Epidemiological Investigations On Sheeppox In Kassala State, Sudan

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To:

My father's soul.
My great mother, Asha.
My sisters, Tammador and Marrwa
My brothers, Isam, Yasir, Shehab, Ammar, Amir and their small families

FOR THEIR PATIENCE, CONSTANT ENCOURAGEMENT AND SUPPORT.
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<table>
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<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<tr>
<td>AGID</td>
<td>Agar Gel Immuno-diffusion</td>
</tr>
<tr>
<td>BEI</td>
<td>Binary Ethyleneimine</td>
</tr>
<tr>
<td>CAM</td>
<td>Chorioallantonic Membrane</td>
</tr>
<tr>
<td>CPE</td>
<td>Cytopathic Effect</td>
</tr>
<tr>
<td>DDW</td>
<td>Distilled Deionised water</td>
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<tr>
<td>DTH</td>
<td>Delayed-type Hypersensitivity</td>
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<tr>
<td>Dr.</td>
<td>Doctor</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>EM</td>
<td>Electron Microscope</td>
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<tr>
<td>GP</td>
<td>Goatpox</td>
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<td>GPV</td>
<td>Goatpox virus</td>
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<tr>
<td>HIS</td>
<td>Hyper immune Serum</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>LSD</td>
<td>Lumpy Skin Disease</td>
</tr>
<tr>
<td>LSDV</td>
<td>Lumpy Skin Disease Virus</td>
</tr>
<tr>
<td>NS</td>
<td>Normal Saline</td>
</tr>
<tr>
<td>Orf</td>
<td>Contagious Pustular Dermatitis</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PHA</td>
<td>Passive Haemagglutination Test</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>SDC</td>
<td>Sodium Deoxycholate</td>
</tr>
<tr>
<td>SNT</td>
<td>Serum Neutralization Test</td>
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<tr>
<td>SP</td>
<td>Sheeppox</td>
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<tr>
<td>SPV</td>
<td>Sheeppox Virus</td>
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<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
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ABSTRACT

The aim of the present study was to determine the situation of sheeppox (SP) in Kassala State, Sudan. Three methods were adopted to achieve the fore-mentioned goal. These methods were questionnaire survey, collection of available data from veterinary services records and detection of antibodies against sheeppox virus (SPV) in the serum samples collected from different localities of the state using agar gel immuno-diffusion (AGID) test.

The questionnaire survey outcomes showed that most of the sheep owners have a good knowledge about SP as an epizootic disease in the state. Moreover, 80% of the owners confirmed the presence of the disease in their sheep flocks. Thus, the disease represents a real problem for the sheep owners in the state. From the previously reported outbreaks of SP, which obtained from the veterinary services records, the control of the disease can be achieved easily by means of vaccination which is possible in the state. In addition, the good infrastructure of the veterinary services in Kassala state may help and push the control program of the disease towards sunlight.

Out of the 502 serum samples, collected from both sexes of sheep, four age groups (1-12, 13-24, 25-36, 37-48 months) and two production system settled and nomad, were tested using AGID test, 319 (63.55%) of them were positive by the test, whereas 183 (36.45%) were negative. High prevalence of antibodies against SPV was in samples from Algash locality (69.57%, n=115) followed by Kassala locality (66.89%, n=151), Nahr Atbara locality (63.79%, n=116) and Setiet locality (53.33%, n=120). Results of risk factors analysis with respect to age groups and sex of sampled animals revealed no statistical significance between disease prevalence and age and sex ($\chi^2= 2.3751$, $P$-value= 0.498) and ($\chi^2= 0.2039$, $P$-value= 0.652), respectively. However, the highest level of the prevalence was observed in females (64.05%, n=253) and the age less than one year (65.63%, n=233).
In conclusion, the findings obtained imply SP is widely distributed in Kassala state localities. It can also be concluded that AGID test is a reliable test to be applied for the detection of SP antibodies under Sudan condition. However, the need for further investigations using more advanced techniques is recommended.
الخلاصة

