep and goats is caused by very closely related isolates of capripoxvirus (Black, 1986). Such analyses not only demonstrate genetic relatedness (Black *et al.*, 1986; Bhat *et al.*, 1993) but are also used as molecular epidemiological tools for the characterization of the viral genomes of capripoxviruses (Bhat and Mishra, 1989).

The extent of nucleotide divergence values within the genomes suggests that the typical sheep and cattle isolates are more closely related to each other than to goat isolates (Gershon and Black, 1988). Divergence between capripoxvirus genomes, however, is smaller than that seen in orthopoxvirus genomes (Gershon *et al.*, 1989b). Furthermore, the capripoxviruses can be used successfully as viral vectors in the preparation of recombinant vaccines by incorporating foreign viral genes, such as those of the rinderpest and the bluetongue viruses (Romero *et al.*, 1993; Wade-Evans *et al.*, 1996).

2.2.6 Sheep pox in the Sudan

In the Sudan, SPP was first reported by Bennet *et al.* (1944). They confirmed that the disease was endemic, of seasonal occurrence in many parts of the country and that the virus was sheep host specific. However, Hajer *et al.* (1988) stated no host specificity of SPPV, it also could infect goats.

SPP in the Sudan is responsible for dramatic economic losses, particularly in animals prepared for export (Losos, 1986). It was assumed to be a predominantly seasonal disease associated with cold winters (Muzichin and Ali, 1979). However, during the last few years, outbreaks of the disease were observed during different seasons of the year (Sheikh-Ali *et al.*, 2004).

In 2008, Sudan reported 30 cases SPP and GTP disease in Bohor Reedbuck (*Redunca redunca*) to the OIE. Bohor Reedbuck is a medium-sized, sandy coloured antelope belonging to the family Bovidae. The diagnosis was based on clinical findings and on post-mortem examination (OIE, 2009).

2.3 Clinical signs of sheep pox

The incubation period is between 8 and 13 days following contact between infected and susceptible animals. It may be as short as 4 days, following experimental infection by intradermal inoculation or by mechanical transmission by insects. Some breeds of European sheep, such as Soay, may die of acute infection before the development of skin lesions. In other breeds there is an initial rise in the rectal temperature to above 40°C, followed in 2–5 days by the development of, at first, macules – (small circumscribed areas of hyperaemia, which are most obvious on unpigmented skin) and then of papules – (hard swellings between 0.5 and 1 cm in diameter) which may cover the entire body or be restricted to the groin, axilla and perineum. Papules may be covered by fluid-filled vesicles, but this is rare. A flat haemorrhagic form of capripox caused disease has been observed in some breeds of European goats, in which all the papules appear to coalesce over the body; this form is always fatal (OIE, 2010).

Within 24 hours of the appearance of generalised papules, affected animals develop rhinitis, conjunctivitis and enlargement of all superficial lymph nodes, in particular the prescapular lymph nodes. Papules on the eyelids cause blepharitis of varying severity. As the papules on the mucous membranes of the eyes and nose ulcerate, discharge becomes

mucopurulent and the mucosae of the mouth, rectum, and prepuce or vagina become necrotic. Breathing may become laboured and noisy due to pressure on the upper respiratory tract from the swollen retropharyngeal lymph nodes or, due to development of lung lesions.

Lambs and kids under 1 month of age may suffer a severe generalized form of SGP. The signs described above for older animals, in comparison, are exaggerated and mortality is increased. If the affected animal does not die in this acute phase of the disease, the papules start to become necrotic from ischaemic necrosis, following thrombi formation in the blood vessels at the base of the papule. In the following 5–10 days the papules form scabs, which persist for up to 6 weeks, leaving small scars. The skin lesions are susceptible to fly strike, and secondary pneumonia is common. Anorexia is not usual unless the mouth lesions physically interfere with feeding. Abortion is rare.

2.4 Pathology

On post-mortem examination of the acutely infected animal, the skin lesions are often less obvious than on the living animal. The mucous membranes appear necrotic and all the body lymph nodes are enlarged and oedematous. Papules, which may be ulcerated, can usually be found on the abomasal mucosa, and sometimes on the wall of the rumen and large intestine, on the tongue, the hard and soft palate, the trachea and the oesophagus. Pale areas of approximately 2 cm in diameter may occasionally be seen on the surface of the kidney and liver, and have been reported to also be present in the testicles. Numerous hard lesions of up to 5 cm in diameter are commonly observed throughout the lungs, but particularly in the diaphragmatic lobes.

2.5 Diagnosis

2.5.1 Specimens and samples collected for laboratory diagnosis

Material for virus isolation and antigen detection should be collected by biopsy or at post-mortem from skin papules, lung lesions or lymph nodes. Samples for virus isolation and for the antigen-detection enzyme-linked immunosorbent assay (ELISA) should be collected within the first week of the occurrence of clinical signs, before the development of neutralising antibodies. Samples for genome detection by polymerase chain reaction (PCR) may be collected when neutralising antibodies are present. Buffy coats from blood collected into EDTA (ethylene diamine tetra-acetic acid) during the viraemic stage of capripox (before generalisation of lesions or within 4 days of generalisation) can also be used for virus isolation. Samples for histology should include tissue from the normal tissue around the lesion and, following collection, should be placed immediately into ten times the sample volume of 10% formalin. Tissues in formalin have no special transportation requirements. Blood samples, for virus isolation from the buffy coat, should be collected in tubes containing anticoagulant, be placed immediately on ice and processed as soon as possible. In practice, blood samples may be kept at 4°C for up to 2 days prior to processing, but should neither be frozen or kept at ambient temperatures.

2.5.2 Enzyme-Linked Immunosorbent Assay (ELISA)

Following the cloning of the highly antigenic capripoxvirus structural protein P32, it is possible to use expressed recombinant antigen for the production of diagnostic reagents, including the raising of P32 monospecific polyclonal antiserum and the production of monoclonal antibodies (MAbs) (Carn, 1995a). Using hyperimmune rabbit antiserum raised by inoculation of rabbits with purified capripoxvirus, capripox antigen from biopsy suspensions or tissue culture supernatant can be trapped on an ELISA plate. The presence of the trapped antigen can then be detected using guinea-pig serum raised against the group-specific structural protein P32, commercial horseradish-peroxidase-conjugated rabbit anti-guinea-pig immunoglobulin and a chromogen/substrate solution. ELISAs for the detection of antibodies against capripox virus have been developed using the expressed structural P32 protein (Heine *et al.*, 1999). More recently, ELISA-based on recombinant capripox antigens (Bowden *et al.*, 2009a) and on inactivated, purified whole sheep pox virus (Babiuk *et al.*, 2009) have been described.

2.5.3 Virus isolation and identification

Culture

Capripoxviruses will grow in tissue cultures of bovine, ovine or caprine origin, although primary or secondary cultures of lamb testis (LT) or lamb kidney (LK) cells are considered to be most susceptible, particularly those derived from a wool sheep breed. The following is an example of an isolation technique: Either 1 ml of buffy coat cell suspension or 1 ml of clarified biopsy preparation supernatant is inoculated on to a 25 cm^2 tissue culture flask of 90% confluent LT or LK cells, and the supernatant is allowed to absorb for 1 hour at 37°C. The culture is then washed with warm Phosphate Buffered Saline (PBS) and covered with 10 ml of a suitable medium, such as General Minimum Essential Medium (GMEM), containing antibiotics and 2% fetal calf serum. If available, tissue culture tubes containing LT or LK cells and a flying cover-slip, or tissue culture microscope slides, are also infected. A constant-virus/varying-serum method has been adopted using serum dilutions in the range 1/4 to 1/500 and fetal calf muscle cells. Because these cells have a lower sensitivity to capripoxvirus than LT cells, the problem of virus 'breakthrough' is overcome (OIE, 2010).

The flasks should be examined daily for 7–14 days for evidence of cytopathic effect (CPE). Contaminated flasks should be discarded. Infected cells develop a characteristic CPE consisting of a retraction of the cell membrane from surrounding cells, and eventually rounding of cells and margination of the nuclear chromatin. At first only small areas of CPE can be seen, sometimes as soon as 4 days after infection; over the following 4–6 days these expand to involve the whole cell monolayer. If no CPE is apparent by day 7, the culture should be freeze–thawed three times, and clarified supernatant inoculated onto fresh LT or LK culture.

Electron microscopy

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

The virions of capripoxvirus are indistinguishable from those of orthopoxvirus, but, apart from vaccinia virus, no orthopoxvirus causes lesions in sheep and goats. However, capripoxvirus is distinguishable from the virions of parapoxvirus, which cause contagious pustular dermatitis, as they are smaller, oval in shape, and each being covered in a single continuous tubular element, which appears as striations over the virion.

Animal inoculation

Clarified biopsy preparation supernatant may also be used for intradermal inoculation into susceptible lambs. These lambs should be examined daily for evidence of typical skin lesions of capripox.

2.5.4 Detection of viral DNA

Conventional or real-time PCR methods can be used to detect capripoxvirus genomes in biopsy or tissue culture samples. Primers for the viral attachment protein gene and the viral fusion protein gene are specific for all the strains within the genus *capripoxvirus* (Heine *et al.,* 1999; Ireland and Binepal, 1998). It is not possible to distinguish between strains of capripoxvirus from cattle, sheep or goats using serological techniques. Strains of virus can be identified using sequence and phylogenetic analysis (Le Goff *et al.,* 2009). Strains can also be characterised by comparing the genome fragments generated by *Hin*dIII digestion of their purified DNA (Black *et al.,* 1986; Kitching *et al.,* 1986b). Using this technique and by sequencing of the genome, differences between isolates from the different species have been identified (Hosamani *et al.,* 2004), but these are not consistent and there is evidence for the movement of strains between species and recombination between strains in the field (Gershon *et al.,* 1989a).

2.5.5 Histopathology

Following preparation, staining with hematoxyline and eosin (H&E) and mounting of the formalin-fixed biopsy material, a number of sections should be examined by light microscopy. On histological examination, the most striking aspects of acute-stage skin lesions are a massive cellular infiltrate, vasculitis and oedema. Early lesions are characterised by marked perivascular cuffing. Initially infiltration is by macrophages, neutrophils and occasionally eosinophils, and, as the lesion progresses, by more macrophages, lymphocytes and plasma cells.

A characteristic feature of all capripoxvirus infections is the presence of variable numbers of 'sheep pox cells' in the dermis. These sheep pox cells can also occur in other organs where microscopic lesions of SGP are present. These cells are large, stellate cells with eosinophilic, poorly defined intracytoplasmic inclusions and vacuolated nuclei. Vasculitis is accompanied by thrombosis and infarction, causing oedema and necrosis. Epidermal changes consist of acanthosis, parakeratosis and hyperkeratosis. Changes in other organs are similar, with a predominant cellular infiltration and vasculitis. Lesions in the upper respiratory tract are characterised by ulceration.

2.6 Prevention and control

2.6.1 Immunity

Susceptible sheep and goats of all ages can be infected with SPPV and GTPV and develop serious clinical disease. The introduction of SGP into a totally susceptible population in a country previously free from the disease results in high mortalities and rapid spread of the disease (OIE, 2004).

2.6.2 Innate immunity

Different breeds of sheep and goats show varying degrees of natural resistance to infection with SPPV and GTPV. Merino and European sheep breeds are very susceptible to SPP. Maternal immunity provides protection from SGP for up to 3 months (Kitching, 1986).

2.6.3 Adaptive immunity and vaccination

Animals that have recovered from capripoxvirus infection do not remain carriers of the virus and have lifelong immunity.

Cell-culture attenuated (live) and inactivated vaccines have been used to prevent SGP. Inactivated vaccines do not provide long-term protection, due to at least in part their failure to induce a cell-mediated immune response, which is the predominant protective response to poxvirus infection (OIE, 2004).

Attenuated vaccines have been shown to induce relatively extended protection, lasting from 12 months to lifetime (OIE, 2004). Instances of live vaccine failure when GTPV strains have been used in sheep have prompted a recommendation for the use of homologous vaccines (Agrawal and Soman, 1997). However, a vaccine made from a SGPV which did affect both sheep and goats in Kenya, was shown to effectively immunize sheep, goats and cattle against infection with a capripoxvirus. This vaccine was found to be stable and safe, and did not transmit horizontally or vertically (Kitching *et al.*, 1986; Davies, 1991b). Immunized animals may not seroconvert to the vaccine.

Recipient species may react differently to attenuated vaccines. Vaccination of susceptible Saanen goats from a disease-free area with a live GTPV vaccine resulted in clinical GTP with 100% morbidity and 41% mortality (Abo-Shehada, 1990).