تهدف هذه الدراسة لتحديد وضع مرض جدري الضان بولاية كسلا، السودان. اعتمدت ثلاثة وسائل لتحقيق الهدف أعلاه: الوسائل هي أجراء مسح استباقي شمل مجموعة من ملاك الضان بالولاية، الاعتماد على البيانات المتوفّرة من الخدمات البيطريّة وتحديد الأجسام المضادة ضد فيروس جدري الضان من عينات المصل المأخوذة من مختلف المحيطات بالولاية باستخدام اختبار الانتشار المناعي في هلامة الاجار (AGID).

نتائج المسح الاستباقي أوضحت أن معظم ملاك الضان على إمام بالمرض ووبائيته في الولاية. علاوة على ذلك، 80% أكدها حدوث المرض في قطعانهم. وبالتالي المرض يشكل مشكلة حقيقية لملاك الضان بالولاية. من تقارير الخدمات البيطريّة السابقة لحدث المرض بالولاية، السيطرة على المرض يمكن أن يتم بسهولة عن طريق التطعيم وهو متوفّر. بالإضافة، ألي أن البنية التحتية التي تتمتع بها الخدمات البيطريّة بولاية كسلا يمكنها أن تساعده وتدفع ببرنامج السيطرة للأمام.

502 عينة مصل. جمعت من الجنسين للضان، ومن أربعة مجموعات مختلفة الأجسام (1-12، 13-24، 25-36، 37-48 اشهر) ومن نظامين للإنتاج مستقر ومتقل. تم فحصهم باختبار AGID (n = 319) (55.36%) من عينات المصل كانت موجبة للاختبار. بينما (36.45%) سالبة للاختبار. أعلى حدوث لل الأجسام المضادة ضد فيروس جدري الضان كانت في عينات محلية القافض (69.57%, n = 115) أعمقها محلية كسهلية و (69.57%, n = 115). محلية نهر عطبرة (79.63%, n = 116) و محلية ستيت (53.33%, n = 120). نتائج تحليل عوامل الخطر بالرجع لأعمار و جنس الحيوانات المختبرة، أوضحت بأنه لا توجد علاقة بين حدوث المرض وعمر وجوهر الحيوان. ومع ذلك أعلى نسبة لحدوث المرض كانت في الاناث والأعمار الأقل من سنها.

لا تضمنت انخفاض نسبة في المحيطات ولاية كسلا. أيضاً استوجب أن نخلص أم أن اختبار AGID أدى إلى أن يكون ولد الأجسام المضادة في ظروف السودان، من ناحية أخرى، الحوجه للمزيد من التحقيق باستخدام التكنولوجيا المتقدمة مطلوب.
INTRODUCTION AND OBJECTIVES

Sheeppox (SP), a contagious disease caused by a capripoxvirus, affects sheep and, occasionally, goats. The disease characterized by generalized pox lesions throughout the skin and mucous membranes, a persistent fever, lymphadenitis, and often a focal viral pneumonia with lesions distributed uniformly throughout the lungs. However, subclinical cases may occur. In addition, all age groups can be affected; pox infections are most severe in young animals. Secondary bacterial infections complicate the disease. It is an OIE list A disease (World Animal Health, 1997).

Sheeppox is widely distributed in northern and central Africa, southwest and central Asia, and the Indian subcontinent. Sheep pox first appeared in Great Britain in 1847, in a flock of imported sheep, and the last outbreak in this country was in 1866. Most of Europe and the Americas are now free from endemic sheeppox, although the disease occurred in Greece in 2000. Transfer of sheeppox among flocks and between countries occurs from the movement of sheep as appears to happen frequently in the countries of the Middle East. The poxviruses of sheep and goat (capripoxviruses) were closely related, both antigenically and physicochemically. They were also related to the virus of lumpy skin disease.

Sheeppox and goatpox viruses are host-specific in general, but cross-infection in some regions of the world may occur. The viruses in Kenya were found to be host-specific for either sheep or goats; however, necrotic skin lesions could be produced experimentally in cattle. There was no evidence of cross-infection under natural field conditions.