Ovination

Ovination involves the inoculation of infected scab material as such, or suspended in glycerine saline, with the false notion that it does prevent the infection. This method had been practiced since centuries (Ten, 1955; Sapre and Kalia, 1980) in many countries including India (Bhanuprakash *et al.,* 2004) by local farmers. It is hazardous as the inoculum does consist of adventious viruses and bacteria. Ovination also causes severe local reactions including nodule formation at the inoculation site, pyrexia and generalized lesions. The virulence, pathogenicity and number of viral particles inoculated are also not known. Further, it could possibly lead to increase in virus pathogenicity as it simulates passaging of virus in natural homologous hosts which, in turn, leads to rapid spread.

There is a need to make the farmers aware of the drawbacks of ovination through extension work and imposing strict restriction on ovination of susceptible livestock (Bhanuprakash, 2001). Earlier workers have employed the intradermal inoculation of dessicated infected scab tissue (Munro, 1947) and ovination as a means for vaccination and have claimed immunity for 14 months with a lyophilized Persian strain (Sabban, 1955). This vaccine has been used in Egypt and its neighbouring countries for a long time. However, this practice is hazardous and hence should be totally banned, wherever it is being practiced.

Sero-clavelization

This is the method of administration of serum from convalescent sheep and/or anti-SPPV serum either at different sites or as a mixture of both at only one site. The prophylactic utility of convalescent sheep serum has been early reported (Red'Ko, 1945). Its protective and curative effects in developing a serum-simultaneous method and a satisfactory response have also been early explored (Borrel, 1903b). However, only mild protection but also failure with immune serum, sensitized lymphocytes and antilymphocytic serum have been reported recently (Prasad *et al.,* 1986).

2.6.4 Control of sheep pox

Affected sheep should be isolated and placed in a hygienically maintained shed. Paliative treatment is indicated along with the feeding of a balanced diet. Debilitated sheep need to be administered with 10% glucose saline intravenously (Rao and Bandyopadhyay, 2000). Lesions should be washed with 1:100–1:10,000 $KMNO_4$ lotion and application of 1:10 boric acid (Rao *et al.,* 1994), a mild antiseptic (Singari *et al.,* 1990) or antibiotic ointment (Nandi *et al.,* 1999), topically with parenteral antibiotic injection to prevent secondary bacterial complications.

Very recently, nucleoside (8-methyladenosine and 2-amino-7-[(1,3-dihydroxy-2-propoxy) methyl]purine (S2242) and nucleotide (S-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine [(S)-HPMPC, cidofovir], (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)- 2,6-diaminopurine [(S)-HPMPDAP] and (S)-6-(3-hydroxy-2-phosphonylmethoxypropyl) oxy-2,4-diaminopyrimidine [(S)-HPMPO-DAPy] analogues have been identified as promising anti-poxvirus agents and there is a ray of hope for use of these agents for therapeutics in the near future (De Clercq and Neyts, 2004).

The most likely manner for SPP to enter a new area is by introduction of infected animals. Restrictions on the movement of animals and animal products are essential to prevent introduction of SPP. Animal products must be subjected to suitable decontamination procedures before entry into non-endemic areas. If a new case is confirmed in a new area before extensive spread occurs, the area should be quarantined, infected and exposed animals should be slaughtered, and the premises cleaned and disinfected.

In enzootic areas, both live attenuated and inactivated vaccines may be useful in the prevention and control of SGP. However, the inactivated vaccines that are available for immunization against GPV and SPV infections only give short-term immunity (Prasad and Datt, 1973; Yadav *et al.,* 1986). The live attenuated vaccines are highly immunogenic

but their usefulness is limited because they lead to death of some of the vaccinated animals because of generalization of the disease.

A carrier state has not been shown for SPPV. However, the virus may persist for many months on contaminated premises. The imposition of quarantine restrictions on areas and premises containing infected or exposed animals is required to prevent disease spread. Depopulation of infected and exposed flocks should be used if limited spread has occurred.

3 MATERIALS AND METHODS

3.1 Study area

The study was conducted in North and South Kordofan States in the western part of the Sudan. Kordofan covers an area of 376,145 km² (146,932 miles²), with an estimated population of 3.6 million people in the 2000 Census. It is largely an undulating plain, with the Nuba Mountains in the southeast quarter. During the rainy season, from June to September, the area is fertile, but in the dry season it is virtually desert. The region's chief town is Al-Ubayyid. Traditionally the area is known for production of gum Arabic. Other crops cultivated include groundnuts, cotton, and millet (UNMIS, 2007).

North Kordofan State (NKS) is located between latitudes 12°15′ - 16°32′N and longitudes 27° and 32° E in an area of 19480 km^2. Al-Ubayyid is the capital of the State. Climate is generally arid and desert. The state is divided into 9 administrative localities each of which encompasses several Administrative Units (AU); amounting to approximately 33 AUs. According to the 2004 population projection, the population of the state is about 1,578,000 (80% are living in rural areas). Umruwaba, Sheikan and Bara localities are characterized by high density populations in contrast to Sodari and Jabratelsheikh localities. More than 79% of the state population (i.e. 1.7 million households) depend on agricultural and pastoral activities for their living. Livestock is estimated at 407,386 heads of animals and consist of cattle, sheep, goats and camels.

Traditional rain-fed farming and grazing systems predominate and are characterized by subsistent small cropping holdings (5-30 *feddans)* and animal herding activities with limited inputs and technologies. Traditional crops and animal production constitute the most important economical activity that contributes to the livelihood, food security and development of both rural and urban areas.

Based on that the state contributes by about 30% to the national non-petroleum exports, the contribution of the state to the gross domestic product by cash crops like water melon seeds is 70%, by Roselle (*karkadeh*) 65%, by camels 30% and by sheep17%.

South Kordofan state (SKS) is located between latitude 11°8′N and longitude 29°53′E. The state has an area of 158,355km² and an estimated population of 1,100,000 people. Kaduqli is the capital of the State. It is centered around the Nuba Hills. The state is divided into the 7 administrative localities Abbassiya, Rashad, Abu Gubeiha, Kalogi, Liri, Talodi and Kadugli, each of which encompasses several Administrative Units.

The vegetation in this area includes semi-desert in the northern parts, followed by poor, medium and wet savannah belts as rainfall increases to the south. There is also some mountainous vegetation on the many *jebels* in the area, where elevation reaches 1,000 metres. In these areas, there is extensive erosion and loss of topsoil.

Kordofan region is characterized by a large livestock population, which represents about 30 percent of the estimated national livestock count. Off-take is below potential, however, for the economy is of subsistence nature, with pastoralists maintaining their perception of animals being both an asset and a social wealth. The prevailing pastoralist strategy is to increase the size of the herds to offset potential hazards, such as outbreaks of disease and occurrence of droughts. The livestock population, as provided by the State Ministries of Animal Resources and Fisheries in 2002, is 2,495,073 cattle, 1,939,881 sheep, 1,804,598 goats and 169,774 camels.

The Hamari desert sheep is one of the main breeds reared in Kordofan region, and are regarded as highest quality for live sheep and the second-best breed for meat. The bulk of Sudan's live sheep exports and meat for local consumption are from this region. In addition, most of the large sheep (average 35–45 kg live weight) and high-quality lambs purchased during the annual sacrifice of Hajj and Ramadan religious festivals originate from Kordofan region.

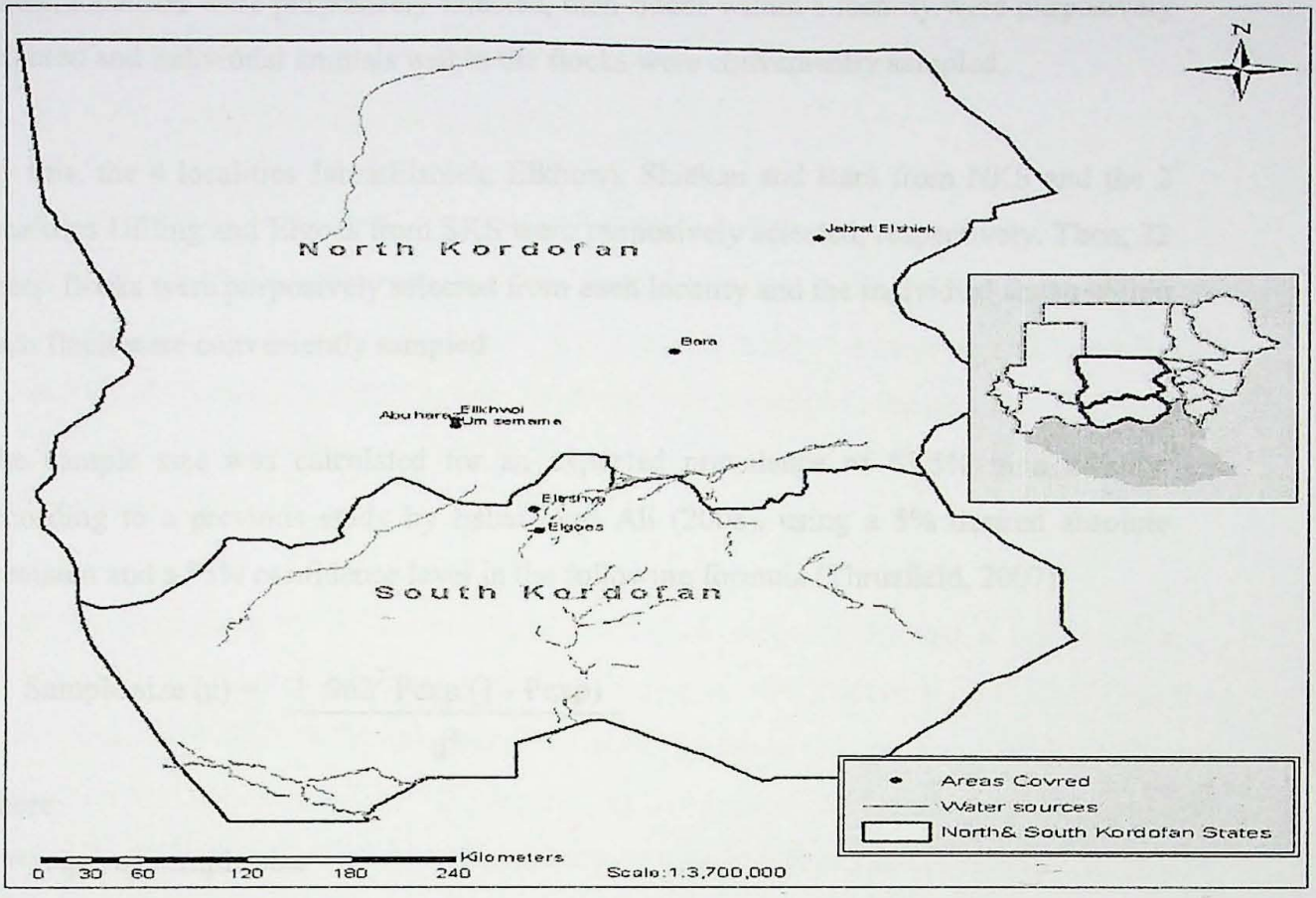


Figure 2: Map of North and South Kordofan States where the study was conducted

3.2 Study design

3.2.1 Study population

The study focused primarily on sheep herds as reference target population. The total population of sheep in Kordofan region is 2,759,124 heads, of which 30% are Hamari sheep and 70% other breeds of local Sudanese sheep (FAO, 1980).

3.2.2 Sample size determination

A cross-sectional study design was carried out from March 2011 to September 2011 to determine the sero-prevalence of SPP in Sudanese sheep flocks.

A multistage sampling technique was carried out. Starting from the region and the 2 states, localities were purposively selected, then flocks within a locality were purposively selected and individual animals within the flocks were conveniently sampled.

By this, the 4 localities JabratElshiek, Elkhowi, Shiekan and Bara from NKS and the 2 localities Dilling and Elgoes from SKS were purposively selected, respectively. Then, 22 sheep flocks were purposively selected from each locality and the individual sheep within each flock were conveniently sampled.

The sample size was calculated for an expected prevalence of 63.5% in a locality, according to a previous study by Eshafi and Ali (2008), using a 5% desired absolute precision and a 95% confidence level in the following formula (Thrusfield, 2007):

$$\text{Sample size (n)} = \frac{1.962^2 \, P_{exp} (1 - P_{exp})}{d^2}$$

where:

n = required sample size

p_{exp} = expected prevalence

d = desired absolute precision

A sample size of n = 357 was calculated. However, to increase the accuracy, the calculated value was multiplied by 3 for 2 States.

2142 serum samples were to be collected, but unfortunately sampling did not accomplish the target sample size, because the field trip coincided with insecurity and civil unrest in SKS. Accordingly, a total of 1500 animals were sampled in the 6 localities in NKS and SKS.

Risk factors considered in this study were individual animal risk factors like sex of animal, age group, weight and breed. Other potential risk factors considered were

location, herd size, movement pattern, mixed herd, family animal care, agri-ecologic zone, and traditional treatment.

3.2.3 Serum sample collection and testing

Per animal, blood samples were collected using plain vacutainer (10 ml.) and sterile needles (19 G; for each animal). The collected blood was allowed to clot for up to 24 hours in the shade. Two aliquots of sera were transferred into cryovials labeled with identified sheep flock numbers and the designated geographic locality in the study area. The cryovials were kept in a -20°C freezer and transferred to the laboratory in cooled containers with ice bags.

Serological diagnosis was carried out on the collected specimens at the Veterinary Research Institute (VRI), Department of Virology, and Department of Viral Vaccine Production, Sudan. SPP antibodies were tested by using a recombinant Enzyme Linked Immunosorbent Assay (ELISA) provided by the Australian Animal Health Laboratory (AAHL) and the Canadian Food Inspection Agency as collaboration project which aimed at evaluating the test performance characteristics of this indirect ELISA in the Sudan by comparing it to the Virus Neutralization Test (VNT). The aim was to validate the ELISA test results as well as to evaluate the test performance in the three different laboratories.

Checker board titration was carried out for antigen versus strong control sera of GTPV (C++), weak control sera of SPPV (C+) and negative control sera of naïve sheep (C-) to determine the optimal dilution for control sera for the ELISA test.

3.2.4. Capripoxvirus antibody detection ELISA

ELISA Test procedure:

Ninety-six-well Maxisorp ELISA plates (Nunc) were coated with 30 ng of each affinity purified recombinant protein (095 and 103) per well in 100µl of 0.05M carbonate–bicarbonate buffer (pH 9.6), and incubated overnight at 4°C. Plates were washed four times with Phosphate Buffered Saline (PBS) containing 0.1% Tween 20 (PBST) prior to the addition of 200µl/well of 10% skimmed milk blocking buffer. The plates were incubated for 1 h at 37°C on a plate shaker and again washed four times with PBST. Control sera of GTPV as a strong positive and SPPV as a weak positive and naïve sheep as a negative control diluted 1:100 in diluent buffer of 2% skimmed milk were added to the plates. Test sera, diluted 1:100 in diluent buffer of 2% skimmed milk were added in duplicates to the plates (100µl/well). Then plates were incubated for 1 h at 37°C with shaking, and then washed four times with PBST. Recombinant protein G conjugated to horseradish peroxidase (protein G-HRP), diluted 1:32,000 in diluent buffer was subsequently added (100µl/well). After incubating at 37°C for 1 h the plates were washed four times with PBST and 100µl of the substrate 3, 3', 5, 5- tetramethylbenzidine dihydrochloride (TMB) ready to use containing 3 µl of hydrogen peroxide was added to each well. An equal volume of 1M H_2SO_4 was subsequently used to stop the enzyme reaction after a 10 min incubation at room temperature, and the optical density (OD) of the reaction product was read at 450 nm on an ELISA plate reader (Immunoskan, BDSL).

ELISA data transfer

The optical density values of the ELISA reader were organized and ordered in a Microsoft Excel spreadsheet for analysis and interpretation after determining the threshold for positive and negative test samples using test properties. Therefore, knowledge of ELISA sensitivity and specificity were important in Receiver Operating Characteristic (ROC) curve analysis which provides an accurate determination of the cutoff value from Area under Curve (AUC) of the sensitivity and specificity curves.

Laboratory analysis and interpretation

In order to enable comparison of data between different ELISA plates, OD values were routinely normalized against a positive control serum and expressed as percent positivity (PP) by calculating mean corrected test OD value/corrected positive control OD value×100 (Wright *et al.*, 1993).

Therefore, the Excel worksheet was designed as template to calculate the result OD given by ELISA reader, for the net mean OD of strong positive(C++) by summation of OD values in A1, A2, B1, B2 based on ELISA plate layout and divided by 4. Then the mean corrected test OD gained by summation of OD of the duplicates in known two wells in the plate layout and divided by 2. For example, if the first serum sample in plate No (1) had a mean OD of 0.089, when divided by 0.7915 of net mean OD of the strong positive control, and multiplied by 100, the percent positivity was 11.25%.

Cut-off values were determined using the mean optical density (OD) values from negative control sera plus two standard deviations (SD). Capripoxvirus antibody titres were calculated using the highest sera dilutions that gave OD values greater than the cut-off. For example, serum sample No 23 was positive, because its calculated mean OD of its duplicates (0.206) was higher than the cutoff value of the negative control sera (0.193) which was derived by summation of results of OD in D1, D2 in the plate layout and dividing by 2 and then adding to standard deviation of D1, D2 multiplied by 2.

Sero-prevalence estimation

SPP sero-prevalence was estimated by calculating the proportion of test positive samples.

3.2.5. Virus Neutralisation Test

A test serum can either be titrated against a constant titre of capripoxvirus (100 TCID50 [50% tissue culture infective dose]) or a standard virus strain can be titrated against a constant dilution of test serum in order to calculate a neutralisation index. Because of the variable sensitivity of tissue culture to capripoxvirus, and the consequent difficulty of

ensuring the use of 100 TCID50, the neutralisation index is the preferred method, although it does require a larger volume of test sera. The test is described using 96-well flat-bottomed tissue culture grade microtitre plates, but it can be performed equally well in tissue culture tubes with the appropriate changes to the volumes used, although it is more difficult to read an end-point in the tubes. The use of Vero cells in the virus neutralisation test has been reported to give more consistent results (Kitching and Taylor, 1985).

Test procedure:

Test sera including a negative and a positive control were diluted 1/4 in GMEM and inactivated at 56°C for 30 minutes. Next, 50 µl of the first inactivated serum was added to columns 1 and 2, rows A to H of the microtitre plate. The second serum was placed in columns 3 and 4, the third in columns 5 and 6, the positive control serum was placed in columns 7 and 8, the negative control serum was placed in columns 9 and 10, and 50 µl of GMEM without serum was placed in columns 11 and 12 and in all wells of row H. A reference strain of capripoxvirus vaccine strain used in the Sudan, with a titre of over log10 6 TCID50 per ml was diluted in GMEM in bijoux bottles to give a log dilution series of log10, 5.0, 4.0, 3.5, 3.0, 2.5, 2.0, and 1.5 TCID50 per ml (equivalent to log10 3.7, 2.7, 2.2, 1.7, 1.2, 0.7, and 0.2 TCID50 per 50 µl). Starting with row G and the most diluted virus preparation, 50 µl of virus was added to each well in that row. This was repeated with each virus dilution, with the highest titre virus dilution being placed in row A. The plates were covered and incubated for 1 hour at 37°C. LT cells were prepared from pregrown monolayers as a suspension in GMEM containing antibiotics and 2% fetal calf serum. Following incubation of the microtitre plates, 100 µl of cell suspension was added to all the wells, except wells H11 and H12, which served as control wells for the medium. The remaining wells of row H were cell and serum toxicity controls. The microtitre plates were covered and incubated at 37°C for 9 days. Using an inverted microscope, the monolayers were examined daily starting at day 4 for evidence of CPE. There were no CPE in the cells of row H. Using the 0240 KSGP vaccine strain of capripoxvirus, the final reading was taken on day 9, and the titre of virus in each

duplicate titration was calculated according to the Karber method (Villegas and Purchase, 1980) as in the following formula:

$$M = xK + 1/2D - D^*(\text{sum } r1)/n$$

where:

M = Titre
xK = Log_{10} of last dilution
D = Log_{10} of interval
Sum r1 = Negative eggs or cell culture
n = Number of tests

If plates are left longer, there is invariably a 'breakthrough' of virus in which virus that was at first neutralised appears to disassociate from the antibody.

Interpretation

The neutralisation index is the log titre difference between the titre of the virus in the negative serum and in the test serum. An index of ≥ 1.5 is positive. The test can be made more sensitive if serum from the same animal is examined before and after infection. Because immunity to capripox is predominantly cell mediated, a negative result, particularly following vaccination in which the response is necessarily mild, does not imply that the animal from which the serum was taken is not protected.

3.2.6 Questionnaire survey

Survey data from NKS and SKS were collected by questionnaires. Questionnaires were developed to evaluate elements of the economic effect as well as potential risk factors associated with SPP epizootics on the livelihood of the respondents.

Twenty five respondents were randomly interviewed to determine potential risk factors associated with SPP, starting from individual animal risk factors like sex of animal, age group, weight and breed. Other potential risk factors considered were location, herd size, movement pattern, mixed herd, family animal care, agri-ecologic zone, and traditional treatment. Further questions addressed the production and management system of sheep, common watering points and the availability of veterinary services and marketing.

Questions were asked on the economic significance of SPP in terms of direct losses like mortality, morbidity as well as indirect losses, like lower productivity which reduces the market value of animals and other expenditures like vaccination and medicines used. Also, seasonality of the disease and how frequent it does occur in a year, as well as how frequent sheep flocks herders vaccinate their animals against SPP was inquired.

Estimation of cost of SPP was calculated based on a simple economic model. The model calculated the monetary value of SPP by a combination of losses, estimated as the lost value of an animal through mortality, direct losses and losses in terms of the effect of the disease on final outputs like lowered productivity, reduced weight gain and lowered milk yield and costs of vaccination and medicines.

3.2.7 Secondary data collection

Retrospective information and secondary data were gathered from the General Planning and Animal Resources Economics Administration, Federal Ministry of Animal Resources and Fisheries (MARF, Khartoum) and from NKS and SKS veterinary authorities to estimate economic losses due to SPP outbreaks in the previous five years.
Also, data from the Sudan Meteorological Authority were reviewed to determine environmental risk factors like temperature, relative humidity, moisture index and rainfall per year, and their association with SPP outbreaks.

3.2.8 Statistical data analysis

Collected data were organized and managed in a Microsoft Excel spreadsheet for analysis by the Statistical Package for Social Sciences (SPSS). Risk factors associated with SPP were analyzed using descriptive statistics and univariate analysis (chi-square test), then by multivariate analysis using Logistic Regression. Test agreement of ELISA versus VNT was calculated using the Kappa test. Sensitivity, Specificity, Positive predictive value and negative predictive value were calculated.

4 RESULTS

4.1 Demographics of questionnaire respondents

All (100%) respondents were male, and the minimum and maximum ages were 27 and 65 years, respectively. In regard to the education level, 64% were illiterate. The largest household (4%) did consist of ≥ 11 people (Table 1). For land ownership, 6.3% of the respondents possessed private lands, while 93.7% of the respondents used communal land. Herds overwhelmingly did consist of sheep (60%).

Table 1: Demographics of households interviewed

Variable	Category	Frequency	%
Gender of the respondents	Male	25	100%
	Female	0	0%
Education level	Illiterate	16	64%
	Primary	4	16%
	Secondary	2	8%
	University	3	12%
Martial status	Unmarried	2	8%
	Married	23	92%
No of people/Household	No children	2	8%
	2-6	15	60%
	7-10	7	28%
	≥11	1	4%
Flocks owned	No animal owned	3	12%
	Sheep	15	60%
	Sheep and goat	2	8%
	Camel	1	4%
	Equine	1	4%
	Different spp.	3	12%

4.2 Description of samples

4.2.1 Description of individual animals

Male and female sheep accounted for 21.7% and 78.3%, respectively, 96% were older than 1 year of age, whereas 4% were younger than 1 year of age (Table 2). In regard to breed, the majority of sheep sampled were Hamari (45.5%), while Zagawi accounted for 3.8% of the sample. Minimum and maximum weights were 8 kg and 60 kg, respectively.

Table 2: Animal's characteristics

Category		Frequencies	Percent(%)
Sex	Female	1175	78.3
	Male	325	21.7
Age	< 1 year	60	4.0
	1-2 years	880	58.7
	3-4 years	406	27.1
	> 4 years	154	10.3
Weight	1-10 kg	17	1.1
	11-20 kg	449	29.9
	21-30 kg	608	40.5
	> 30 kg	426	28.4
Breed	Khabashi	260	17.3
	Hamari	683	45.5
	Zagawi	57	3.8
	Gharaj	500	33.3

4.3 Sero-prevalence of SPP in NKS and SKS

The overall sero-prevalence in NKS and SKS was 20.1 % (95% CI=18.1%-22.1 %). The highest prevalence was recorded in Elgoes locality with 44.5% (CI=42.0%-47.0%), followed by Dilling locality with 25 % (CI=22.8%-27.2%) in SKS. In NKS the prevalence was 20% (CI=18.0%-22.0%) in Bara locality, 14.3% (CI=12.5%-16.0) in

Elkhowi locality, 13.6% (CI=11.9%-15.3%) in JabratElshiek locality and 11.4% (CI=9.8%-13.0%) in Shiekan locality (Table 3). Based on the CIs there was no significant difference in prevalence for JabratElshiek, Elkhowi and Shiekan, while Bara did have a higher prevalence. Prevalence in both locations in SKS was higher than in any location in NKS, with Elgoes showing a higher prevalence than Dilling.