In Sudan, the first scientific investigation on sheeppox was stimulated by Bennet et al. (1944), who affirmed the endemicity of the disease in the country and host specificity of the virus. Sheeppox in the Sudan was,
generally, known to be a seasonal disease associated with cold winter. However, during the last few years outbreaks of the disease were observed at different times of the year. The occurrence of sheeppox in some vaccinated animals has also been reported by some stock owners.

Sheeppox is considered one of the major constraints of sheep production due to it is impacts in the losses of reproduction functions and damage to wool, hair, and leather. In addition, high morbidity and mortality in young lambs and abortion among pregnant ewes were reported.

Vaccination of sheep using sheeppox vaccine is routinely adopted for exported flocks, and for the disease spreading control program. Epidemiological studies on sheeppox in Sudan are scarce; most of the reviewed works were on isolation and identification of the causative virus.

**Objectives**

Due to these evidences, this study was carried out with reference to the disease in Kassala State to determine the following

- The prevalence of sheeppox in Kassala State.
- The sex and age of sheep relatedness to spread of the disease were also investigated.
- To evaluate the control program involved in the state towards the disease.
CHAPTER ONE

LITRETURE REVIEW

1.1 Definition

Sheeppox was a contagious viral infection causing mortality in lambs and mastitis and abortion in ewes (Losos, 1986). It is caused by a capripoxvirus. It’s one of the most economically important and endemic disease of the sheep in northern and central Africa, Southwest and central Asia, and the Indian subcontinent (Carn, 1993; Esposito and Fenner, 2001). The disease manifests itself in pyrexia, cutaneous and lung lesion and lymphadenopathy (Munz and Dumbell, 1994; Esposito and Fenner, 2001).

1.2 Aetiology

This disease is caused by a virus belong to the family Poxviridae and genus Capripox. Poxviruses were the largest and most complex of all viruses (Fenner et al., 1999). They are deoxyribonucleic acid (DNA) viruses responsible for disease in most domesticated, wild mammals and birds. They multiply in the cytoplasm of the host cell, whereas most other animals DNA viruses multiply in the cell nucleus (Oxford concise veterinary dictionary.1st.Indian reprint, 1992).

Capripoxviruses represent one of eight genera within the Chordopoxvirus subfamily of the Poxviridae. The Capripoxvirus genus is currently comprised of sheeppox virus (SPV), goat pox virus (GPV), and lumpy skin disease virus (LSDV), causing disease in sheep, goats and cattle respectively (Carn, 1993; Esposito and Fenner, 2001). There was only one serotype of SPV. Various strains of SPV and GPV cause disease only in sheep, others only in goats, and some in both sheep and goats (Kitching and Taylor, 1985a). These viruses were morphologically and serologically indistinguishable but have, different host species adaptation and cross protection had been observed (Kitching, 2003).

1.3 Morphology
Because they were the largest of all viruses and could be easily visualized with an electron microscope, the poxviruses were the first viruses to be intensively studied in the laboratory (Fenner, 2000). The poxviruses were identified by their brick-like or ovoid shape and their ability to induce skin eruption. They consist of a single linear double-stranded DNA molecule; the two polynucleotide strands are joined by short hairpin loops, and substantial numbers of genes per genome were detected (Austin and Robert, 2005). Virions have a complex construction and consist of an envelope and mature naturally by budding through the membrane of the host cell, a surface membrane, a core, and lateral bodies. Virion surface is characterized by tubular units. The core is biconcave, with two lateral bodies, and nested between the core membranes (Büchen-Osmond, 2003). Sheep pox virus, morphologically resemble the other pox viruses but, smaller and more elongated (Losos, 1986).

1.3.1 Sheep pox virus genome

Comparative genomic data indicate the close genetic relationship among capripoxviruses, and they suggest that SPV and GPV are distinct and likely derived from lumpy skin disease virus-like ancestor (Tulman et al., 2002). SPV and GPV genomes are approximately 150 kbp and are strikingly similar to each other, exhibiting 96% nucleotide identity over their entire length. Wild-type genomes share at least 147 putative genes, including conserved poxvirus replicative and structural genes and genes involved in virulence and host range (Tulman et al., 2002).

1.3.2 Resistance of SPV to physical and chemical agents

Poxviruses were sensitive to lipid solvents and acid, and they survive in the adverse environmental condition for long period of time (Losos, 1986). Sheep pox virus had little resistance to sun radiation and putrefactions but, can survive in dark cool rooms up to 2 years and in the fleece of sheep for 2 months (Seifert, 1996).

1.4 Cultivation of SPV

The poxviruses were adapted to developing eggs embryo with some difficulties, to growth on chorioallantonic membrane (CAM) (Sabban, 1957). The
sheep pox virus can not be cultivated on the CAM or on the fetal lung cells (Sheikh Ali, 1997). Nonetheless, two paradoxical reports of SP successfully infecting the CAM of embryonated hen’s egg were given by Sabban (1957), and Baharsefat and Yamini (1967).

Sheep pox and goat pox viruses grow in many ovine, caprine and bovine tissue cultures. Thus propagation of SPV and GPV in kid kidney, kid testis and sheep kidney were successfully carried out by Ramyer (1966). Beside the kidney and testicle, SPV was also adapted to embryo muscles cell culture (Vigario and Ferrez, 1967). The optimum pH and incubation temperature were 8.2 and 36 to 37°C, respectively (Soman and Singh, 1981).

Using sheep thyroid cell culture, SPV was isolated from field samples in the first passage, at the same time this system was found useful for the titration of the virus (Nitzschke et al., 1967). Calf kidney cell culture was used to attenuate SPV in the process of vaccine production (Chifney et al., 1973). Fetal bovine lung fibroblast cell culture was used to produce sheeppox vaccine in Somalia (Tozzini et al., 1987).

1.4.1 Cytopathic effect of SPV in culture

Sheep pox virus produced cytopathic effect (CPE) in all susceptible cell culture systems, which mainly characterized by cytoplasmic granulation and formation of intracytoplasmic inclusions, vaculation and necrosis of the nucleus and cellular degeneration (Cilli and Baldelli, 1958). The variant form of sheep pox CPE in lamb testicle cell cultures is the cytoplasmic bridges connecting separated clusters of the cells (Srivastava and Singh, 1980a).

The type of CPE produced by SPV has correlated to the passage level of the virus. The low passaged virus CPE was characterized by rounded referactile cells in isolated foci which could be seen after 72 hours, whereas both rounded and spindle-shape cells with a tendency to a diffused CPE was produced by the mid-passaged virus. In the high passaged virus both types of cells and generalized CPE occurred in 48 hours, and sloughing of the monolayer ended earlier (Soman and Singh, 1980).
1.5 Host range

Information about host range of SP is conflicting by many researchers, as mentioned by Bennet et al. (1944) that SP did not infect goats while GPV caused a disease in sheep which was more acute than that caused by SPV in sheep. Hajer et al. (1988), reported non-host-specific by which SP and GP isolates cross infected both species and showed similar pathogenicity.

On the other hands Murty and Singh (1971); Munz and Dumbell (1994); Rao and Bandyopadhyay (2000) reported that SPV generally considered being host specific as the disease outbreaks or virus isolates noted to cause disease in sheep only. Investigation in Sudan affirmed the host specificity of the SPV, which could neither naturally nor experimentally infect goats; but was lethal to sheep. Sheeppox virus displayed different characteristic and thus, heterogeneity within the virus in Sudan was recognized (Sheikh-Ali et al., 2004).

Kitching and Taylor (1985a) described variation in the host between different strains of SP and GP. In goats, Nigerian SPV produces a transient fever and no more than a local reaction at the site of inoculation although it was particularly virulent to sheep. Indian GPV which highly virulent for goats was mild in sheep. Yemen GPV was equally lethal to both sheep and goat, whereas Sudan isolate was more lethal for goats what ever it derived from goats or sheep.