Table 3: Sero-prevalences in individual sample localities in NKS and SKS using iELISA

State	Locality	Total of samples	Positive	Prevalence (%)	95% CI
N.Kordofan		1000	137	13.7	12.0%- 15.4%
	JABRAT ELSHIEK[a]	250	34	13.6	11.9%-15.3%
	ELKHOWI[a]	300	43	14.3	12.5%-16.0
	SHIEKAN[a]	350	40	11.4	9.8%-13.0%
	BARA[b]	100	20	20	18.0%-22.0%
S.Kordofan		500	164	32.8	30.4%-35.2%
	DILLING[c]	300	75	25	22.8%-27.2%
	ELGOES[d]	200	89	44.5	42.0%-47.0%
	OVERALL PREVALENCE	1500	301	20.1	18.1%-22.1%

Different superscripts signify statistical differences in sero-prevalences at $p<0.05$

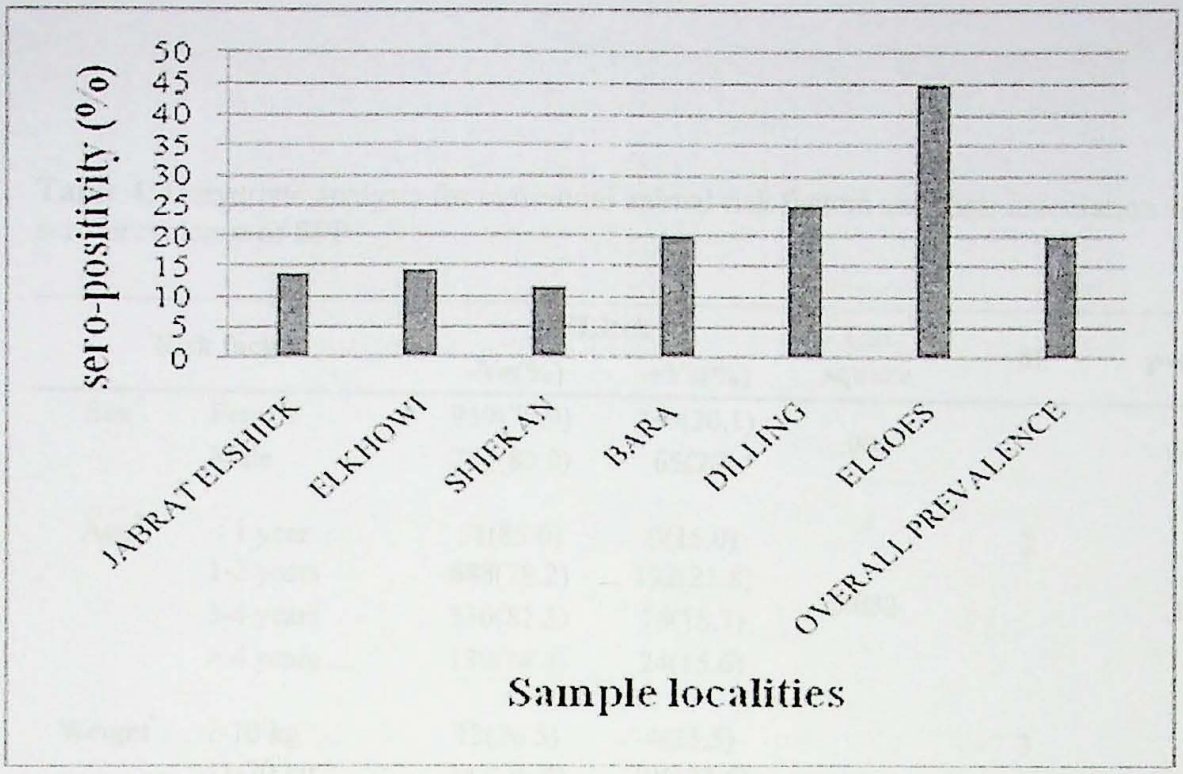


Figure 3: Percentages positive samples in sample localities in NKS and SKS from March to May 2011

4.4 Risk factors associated with SPP

4.4.1 Individual animal risk factors

Individual animal risk factors associated with the sero-prevalence of SPP are listed in Table 4. Sex, age and weight were not associated with SPP sero-prevalence, whereas breed was. The Gharaj breed with a high sero-prevalence of 54.5% did particularly contribute to this association. Differences between categories of risk factors in Table 4 are indicated by superscripts.

Table 4: Univariate analysis for individual animal risk factors and their association with sero-prevalence of SPP

Risk factor		ELISA		Chi square	df	p-value
		-Ve(%)	+Ve(%)			
Sex*	Female	939(79.9)	236(20.1)	.001	1	.973
	Male	260(80.0)	65(20)			
Age*	< 1 year	51(85.0)	9(15.0)	5.032	3	.169
	1-2 years	688(78.2)	192(21.8)			
	3-4 years	330(81.3)	76(18.7)			
	> 4 years	130(84.4)	24(15.6)			
Weight*	1-10 kg	13(76.5)	4(23.5)	5.457	3	.141
	11-20 kg	343(76.4)	106(23.6)			
	21-30 kg	493(81.1)	115(18.9)			
	> 30 kg	350(82.2)	76(17.8)			
Breed**	Khabashi	224(86.2)	36(13.8)	79.047	3	.001
	Hamari	595(87.1)	88(12.9)			
	Zagawi	44(77.2)	13(22.8)			
	Gharaj	336(67.2)	164(32.8)			

** Significant by univariate analysis using Chi square
*not significant by univariate analysis using Chi square

4.4.2 Other potential risk factors

Of the 14 investigated putative husbandry and ecological risk factors, 12 (state, locality, mixed herd, herd size, movement pattern, agri-ecological zone, family animal care, insect bite, temperature, relative humidity, rainfall, traditional treatment) were found to be significantly ($p<0.05$) associated with the sero-prevalence of SPP. Only the factors seasonality and landownership were found to be not significantly associated with SPP ($p> 0.05$) (Table 5).

Table 5: Husbandry and ecological risk factors and their association with sero-prevalence of SPP

Risk factor		ELISA No.-ve (%)	ELISA No. +ve (%)	Chi square	df	p-value
State*	N.Kordofan	863(86.3)	137(13.7)	75.810	1	.001
	S.Kordofan	336(67.2)	164(32.8)			
Locality*	JabratElshiek	216(86.4)	34(13.6)		5	
	Elkhowi	257(85.7)	43(14.3)			
	Shiekan	310(88.6)	40(11.4)	107.937		.001
	Bara	80(80.0)	20(20.0)			
	Dilling	225(75.0)	75(25.0)			
	Elgoes	111(55.5)	89(44.5)			
Mixed herd*	Pure sheep	977(81.5)	222(18.5)	8.964	1	.003
	Mixed	222(73.8)	79(26.2)			
Herd size*	51-100	499(76.8)	151(23.2)	14.152	2	.001
	101-150	314(78.5)	86(21.5)			
	>150	386(85.8)	64(14.2)			
Movement pattern*	Nomadic	336(67.2)	164(32.8)	75.813	1	.001
	Sedentary	863(86.3)	137(13.7)			
SPP seasonality	Dry hot season	17(81.0)	4(19.0)		3	
	Dry cold season	64(81.0)	15(19.0)	.113		.990
	Rainy season	18(78.3)	5(21.7)			
	Different seasons	1100(79.9)	277(20.1)			
Agri-ecologic zone*	Arid	216(86.4)	34(13.6)	75.815	2	.001
	Semi-arid	647(86.3)	103(13.7)			
	Sub-arid	336(67.2)	164(32.8)			
Landownership	Tenure	77(81.1)	18(18.9)	.0790	1	.778
	Communal	1122(79.9)	283(20.1)			
Family animal care*	Man	387(82.9)	80(17.1)		3	
	Children	335(69.4)	148(30.6)	52.514		.001
	Man+woman	77(89.5)	9(10.5)			
	Man+children	400(86.2)	64(13.8)			
Insect bite*	Yes	277(88.8)	35(11.2)	30.494	2	.001
	No	378(82.5)	80(17.5)			
	I don't know	544(74.5)	186(25.5)			
Temperature*	High	216(86.4)	34(13.6)	7.821	1	.005
	Moderate	983(78.6)	267(21.4)			
Relative humidity*	Moderate	983(78.6)	267(21.4)	7.821	1	.005
	Low	216(86.4)	34(13.6)			
Rainfall*	Heavy	646(76.0)	204(24.0)		2	
	Moderate	372(83.6)	73(16.4)	20.850		.001
	Low	181(88.3)	24(11.7)			
Traditional treatment*	Yes	154(95.1)	8(4.9)	25.914	1	.001
	No	1045(78.1)	293(21.9)			

* Significant by univariate analysis using Chi square

4.4.3 Multivariate analysis of risk factors and their association with sero-prevalence of SPP

Multivariate logistic regression analysis (Table 6) of the risk factors to explain SPP sero-positivity identified the combination of locality, herd size, insect bite, rainfall/year and traditional treatment to be respective statistically significant risk factors ($p<0.05$). The regression coefficients (Exp (B) represent Odds Ratios (OR) which indicate by how much the likelihood of seropositivity will increase (OR>1) or decrease (OR<1). The final regression model derived for SPP sero-positivity in Kordofan region, based on the major risk factors identified, was: Log (P) = 1.782 constant + 0.791 locality + 0.740 herd size + 1.222 insect bite + 2.169 rainfall + 1.421 traditional treatment. This model suggests that within the combination of five factors, the probability of sero-positivity increased by insect bite, rainfall and traditional treatment, while it was reduced by locality and herd size, serving as a kind of 'protective factors, because their values less than 1 in the model.

Table 6: Multivariate logistic regression model of risk factors and SPP sero-positivity

Risk factor		Exp(B)	95% CI for Exp(B)		p-value
			Lower	Upper	
State	N.Kordofan (ref)				.077
	S.Kordofan	.484	.094	2.485	.384
Locality*	Shiekan (ref)				.014
	Elkhowi	3.835	.398	36.951	.245
	Bara	.864	.363	2.053	.740
	Dilling	.659	.198	2.200	.498
	Elgoes	.454	.272	.757	.002
Breed	Zagawi (ref)				.393
	Gharaj	.399	.061	2.594	.336
	Hamari	.562	.235	1.340	.193
Mixed herd	Mixed (ref)				.077
	Pure sheep	1.260	.811	1.957	.303
Herd size*	>150 (ref)				.091
	51-100	.602	.363	1.001	.050
	101-150	.477	.237	.962	.039
Family Animal care	Man+woman (ref)				.057
	Man	.901	.286	2.834	.858
	Children	1.014	.259	3.977	.984
	Man+children	.300	.074	1.219	.092
Insect bite*	Yes (ref)				.081
	No	.914	.361	2.311	.849
	I don't know	.295	.087	.995	.049
Rainfall/year*	Low (ref)				.011
	Heavy	8.754	2.094	36.597	.003
	Moderate	4.048	1.097	14.930	.036
Traditional treatment*	Yes (ref)				.007
	No	4.143	1.130	15.183	.032