As stated by Davies (1976), who reported that SPV and GPV isolated in Kenya are not host specific as in the Middle East and India. Moreover, the virus strains from one species are of a similar pathogenicity for the other, and the same virus appears to occur in the field outbreaks in mixed flocks. In addition, a silent infection with no skin lesions occurred in the field outbreaks.

1.6 Transmission

Sheeppox virus transmitted by direct contact with infected animals or indirect by contaminated objects. Moreover, Inhalation of aerosols from acutely affected animals, aerosols generated from dust contaminated from pox scabs in
barns and night holding areas, and contact through skin abrasions either by fomites or by direct contact are the natural means of transmitting SPV. Spread of the virus via air can occur (Kitching and Taylor, 1985b). Biting insects like Stomoxys may also involved in mechanical transmission of capripoxviruses (Kitching and Moller, 1986).

In endemic areas, capripoxviruses are probably maintained through short-cycle transmission and their ability to resist desiccation and other reverse environmental factors exist. These viruses can survive in scab materials for period of 3 to 6 months (Singh et al., 1979). Thus, recovered animals were a source of infection to susceptible population with which they come into contact during seasonal grazing and trade movement (Davies, 1981). The virus can cause infection experimentally by scarification, intravenous, intradermal, intranasal, or subcutaneous inoculation (Katiyar, 1961).

1.7 Clinical signs

According to the virus strain and host species involved; the clinical syndromes exhibited by SP vary in different geographical regions. They were characterized by the formation of pustules on the skin; signs of systemic infection may also present. The incubation period of SP in sheep is 4 to 7 days (Losos, 1986). Although all age groups can be affected while it is more sever in young animals and the mortality of the animals under four months of age approximately 100% (Losos, 1986).

The acute form of SP characterized by severe systemic response with widespread vesicular lesions affecting the head, nostril, lips, buccal surfaces and wool-free skin. The vesicular stages followed by the development of pustules which eventually become covered by scab (Losos, 1986). Mortality was high when the disease was systemic and lesion develop in respiratory and alimentary canals (Davies, 1981), and death occurred within 3 to 5 days.

The classical form of SP developed skin nodules which do not change into vesicles and pustules, this nodular form of SP prevalent in Sudan (Losos, 1986; Sheikh-Ali et al., 2004). Within 8 to 12 days the nodules become necrotic
and sloughed off, leaving shallow clearly demarcated ulcer. Completed healing took place in 5 to 7 weeks and terminated in recovery or death (Katiyar, 1961). Rare subcutaneous form of SP was also diagnosed in Sudan (Sheikh-Ali et al., 2004).

1.8 Pathology

A number of researchers (Vegad and Sharma, 1973; Davies, 1976) described a typical SP infection in which the vesicular and pustular stages of lesion development were absent, and necrotic nodules most commonly observed. Pustules formation involved accumulation of purulent material between the necrosed crust of epidermis and the underlying granulation tissue (Vegad and Sharma, 1973). Micro vesicles formation in the epidermis was reported by Murray et al. (1973). The main histological changes seen in the infected dermis are edema and production of large number of characteristic sheeppox cells showing intranuclear and intracytoplasmic inclusion bodies (Murray et al., 1973).

The increased thickness of the epidermis was due to acanthosis, hyperkeratosis and hydropic degeneration of prickle cells. Because the hydropic degeneration did not result in the rupture of the cells, there was no vesicles formation (Murray et al., 1973).

Systemic SP shows lesions in the respiratory and gastrointestinal tracts. Lungs may show depressed gray areas on their surface (small pale nodules) and secondary bacterial bronchopneumonia was often a complication (Losos, 1986). Desquamation of the nodules led to formation of ulcers in the mucosa of duodenum and large intestine (Katiyar, 1961).