*significant by multivariate analysis using logistic regression

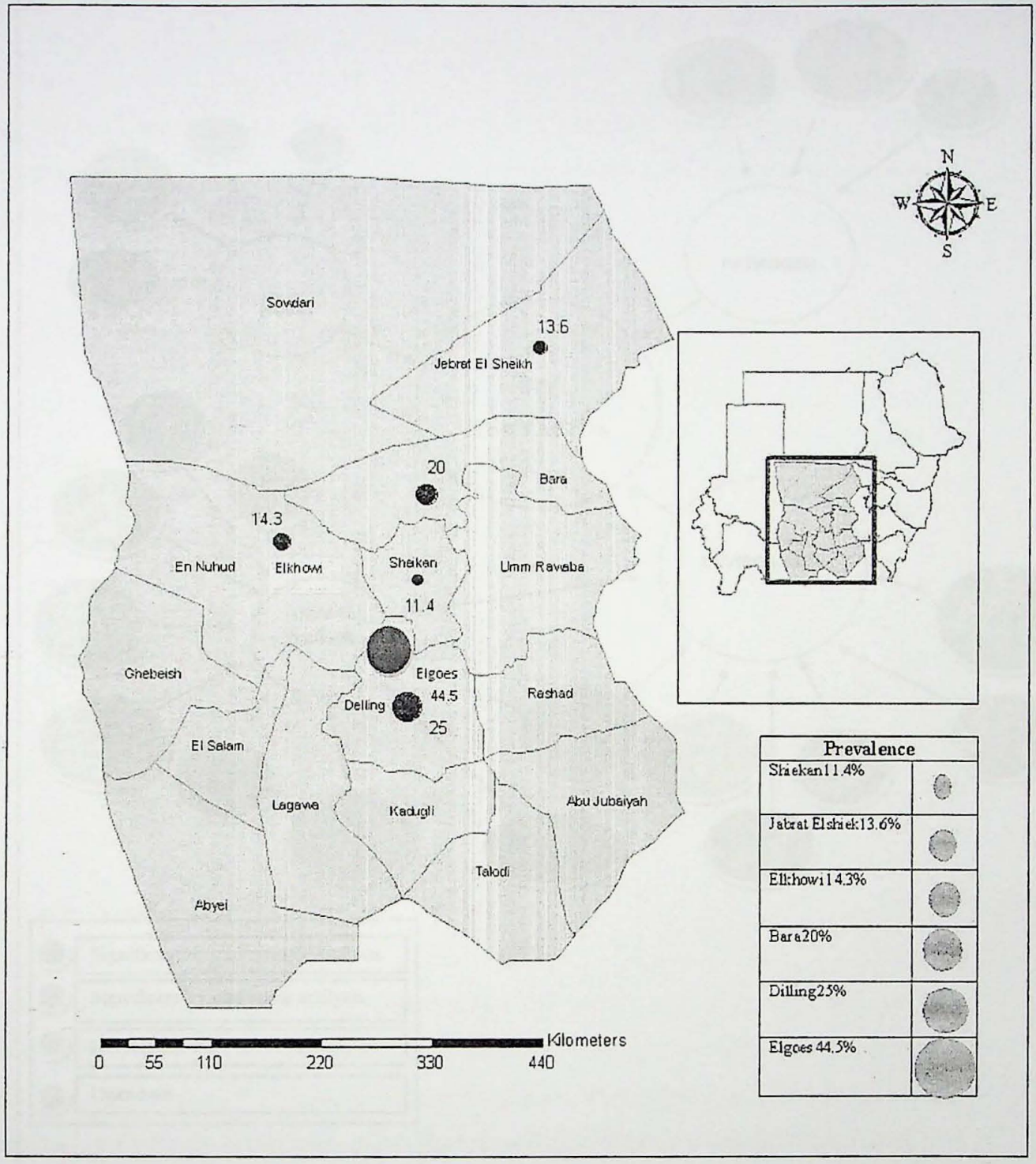


Figure 4: SPP sero-prevalence geographical distribution based on ELISA test in localities Shiekan, JabratElshiek, Elkhowi and Bara in North Kordofan state and Dilling and Elgoes localities in South Kordofan state

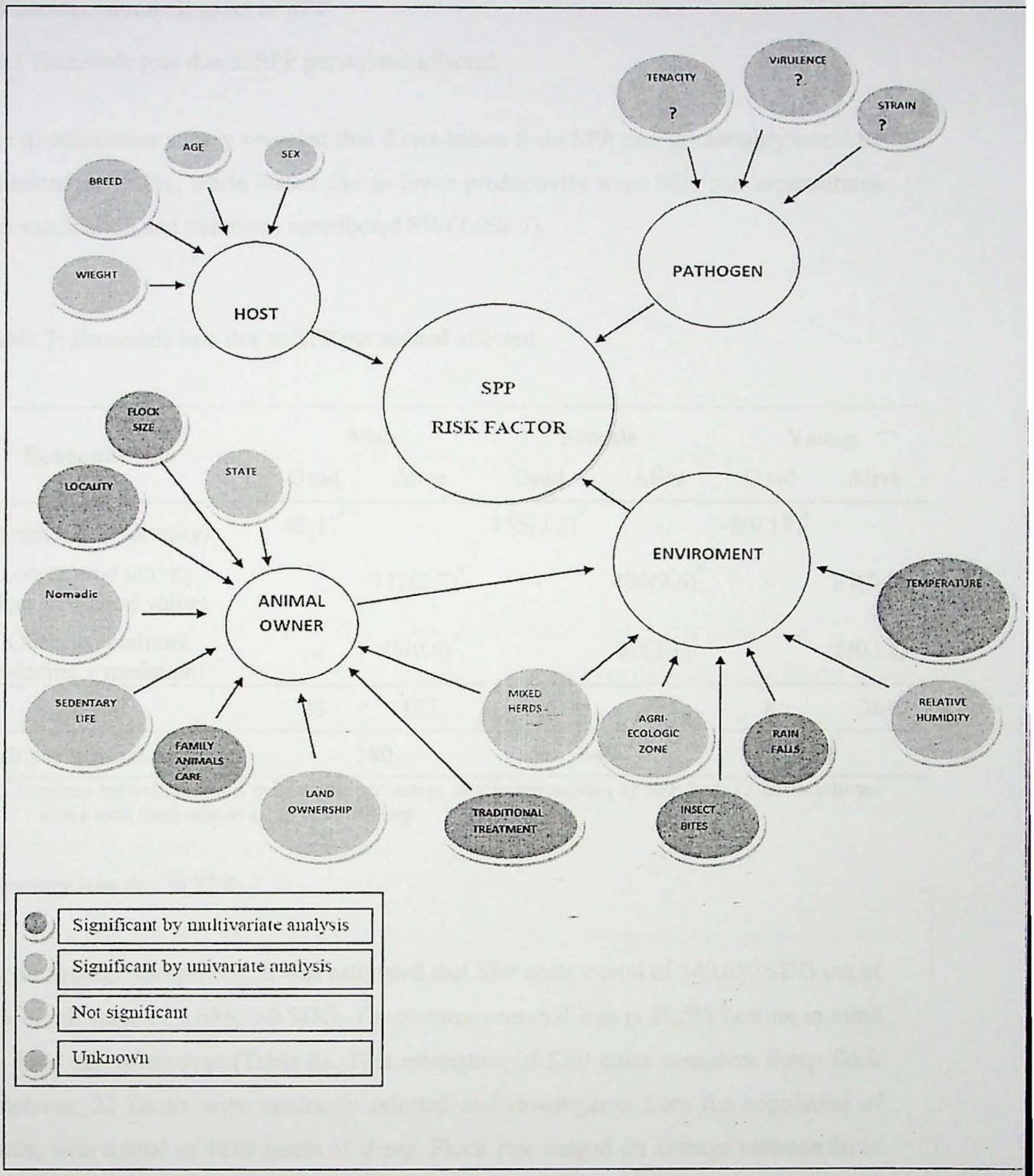


Figure 5: Hypothetical risk factor diagram of SPP sero-prevalence in NKS and SKS with associations of Host, Agent, and Environment

4.5 Socioeconomic impact of SPP

4.5.1 Economic loss due to SPP per animal affected

The questionnaire survey revealed that direct losses from SPP due to mortality could be estimated as 13.8%, while losses due to lower productivity were 36% and expenditures like vaccination and treatment contributed 8% (Table 7).

Table 7: Economic loss due to SPP per animal affected

Economic loss	Male		Female		Young	
	Dead	Alive	Dead	Alive	Dead	Alive
Direct loss (mortality)	43(1)*	-	155(3.8)*	-	8(0.19)*	-
Lower productivity (loss in market value)	-	112(2.7)*	-	406(9.6)*	-	21(0.5)*
Other expenditure (vaccine + medicine)	-	25(0.6)*	-	90(2.1)*	-	5(0.12)*
Total	43	137	155	494	8	26
Overall per animal average	**180**		**649**		**34**	

( )* Numbers between brackets indicate the percentage of affected animals by SPP from 22 flocks selected with a total flock size of 4,215 head of sheep

Monetary loss due to SPP

For an average sheep flock, it was estimated that SPP costs a total of 540,050 SDG out of total flock value of 2,636,350 SDG. The average seasonal loss is 20.5% bearing in mind the time gap to recover (Table 8). This estimation of SPP costs considers sheep flock structures; 22 flocks were randomly selected and investigated from the population of flocks, with a total of 4215 heads of sheep. Flock size ranged on average between 50 to more than 150 animals, small flocks (50 -100) represented 43%, medium flocks (101-150) 27% and large flocks (>150) 30% of the flocks.

Table 8: SPP costs per animal affected in monetary value (Sudanese Pounds, SDG)

Category	Average price/unit (SDG)	No of animals	Total loss
Young	300	34	10,200
Male	600	180	108,000
Female	650	649	421,850
Total	**1,550**	**863**	**540,050**

The current Sudanese currency is Geneh (SDG), equals to pound (USD 1.00 =2.7 SG)

Production and income

The production system of the study area was traditional, with sheep representing the main source of income of 73% of the study households. For the respondents, 91% of them were found to employ mixed farming practices. The questionnaire showed that 14% of respondents considered the impact of SPP on their animal production as low, 27% as moderate, and 59% as severe. No detailed information was recorded for sheep production as source of income from skins and hides, milk and manure.

Animal-raising in the study area was found to be, after crop production, the second most important source of livelihood and economic activity in NKS according to the Sudan Social Development Organization's NKS Survey from 2008. For the people in the state, animals are an important insurance against crop failure and other shocks. The percentages of households owning sheep and goats as source of income were estimated to be 49% in urban and 48% in rural areas.

4.5.2 Seasonal economic activities in Kordofan region

Table 9 lists features of nomadic life in some areas of Kordofan region; the households being busy all through the twelve months of the year is the predominant feature of this livelihood. Starting from December and January, the cold dry season, herders try to accommodate the scarcity of feed; animals do have to increase feed uptake to maintain body temperature which leads animals to congregate, which poses a risk factor to contract SPP in the cold dry season (Table 9). In the same period, some herders move back to the area they cultivated in order to harvest, which might also constitute a risk of SPP, because of insufficient personnel available to care for the animals at the flock level. In February and March, herders have to make their flocks ready for breeding (lambing) which carries a risk of transmission of SPP through direct contact from infected to susceptible animals during natural mating of animals. Conversely, in April and May, the only job for the nomads is the management of their flocks when they are moving to the rain grazing area towards the North. August, September and October are relatively free months compared to the rest of the year. In October, nomads start moving South with their flocks.

Table 9: Seasonal economic activities of some sheep herders in Greater Kordofan region

Activities	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Movement to												
North			×	×	×							
South										×	×	×
Cultivation						×	×					
Harvesting	×											×
Herding in												
Dry season	×	×	×									
Wet season							×	×	×			
Breeding (lambing)		×	×	×								

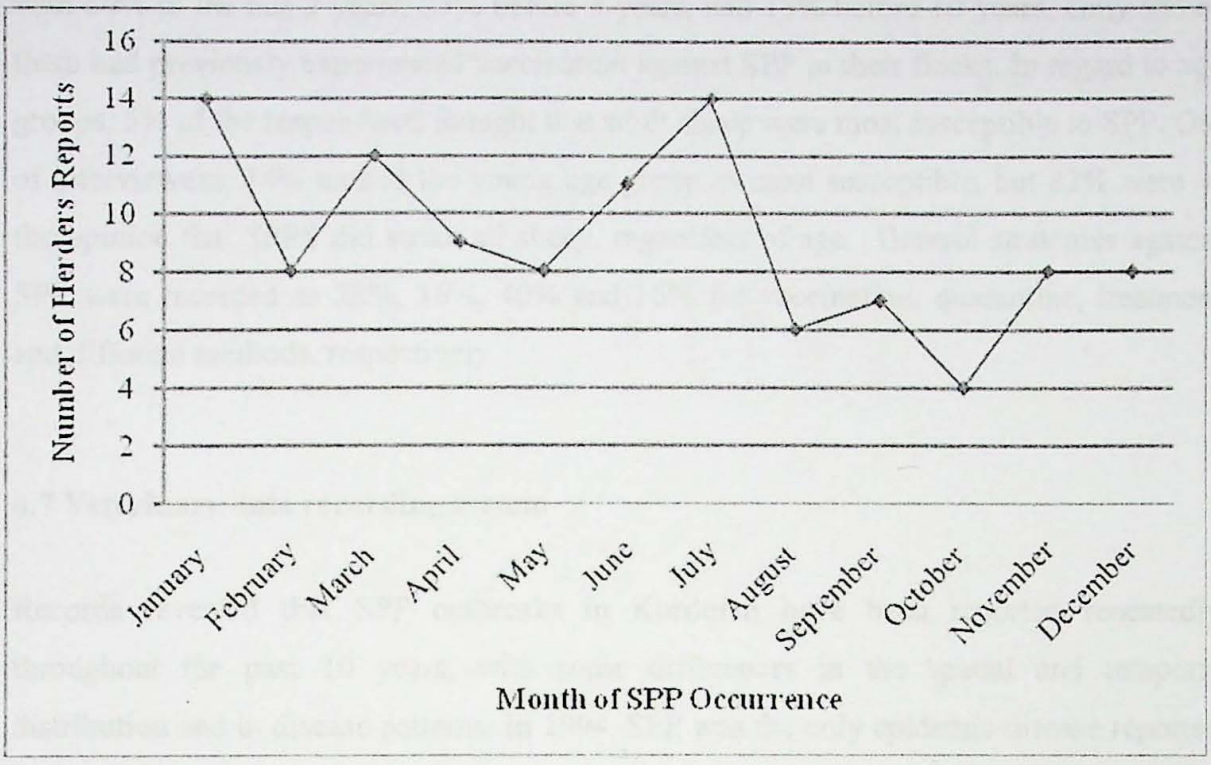


Figure 6: Frequency of reporting incidence of SPP in the Sudan coincides with some of the seasonal economic activities of some of sheep herders in Kordofan region. There is also a significant increase in incidence in the dry season from January until July, related to the lack of feed reserves and the lambing period. Data collected from 2005 to 2011 (OIE, 2011)

4.6 Questionnaire survey

The results of the questionnaire survey demonstrate that the production system of sheep herders in NKS and SKS comprised of 77% sedentary and 23% nomadic herds. All the sheep herders interviewed were found to be aware of SPP signs in general, but 68% of them were aware of skin lesion, and 13% aware of respiratory si, while 9% were aware of digestive distress, and 9% that was aware of the complex form of SPP.

All sheep herders recorded that they have experienced SPP occurrence in their flocks, with 59% in the last 2 years, 23% before 5 years, and 18% before 10 years. Only 9% of them had previously experienced vaccination against SPP in their flocks. In regard to age groups, 5% of the respondents thought that adult sheep were most susceptible to SPP. Out of interviewees, 14% named the young age group as most susceptible, but 82% were of the opinion that SPPS did strike all sheep, regardless of age. Control strategies against SPP were recorded as 28%, 16%, 40% and 16% for vaccination, quarantine, treatment and different methods, respectively.

4.7 Veterinary data recording system

Records revealed that SPP outbreaks in Kordofan have been reported repeatedly throughout the past 10 years, with some differences in the spatial and temporal distribution and in disease patterns. In 1994, SPP was the only epidemic disease reported in NKS and this was due to the lack of sheep pox vaccine at that time. In 2001, 3 outbreaks were reported in West Bahar Elghazal state, with a morbidity of 1.1% and a mortality of 0.16%. In 2003, 3 SPP outbreaks were made known in Kadugli locality in SKS, with a morbidity rate of 2.68% and a mortality rate of 0.37%, while for 4 outbreaks occurred the same year in Rashad locality, the morbidity rate was 5.52% and the mortality rate 1% there. Also in 2003, one outbreak was recorded in West Kordofan state (WKS) with a morbidity rate of 3.17% and a mortality rate of 0.67%. In 2005, 4 outbreaks of SPP were recorded in SKS, the morbidity was reported as 14.2% and the mortality as 2.9%, while in the same year one outbreak occurred in WKS with morbidity of 10.5% and mortality of 0.7%. In 2006, 6 outbreaks were reported for NKS, one in the Gheebesh locality with 6% morbidity and 0% mortality, one in Dilling locality with morbidity of 5.5% and mortality of 1.6%, two in Talaodi locality with morbidity of 8.4% and mortality of 0.9%, one in Rashad locality with morbidity of 4.6% and mortality of 0.8%, and one outbreak in Abu Jebeha with 5.4% morbidity and 0.4% mortality (Figure 7). The morbidity rates underline that in each outbreak a proportional larger number of animals were affected in comparison to mortality, which was usually low.

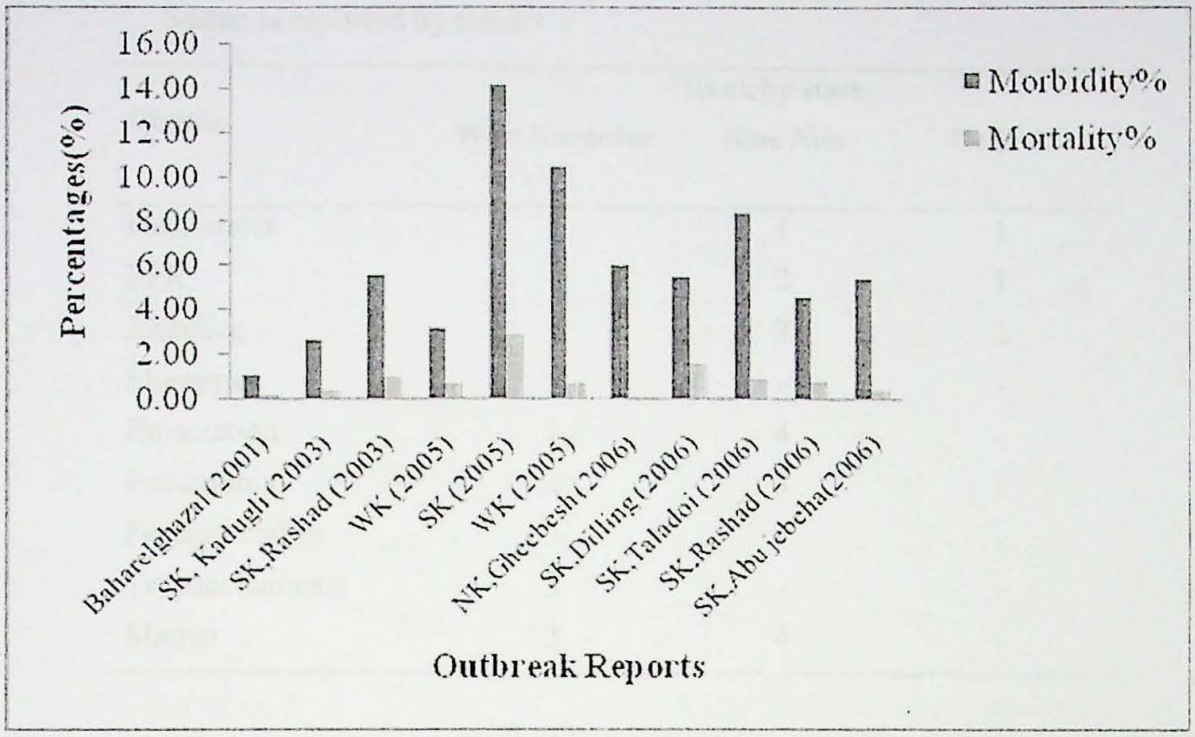


Figure 7: Morbidity and mortality rates of 23 SPP outbreaks reported for Kordofan and neighbouring states between 1994 and 2006

Table 10 explained the relative importance of SPP in West Kordofan, Blue Nile and Gedarif states. They ranked SPP as top priority in WKS, followed by Pneumonia and Heart water, in contrast to Blue Nile and Gedarif states where Heart water and PPR were ranked as top priorities, followed by Sheep pox and Pneumonia.

Table 10: Ranking of most common diseases for which animals were banned by veterinary inspectors from being sold at primary markets in three states of Sudan as reported by traders

Disease	Rank by state		
	West Kordofan	Blue Nile	Gedarif
Heartwater	3	1	1
PPR	-	2	1
Jaundice	-	2	2
Sheeppox	1	-	-
Emaciation	1	4	-
Pneumonia	2	3	3
Foreign bodies	3	-	-
Trypanosomosis	3	-	-
Mange	3	4	-

4.8 Evaluation of test performance characteristics

4.8.1 Optimization of indirect ELISA test

A 1:100 dilution was used as optimum concentration of recombinant antigen versus serum according to checker board titration. Plate-to-plate variation in readings was recorded with an average OD of 0.153 for control sera at a wavelength of 450 nm. There were some extreme values, higher than the average values, on plates 11 and 12 for which average ODs of 0.76 and 0.38 were recorded, while values lower than the average OD were recorded for plates 16, 18 and 24, which showed ODs of 0.043, 0.049 and 0.043, respectively (Figure 8). For the test sera, the average OD values (with 95% Confidence Intervals (CI) in Table 11) by State were 0.108 and 0.135 for NKS and SKS, respectively. The average OD values by age groups were 0.107, 0.120, 0.114, and 0.114 for animals of age groups < 1 year, 1-2 years, 3-4 years and > 4 years, respectively, (Table 12).

Table 11: Mean OD values with 95% CI by localities in NKS and SKS

State	Locality	Mean OD Values	95% CI for mean	
			Lower Bound	Upper Bound
N.Kordofan		0.108	0.104	0.112
	JabratElshiek	0.106	0.097	0.115
	Elkhowi	0.106	0.101	0.112
	Shiekan	0.101	0.095	0.108
	Bara	0.145	0.133	0.157
S.Kordofan		0.135	0.130	0.141
	Dilling	0.124	0.117	0.131
	Elgoes	0.152	0.142	0.161

Table12: Mean OD values with 95% CI by age groups in NKS and SKS

Age groups	Mean OD Values	95% CI for mean	
		Lower Bound	Upper Bound
< 1 year	0.107	0.094	0.120
1-2 years	0.120	0.115	0.124
3-4 years	0.114	0.108	0.121
> 4 years	0.114	0.104	0.123

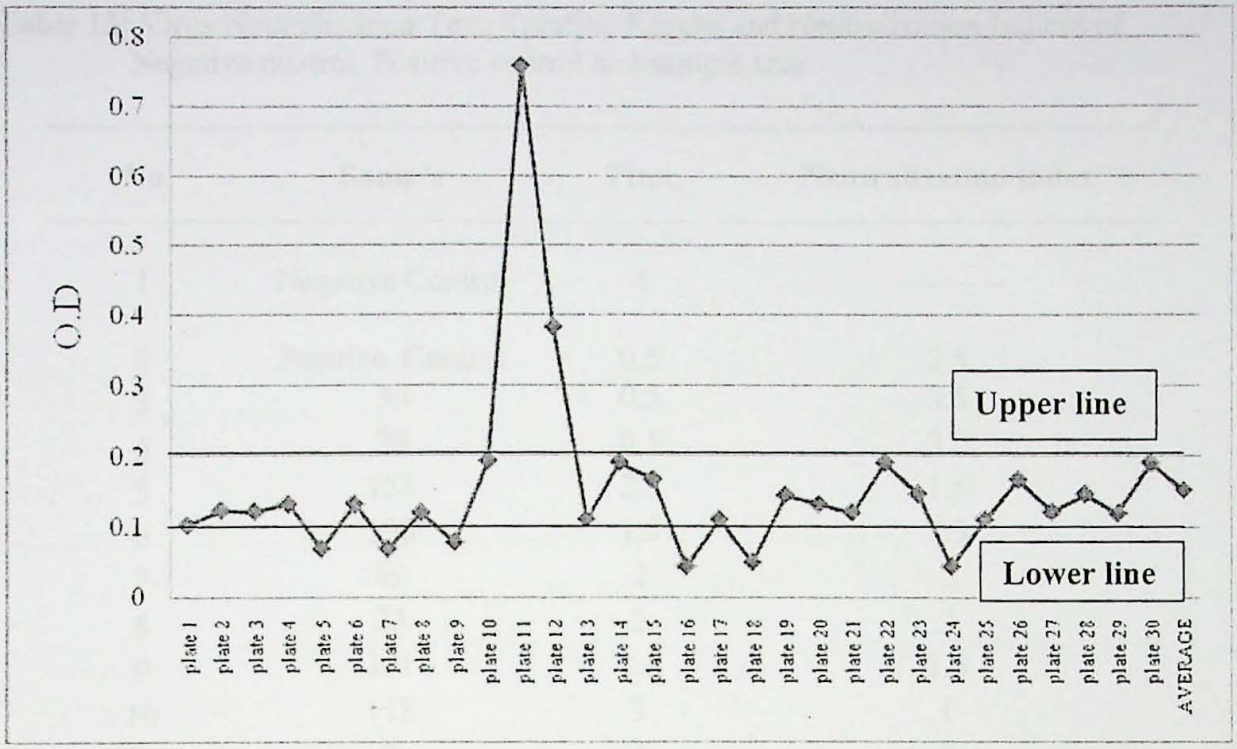


Figure 8: Determination of cutoff value from individual ODs of plates of the indirect ELISA based on control negative(C-) serum used to detect antibodies to capripox virus

4.8.2 Virus Neutralization Test (VNT)

VNT was carried out for 22 serum samples, including the control positive and control negative sera of the ELISA test. Out of the 22 samples 12 were positive, having Neutralization Indices (N.I $\geq$ 1.5) of 3.5, 3, 2.5, 2 and 1.5 recorded for 25%, 8.3%, 8.3%, 25% and 33.3 % of positive samples (Table 13).