1.9 Epizootiology

Sheeppox and goatpox were the two of most important viral infection of domestic animals due to their impacts in causing considerable economic losses, not only because of the high morbidity and mortality rates but also because of their reduction of reproduction and the effect on the wool and leather (Losos, 1986). Pox diseases are relatively rare among domestic animals in Europe and North America.
They were endemic in Africa, Near, Middle and Far East and were reported in many countries belonging to the above mentioned regions (Losos, 1986).

In the enzootic areas, the morbidity rate was 5 to 10% with a very low mortality. However in regions free of antibodies to SPV the morbidity rate may reach 60% especially in young lambs and the rest of animals develop inapparent infections (Losos, 1986).

An outbreak of SP in Nigeria resulted in 100% morbidity and 30% mortality (Aasagba and Nawathe, 1980). In Jordan with morbidity and mortality rates 100% and 70% respectively (Hailat et al., 1994). In Indian with average morbidity and mortality rates of 63.5% and 49.5%, respectively. Due to this report modeling studies suggested it would take about 6 years for a flock or herd to recover from an outbreak, with average annual losses in income of 30-43%, depending on flock type and the owner's actions (Garner et al., 2000). The nodular form of sheeppox has caused serious problems in Sudan; where a mortality rate of 6% had been observed, some outbreaks of SP coincided with outbreaks of bovine lumpy skin disease (Muzichin and Ali, 1979).

There was no statistically significant (P > 0.05) association of SP with age and sex of the sheep. Whereas, the occurrence and spread of the diseases were associated with poor management, climatic factors, feed scarcity and inadequate veterinary services (Woldemeskel and Ashenafi, 2003).

Sheeppox was known to be associated with the cold winter season in some reports (Muzichin and Ali, 1979). Other reports, the outbreaks of SP occurred at any time of the year, so the disease occurrence has no relationship with certain season, and this refer to the change in the rearing system (Sheikh-Ali et al., 2004).

As stated by Losos (1986), the virus was hardly and resistant to desiccation and particles surviving in the scabs which had fallen during the previous outbreaks cause a new epidemic to occur whenever fresh animals were introduced to the farm, also the high density of the animals in the farm act as a predisposing factor for the disease occurrence.
1.10 Zoonosis

In Sweden and India mild lesion of small red papules followed by vesicles on the hands and arms have been reported in human working with capripoxvirus, no generalization occurred. These were the only two human cases reported so far (Sawhney et al., 1972).

1.11 Immunity and Antigenic relationship

The immunity induced by infection with SPV results in solid and enduring immunity, and appears to depend on both arms of immune system, humoral and cellular components (Merck’s and Company, 1998).

This observation was made by passively transferring antiserum against extracellular and intracellular SPV to sheep, and on challenge the incubation period was prolonged. Additionally, lambs hyper immunized with SPV developed delayed-type hypersensitivity (DTH) reactions, which were very important in protection (Srivastava and Singh, 1980b). Kalra and Sharma (1984) confirmed that cellular immunity played a major role in the defense mechanism against SP. On the other hand, the role of the humoral immunity was emphasized by Kitching (1986) who stated that both sheep immunized with immune sera and lambs born to recovered ewes resisted challenge.

Animals that have recovered from capripoxvirus infection do not remain carriers of the virus. Maternal immunity provided protection from SP and GP for up to three months (Kitching, 1986).

Generally, poxviruses have strong immunogenic properties. Poxviruses modulate the immune response in infected hosts by inhibiting the synthesis and release of IL-1 from infected cells; encoding soluble cytokine receptors for tumor TNF-α, TNF-β, IL-1, and importantly, IFN-γ; synthesizing virus-encoded cytokines like epidermal growth factor and transforming growth factor, which antagonize the effects of host cytokines mediating the antiviral process (Pickup, 1994; Haig,
In addition, inducing apoptosis in a significant number of antigen-presenting cells (Kruse and Weber, 2001), as well as inducing IL-10 release that has the capacity to impair the initiation of an acquired immune response (Haig, 1998; Lateef et al., 2003). Sheeppox virus showed evidence for an immune escape mechanism that alleviates the host's immune response to viral proteins and therefore generates the possibility of replicating in the host in spite of vaccination. Such suggested enhanced the postulate of replication strategy appears to be essential for the continued existence of SPV. However, the inactivated poxviruses possessed immunostimulating capacity and were used as a prophylactic or metaphylactic application that efficiently reduced susceptibility to infectious diseases in different species (Abdel-Aziz and Ahmed, 2005).