Table 13: Virus Neutralization Test: Titration Results and Neutralization Indices of Negative control, Positive control and sample sera

No	Sample	Titer	Neutralization index
1	Negative Control	4	----------
2	Positive Control	0.5	3.5
3	30	0.5	3.5
4	70	0.5	3.5
5	153	2.5	1.5
6	100	1.5	2.5
7	89	2	2
8	23	2	2
9	101	2.5	1.5
10	118	3	1
11	2	4	0
12	3	2.5	1.5
13	4	3.5	0.5
14	28	3	1
15	13	4	0
16	16	3	1
17	29	2	2
18	86	1	3
19	54	4	0
20	96	2.5	1.5
21	56	3	1
22	25	3	1

Titer expressed in Log_{10} $TCID_{50}$ /50 μl

Positive N.I. ≥ 1.5
Negative N.I. <1.5

4.8.3 Measurement of agreement of indirect ELISA with VNT for SPP

Out of 22 serum samples 8 were positive by the indirect ELISA, while 12 out of the 22 samples were positive by VNT. The degree of agreement between the two test was measured by the Kappa test (value = .065) (Table 14).

Table 14: Kappa test for the degree of the agreement between indirect ELSIA and Virus Neutralization Test

		Value	Asymp. Std. Error	Approx. Tb	Approx. Sig.
Measure of Agreement	Kappa	0.065	0.200	0.324	0.746
N of Valid Cases		22			

4.8.4 Indirect ELISA test properties

A 2×2 table was formulated by VNT as true status (gold standard) and indirect ELISA as test under experiment. For the 8 positive samples tested by indirect ELISA, 4 were truly positive, while 4 were falsely negative. For the 14 negative samples tested by indirect ELISA, 8 were falsely negative, while 6 were truly negative. The test properties of Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV), Negative Predictive Value (NPV) were found to be 33.33% , 60% , 50%, and 42.9%, respectively (Table 16) in annex.

5. DISCUSSION

This study was undertaken to provide more information about the prevalence of SPP, to determine potential risk factors and their association with SPP, to assess the economic effect of SPP in NKS and SKS, as well as to evaluate the test performance characteristics of the employed indirect ELISA to detect antibodies to capripox virus. The study was based on a cross-sectional sample survey of SPP in sheep flocks in the Kordofan region with a questionnaire survey of study herders to collect information about the production system, management practices and economic activities in the study area, besides information about SPP and on knowledge and awareness of the respondents about it. Also, retrospective information was reviewed in order to provide a past picture of SPP in Kordofan region and to compare it with the current study.

5.1 Epidemiological survey

The overall sero-prevalence of SPP in Kordofan region using indirect ELISA was 20.1 %. This prevalence is lower than that of a previous study by Dirani *et al.* (2009) who reported SPP prevalences in West Kordofan region of 53.6%, 28.3% and 33% for 2003, 2004 and 2005, respectively. The difference between the sero-prevalences of the current and the previous study in all likelihood are due to differences in study designs, sample size determination, target populations and study areas. However, we would also conclude that the ELISA is not yet optimized for use in Sudan based on most recent checkerboard titrations that were undertaken. The current estimates (for seroprevalence) using recombinant antigen ELISA are therefore likely to be lower than the true sero-prevalence.

Generally, the SPP sero-prevalence was higher in SKS than in NKS which might be attributed to host factors, like, for example, flock size for which the sero-prevalence was statistically significantly different between different sized flocks; thus, the smaller the

flock size was, the greater risk it could have lead to the spread of SPP in close flock space. This is in agreement with the report of Mohamed *et al.* (1982) on the outbreak which occurred in 60 goats in a flock in the Shambat and Halfaya area (Khartoum North), where animals were kept under poor husbandry conditions in semi-shaded enclosures made of thorn trees, entrapping the animals and increasing the probability of closer contacts between them as well as facilitating faster spread of infection in closer space.

Vaccination coverage against SPP in the Sudan from 2005-2010 was estimated as 11.34% and 0.04% for sheep and goats, respectively (OIE, 2010). Only 9% of the respondents in this study had previously experienced vaccination against SPP in their flocks. It has to be noted that the serum samples were collected only from non-vaccinated flocks, based on the credibility of answers of the sheep herders that they had not vaccinated the sampled animals. If reportedly having been vaccinated any vaccinated flock was excluded from sampling. It is not possible to differentiate between vaccinated and infected animals serologically, however, it was even found that the indirect ELISA was unable to detect antibodies reliably in vaccinated sheep and goats (Bowden *et al.,* 2009). Thus, if it is assumed that sheep flock owners were not credible in giving correct information about vaccination of their flocks, the high sero-prevalence in tested flocks could, at least in part, be attributed to previous vaccination.

Insect bites were found to be a statistically significant risk factor associated with SPP. It is known that pre-infected flies do transmit the virus to susceptible animals and that the virus remains alive for about 4 days in some flies. The high concentrations of virus in the skin may also contribute to the spread of SPP and GTP via insect vectors (Bowden *et al.,* 2008). Mellor *et al.* (1987) reported that *Stomoxys calcitrans* is an efficient mechanical vector of capripox viruses. Also Kitching *et al.* (1986) reported that Nigerian and Oman isolates of SPPV could be transmitted to sheep by *S. calcitrans*, but not by biting *Mallophaga* species, sucking lice (*Damalimia* species), sheep head flies (*Hydrotaea irritans*) and midges (*Culicoides nubeculosus*), although it was possible to isolate SPPV from previously infected sheep which were hosts on which the aforementioned flies did feed.

Regarding traditional treatment, only 12% of respondents did use it and probably they do not have access to veterinary services; traditional treatment was claimed to be as efficient as modern veterinary practices like vaccination and treatment with different drugs. In fact, some respondents strongly did believe in traditional medicine, for example the use of *Acacia* species emulsion against SPP and the use washing soap with carbonate stone for treatment of SPP were mentioned in the interviews, as were certain perfumes said to have an effect against SPP skin lesions, or the belief that once an animal with SPP is in a flock, it is better to leave this infected animal in the flock because the flock will be free of SPP for life, if the sick animal(s) recover. The key issue is that all the previously mentioned ethnoveterinary practices could be right or wrong, based on the indigenous knowledge built by a particular community in the study area over time. Such practices may deserve to be evaluated by scientific studies.

5.2 Questionnaire survey

The questionnaire survey revealed that in regard to SPP seasonality, 88% of the respondents agreed that SPP occurs in different seasons of the year, by this agreeing with the study done by Shiek-Ali *et al.* (2004); Muzichin and Ali (1979), however, disagree in that they place SPP into the cold winter season. Clinically, some cases of SPP were observed with different severity and it was mostly mild to moderate fever and skin lesion covering the skin especially around head region, perineum and udder region.

As for awareness of herders of SPP signs, the questionnaire survey revealed that 68% of the respondents were familiar with the skin lesions of SPP, which agrees with Losos (1986) who reported that the nodular form of SSP did prevail in the Sudan, as seen in the Rawasi, Bagir and University farm SPP outbreaks. Concerning control strategies against SPP, 40% of the respondents use antibiotic treatment as first choice, which might save secondary infections. Concerning the use of vaccination, the picture remains fuzzy. On one hand, 28% of herders interviewed reported to use vaccination, on the other hand only

9% of them reported that they previously had experienced vaccination against SPP in their flocks. Beyond doubt, use of vaccination is more effective than the spurious treatment used. It may be however, that herders are of the opinion that the cost of treatment is cheaper than that of vaccination. Quarantine is rarely applied, only 16% of the respondents use quarantine as control measure against SPP; 16 % responded that they adopt a mix of strategies like isolation of infected animals, treatment and restricted movement.

5.3 Economic effect of SPP

The economic effect of SPP was demonstrated by two simple models, the estimation of the cost of SPP in affected flocks and the effect of SPP on the seasonal economic activities of sheep herders, when it occurs. Average SPP costs were calculated to be total of 540,050 SDG, 20.5% of the total average flock value of 2,636,350 SDG. This average cost includes the time for a sick animal to recover. It is a very crude estimate. Detailed information about the days of sickness which could have refined the estimate was not available. With such information it would have been possible to arrive at regression coefficients in linear models which could be used to give more accurate estimate of cost. Through observation of the seasonal economic activities of sheep herders in Kordofan region it was found that SPP frequently is reported from January until July, months when sheep herders are busy with different activities like herding, cultivating, harvesting and breeding their animals. This seasonal concentration of SPP is in accordance with the OIE report which stated that in sub-Saharan Africa two waves of SPP could be distinguished, one in February and March during the cool period and one from June to October during the rainy season; the reference data for this report were from 1996 to 2008 (OIE, 2008).

5.4 Laboratory testing

One objective of this study was to evaluate the test performance characteristics of the employed new indirect ELISA, which bases on recombinant antigen to detect antibodies to capripox viruses in comparison to the standard Virus Neutralization Test (VNT).

A checker board titration was done to determine the optimum dilution of antigen versus serum. One thousand and five hundred (1500) serum samples of sheep collected from the Kordofan region subsequently were tested using the indirect ELISA. Plate-to-plate variations were noticed, which was expected and could be attributed to random and systematic errors like volume of the reagents, interpersonal variation in performing the test in the same laboratory as well as to different laboratories conditions, machine errors (old ELISA reader might need calibration). In order to somewhat reduce random and systematic errors, samples sizes were adjusted by multiplying the sample size by three for each study state as well as by testing samples in duplicate for all localities with the exception of Dilling and Elgoes. Also, control sera in each plate were compared with test sera in order to achieve at least some degree of internal quality control for each individual test plate.

Generally, test results were lower than expected and as reported by the test constructors (Bowden *et al.,* 2009). Reasons can only be speculated, ranging from failures to optimize and standardize the ELISA testing system under the laboratory conditions in the Sudan to lack of the availability of all required and recommended reagents. Noteworthy is, that the ELISA test was done on field sera, whereas test constructors did it on sera from animls that had been infected experimentally. Subsequently, the test constructors will test a subset of the field sera which are already tested as a part of this study in Australia and Canada. These data, once obtained, will provide a comparable base of ELISA performance in all three laboratories.

The *Kappa* test was employed for 22 serum samples which were first tested by VNT and results then compared to those of the indirect ELISA, in order to measure the degree of agreement between the two tests, Subsequently the indirect ELSIA test properties were calculated to be 33.3% sensitivity, 60% specificity, 50% positive predictive value and 42.9% negative predictive value. Specificity was by far lower than the diagnostic specificity reported for the test by Bowden *et al.* (2009), which was 94% for sheep and 95.1% for goats. Sensitivity also was by far lower than that of experimental infections,

where it resulted in sensitivities of 97.1% and 94.7% for sheep and goats, respectively. Therefore, the lower sensitivity of ELISA test contributed in increased false negative results. However, sera collected from day 14 following vaccination or experimental challenge lead to a sensitivity of 11.5% post vaccination or 97.1% post infection, for sheep, and to 0.0% sensitivity post vaccination or 94.7% post infection for goats (Bowden *et al.*, 2009).

Although these data, which represent repeated observations from a limited number of animals, are not considered acceptable for establishing estimates of diagnostic sensitivity (Jacobson and Wright, 2008), they give a preliminary indication of assay sensitivity following experimental infection or vaccination.

This means that the performance of the employed test was disappointing; the test considerably has to be improved to suit the conditions in the Sudan.

6 CONCLUSIONS AND RECOMMENDATIONS

The current study revealed that SPP prevails both in SKS and in NKS. Also, a number of risk factors like locality, herd size, insect bites and rainfall/year were found to be significantly associated with SPP seropositivity in the Kordofan region. The average cost of SPP was estimated at 20.5% of average sheep flock production costs. Indirect ELISA test performance was not adequately valid.

According to this study, SPP is considered one of the serious health and production problems of sheep in the Kordofan region. As Kordofan is one of the major production areas in the Sudan, SPP affects both domestic and international trade in livestock and livestock products. Therefore this study recommends the following:

- Reducing the incidence of SPP in Kordofan region by employing different control strategies like vaccination, strict movement control and quarantine, besides mitigating identified risk factors which predispose for the occurrence of SPP should be carried out.
- Raising awareness of the sheep flock owners about the socioeconomic importance of SPP as a priority disease in regards to exports which affects their livelihood and the national economy should be done.
- The role of wild ruminants in epidemiology of the SPP, where they act as a reservoir or carrier for the virus should be studied
- Conducting the serosurveillance in other States in the Sudan where small ruminant contribute in the livelihood of people should be carried out.
- Isolation and characterization SPPV strains circulating in Kordofan region and genetically compare it with other isolates in the Sudan should be done.
- Investigation of the potential role of the insect vectors like the ticks and other blood sucking flies in the transmission of SPP in Kordofan region is necessary.

- To further the study of the economic impact of SPP, a more comprehensive design for generation of regression coefficients of linear models regarding the impact of different factors over time is needed.
- Improving of the test performance characteristics of the indirect ELISA employed to detect antibodies to capripox viruses should be carried out.

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8. ANNEXES

Annex 1 Questionnaires survey for sheep flock herders and professionals

Questionnaire format (1) to collect data related to sheep pox disease in North and South Kordofan states from 30/04 /2011 until 24/05/2011.

Date of visit: ..... **Investigation No: .....**

1. Sheep's owner primary data:

Name: .....Age .....

Marital status:.....

Sex: .....

Number of people living in the household .....

Education level: illiterate (...) primary (...) secondary (...) university (...)

Flocks size .....

What are the other animal species the owner has?

Goats (.....) cattle (....) camels (.....) equines (.....)

- Are you aware of SPP clinical signs?
  Yes (....) No (....)

  If yes what are they?
  .....
- What is the mortality (%) of SPP in your flocks?

  .....
- What is the morbidity (%) of SPP in your flocks?
  .....
- What are the clinical signs of SPP you are familiar with?

Skin lesion around the muzzle and face region (….)

Skin lesion in the tail and around the prenium region (….)

Respiratory signs (…..)

Diarrhea (…..)

Emaciation (…..)

Abortion (……)

Others ……

- What is the most affected Age group in your flocks?
  Young (….) Old (…..)
- Is there any traditional treatment due to the SPP infection?
  ……..
- Are your animals vaccinated against SPP?
  Yes (……) No (…..)

Is SPP getting reduced after vaccinating sheep flocks?

Yes (…) No (…)

And if yes for how long animals remained unsusceptible?
……..

- Are there any local practices that keep animal protected from SPP?
  ……..
- Is SPP disease transmissible by biting flies?
  Yes (….) No (….)

  If yes please identify the fly(s)?
  ……
- What is the toll of SPP on the production of your flocks?
  Mild (…..) Moderate (…..) Severe (….)
- What are SPP disease management strategies you are adopting?

Vaccination (….) restricted movement (….) quarantine (….) Treatment (….)
Others ……

2. Study area data:

State .....Locality .....village or location .....

Geographic coordinate (longitude ....., latitude .....), Sheep population .....
Human population .....

- Has the SPP been reported before in the area?

Yes (.....) No (.....)
and if yes when?
.....

- What is the migration pattern in the area which is associated with sheep pox prevalence?
  From the area (.....) Into the area (.....)

  .....

3. Animal's primary data:

Sex .....

Age .....

Ecotype.....

Animal size .....

Color .....

Health condition: sick (...) healthy (.....)

Body condition: poor (.....) fair (.....) good (.....)

4. Climatic factors:

Temperature °C .....

Relative humidity (%).....

Soil type .....

Rainfall /year.....

Plant cover: ....

Agri-ecologic Zone: dry (.....) Arid (.....) semi-arid (.....) sub-arid (.....)

5. Production system

Traditional system (.....), intensive system (.....), extensive system (.....)

- Are you practicing animal breeding only or having mixed farming?
  Yes (.....) No (.....)

  and if yes to what extent is it practiced?

  .....

- Are there any activities you do beside breeding sheep?
  Yes (....) No (....),

  If yes what are they?
  .....

- Which are the productions inputs basically you are depending on?
  Land (....) labours (....) capital (....) others.....

- Who is taking care of animals while they are let out to graze (free range)?
  Man (.....) Woman (.....) Children (.....)

- Who is taking care of the animals when it is back from grazing?
  Man (.....) Woman (.....) Children (.....)

- What is the breeding season of your sheep flocks?
  Dry hot season (....) dry cold season (....) rainy season (....)

- How do you breed your sheep flocks ?
  Naturally (....) artificial insemination (....)

- Are you using embryo transfer technology in your sheep flocks?
  Yes (....) No (....)

- Are the family members having a role to play in rearing and treating the sick animals? Yes (.....) No (.....)
  And if yes, please identify them with their duties?

  .....

- Is animal-raising the only source of income for the family?
  Yes (.....) No (.....)

  And if No what are the others sources?

  .....

- What kind of land ownership you have?

  Absolute ownership (....), tenure land (....), communal land (.....)

- **Remark :**
  .....
  .....
  .....
  .....
  .....

Investigator name: ..... Position .....
Signature ..... Date submitted .....

Questionnaire format (2) for the professional about sheep pox disease in North and South Kordofan states from 30/04/2011 until 24/05/2011.

1. Professional's data:

Name ..... Status .....Working at .....

For how long..... Academic qualification.....

- What are the kinds of the veterinary service in the area?

  Vet clinic (...) quarantine (...) abattoir (...) livestock market (...)

- Are there any records for SPP in the area?
  Yes (...) No (...)

  And if yes in which locality? .....

- What type of diagnosis is considered for diagnosing SPP?

  Clinical sings (...), serology (...), virus isolation (...) PCR (...)

- What is commonly similar disease to SPP do you know ?
  a..... .b.....
  c..... d.....
  e..... f.....

- Is there any vaccination program against SPP?

  Yes (...) No (....)

- If yes to what extent is the vaccination coverage in the area (%) .....

- What are the sheep movement patterns?

  Sedentary (...) Nomadism (...)

- In which season of the year SPP outbreaks occurred?
  Hot dry season (….) dry cold season (….) rainy season (….)
- Are there any watering points facilitate mixing of different flocks in the area?
  Yes (…) No (….),
- What are the main routes of transmission of SPP among sheep flocks?

  Direct contact (…) feed and drinking water (…) biting flies (…) sexually (…)
- What is the incubation period of SPP?.....
- Are there any susceptible animals to SPP in the area ?
  Yes (…) No (…)

  And if yes specify them?

  ……
- How regularly animal get vaccinated against SPP disease?
  Periodically (…) non periodically (…)
- Are there any human cases due to SPP infection?
  Yes (…) No (…)

  If yes what are the symptoms?

  ……
- Are there any contacts with wildlife?
  Yes (…) No (…)

  If yes what type?

  ……
- To what degree of severity does SPP affect local and export market?
  Mild (….) moderate (….) severe (….)

2. Secondary data about SPP

- Year of the investigation ……

- Were there any outbreaks of SPP in that year?

Yes (……) No (……)

And if yes how many were they? ……

- Location of the outbreaks ……..

- In which season SPP outbreak occurred?
  Summer (….) winter (….) autumn (….) other ……..

- What is mortality rate (%) due to SPP? ……

- What is morbidity rate (%) due to SPP?.....

- Which kinds of animals are involved in the outbreak?

  Sheep (….) goats (….) sheep and goats (….) cattle (….) camels (….)

  Others ……

- What were the common signs associated with the outbreak?

Skin lesion on the face (….)

Skin lesion around the inguinal region and the tail (….)

Respiratory signs (….)

Diarrhea (….)

Abortion (….)

Nodules around chest and belly (….)

Others ……

- What were the most affected sex age groups?
  Male (….) Female (….)

  Young (….) Old (….)

What were the most affected in different physiological status ?

Dry animals (….) pregnant (….) lactating animals (….)

- What were the measures to control the SPP outbreak?

  Vaccination (….) restricted movement (….) quarantine (….) Treatment (….)
  Others ……

- How likely did SPP outbreak affect the livelihood of the household in the area?

  Very unlikely (….) Negligible (….) likely (….) very likely (….)

- What was the monetary loss from SPP in this year per capitae (sheep)?
  ……
  Which were the other expenditures to control SPP outbreak?

  Veterinary care (….) drugs (….) vaccination (….) other losses ……

- **Remarks:** ……
  ……
  ……
  ……
  ……
  ……

Investigator name: …… **Status** ……
Signature …… **Date submitted** ……

Annex 2 Table 15: Comparison of indirect ELISA and VNT results

No	Sample number	VNT*	iELISA*
1	Negative Control	0	0
2	Positive Control	1	1
3	30	1	1
4	70	1	0
5	153	1	1
6	100	1	0
7	89	1	0
8	23	1	0
9	101	1	0
10	118	0	0
11	2	0	0
12	3	1	1
13	4	0	1
14	28	0	1
15	13	0	0
16	16	0	0
17	29	1	0
18	86	1	0
19	54	0	1
20	96	1	0
21	56	0	0
22	25	0	1

*Note: For VNT and iELISA
0 = Negative
1= Positive

Annex 3 Table 16: 2×2 table for test results of VNT and indirect ELISA

		True status VNT		
		+ve	-ve	
IELISA	+ve	4 a	b 4	8
	-ve	8 c	d 6	14
		12	10	22

Se ELISA = a/a+c*100= 4/12*100=33.33%

Sp ELISA =d/b+d*100=6/10*100=60%

PPV=a/a+b*100 =4/8*100=50%

NPV=d/c+d*100= 6/14*100= 42.9%

9. CIRRICULUM VITAE

Personal information

Name: Mohammed Elsadig Ahmed Mansour

Nationality: Sudanese

Place of birth: Omdurman, Khartoum

Date of birth: August 23rd, 1982

Marital status: unmarried

Address: Federal Ministry of Animal Resources and Fisheries

Animal Resources Research Corporation

Veterinary Research Institute, (VRI), Soba,

P.O.Box.8067, (Alamarat), street No (1), Khartoum, Sudan

Mobile No: +249 920036574

Email: hamo152000@yahoo.com

Qualification

- Bachelor of Veterinary Medicine (BVM), Faculty of Veterinary Medicine, University of Khartoum, October, 2004.

Work experience

- Worked for Veterinaries Sans Frontieres (VSF), Germany, Khartoum office on the livestock profiling program in Omdurman area.
- Participating in annual vaccination campaign teams against epidemics & outbreaks in West Omdurman area organized by Federal Ministry of Animal Resources and fisheries.
- Participating in veterinary orientation programme for the target community in West Omdurman Area.
- Trainer of trainee in poultry production in livelihood program for beneficiaries of World Vision organization, North Sudan program.
- Trainer in veterinary diagnostic laboratory workshop held in Juba, South Sudan, sponsored by Food and Agriculture Organization of United Nations (FAO).

Training courses attained

- Training course held in Goats Breeding Center in Omdurman Veterinary Hospital about Artificial Insemination by (NIFA instrument) THE NETHERLANDS.
- Training course in National Center for Skin & Hide Processing.
- Training course on Race horse foots trimming & shoeing, and ultra – sound pregnancy diagnosis in mare, in Equestrian club, Khartoum.
- Training course on serodiagnosis of Rift Valley Fever as an emerging disease held at VRI, Soba.
- Training course on research methodology and its general principal held at kuku Animal Production Research Centre, Khartoum North.
- Training course on Geographic Information System (GIS) and remote sensing and their application in livestock Disease Diagnosis and Research at VRI, Soba from 15-25 , 11, 2009.
- Participated by poster presentation titled -Epidemiological studies on Rift valley fever in the Sudan- in integrated workshop for controlling of Rift valley fever in Middle East and Africa held in Cairo , Egypt from 27- 29 , January 2009 .
- Participated in regional (AFRA) training course organized by Department of Technical Cooperation of International Atomic Energy Agency (IAEA) on Transboundary Animal Disease Surveillance held in Entebbe, Uganda from 7-11 December, 2009.

Work History

- 2007- up to date — Veterinary Research Institute, (VRI), Soba
  Laboratory researcher assistant

- June - July 2005 — VSF , Germany, Khartoum office
  Veterinarian

- Oct 2004- Oct 2005 Omdurman Veterinary Hospital
  Veterinary officer

Languages

- Arabic language; Mother tongue.
- English language; Fair communication skills.
- French language; Niveau A2.
- German language; Beginner.

Computing skills

- Computer literate.
- Familiar with statistical analysis software like SPSS.
- Familiar with spatial mapping software like ArcGIS, map maker.
- Familiar with surveillance software like survey toolbox, WinEpisocpe, @ risk.

References

- Dr. Tamador Mohammed Abdullah, PhD, (Associate professor)
  Veterinary Research Institute, Soba
  Head of RVF Unit,
  Email: tamadormohamed@yahoo.com
  Mobile: +249912950987

- Dr. Yahia Hassen Ali, PhD, (Associate professor)
  Veterinary Research Institute, Soba
  Head of Department of Virology
  Email: y_beik@ hotmail.com
  Phone: +249 183 380 015

10. SIGNED DECLARATION SHEET

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

Candidate name: Mohammed Elsadig Ahmed

Signature: ..... Date of submission 1/12/2011

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

ADDIS ABABA UNIVERSITY

DR. YILKA ASFAW

Signature ..... Date.....

DR. HAILELEUL NEGUSSIE

Signature ..... Date.....

FREIE UNIVERSITÄT -BERLIN

PROF. KARL-HANS ZESSIN

Signature ..... Date.....

DR. MAXIMILLIAN BAUMANN

Signature ..... Date.....

SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

PROF. ABDELHAMID ELFADIL

Signature ..... Date.....