Antigenic relationship between SPV and GPV looked to be very close related, but the relationship of this virus to other pox viruses has not been well defined because the serological methods used in such determination vary in their specificity (Losos, 1986). SP and GP viruses share common precipitating antigen detectable by agar gel immuno-diffusion test. Sheeppox virus protects goat against GP infection, GPV also protects sheep against infection with SPV (Sharma and Dhanda, 1971a).

According to Hosamani et al. (2004), P32, one of the major immunogenic genes of capripoxvirus, was isolated and sequenced from two Indian isolates of GPV and a vaccine strain of SPV. The sequences were compared with other P32 sequences of capripox viruses available in the database. Sequence analysis revealed that SPV and GPV share 97.5% and 94.7% homology at nucleotide and amino acid level, respectively. A major difference between them was the presence of an additional aspartic acid at 55th position of P32 of SPV that was absent in both GPV and LSDV.

Phylogenetic analysis showed that members of the capripoxvirus could be delineated into three distinct clusters of GPV, SPV and LSDV based on the P32 genomic sequence. Using this information, a PCR-RFLP method has been developed for unequivocal genomic differentiation of SPV and GPV (Hosamani et al., 2004).
Sharma and Dhanda (1971b) reported some cross reaction in precipitation between SP and contagious pustular dermatitis (Orf), and GP and Orf. However, Rao and Malik (1979) have reported that GP was nearer to Orf than to SP, by neutralizing titer obtained in the reaction of contagious pustular dermatitis virus with GP hyperimmune serum. Elaborated study by Sharma et al. (1988) observed cross reaction between SPV, GPV and Orf viruses.

1.12 Diagnosis

It was not difficult to diagnose clinically SP and GP when generalized infection occurred during epizootics. In enzootic areas, these diseases clinically resemble Orf, as they may be mild and produce lesion confined to the mouth and udder, however, the three diseases can resemble each other and can be mistaken (Merck’s and Company, 1998).

Demonstration of the virus particles under the electron microscope (EM) was a rapid and reliable method of diagnosis for SP (Muzichin and Ali, 1979). The laboratory methods required for the definitive diagnosis of the SP can be carried out through different methods. Isolation of the virus and inoculating culture with this isolate to observed CPEs in the culture, detection of the viral antigens in the skin lesions, and demonstration of serum antibodies (Losos, 1986).

Many serological tests were conducted to detect the antibodies against SPV in the serum of the infected sheep. Serum neutralization test (SNT) has been most specific but less sensitive test (Kitching and Carn, 1996), agar gel immuno-diffusion (AGID) (Kitching et al., 1986), counter immuno electrophoresis (Sharma et al., 1988), enzyme linked immuno-sorbet assay (ELISA) (Tiwari et al., 1996), and reverse passive haemagglutination test (PHA) for detecting sheeppox antigen (Tiwari et al., 1995) were performed. Agar gel immuno-diffusion test and passive haemagglutination test were the best techniques to be used for detecting sheeppox antibodies under Sudan conditions (Ali et al., 2004).

Although sensitive, ELISA and virus isolation in cell culture failed to detect virus particles that were bounded to neutralizing antibody (Ireland and
Binepal, 1998), and the sensitivity of a precipitation or an agglutination test was usually low. Hence, the highly sensitive molecular biology techniques based on polymerase chain reaction (PCR) was routinely employed for the identification of capripoxviruses in skin biopsies and cell cultures (Ireland and Binepal, 1998; Mangana-Vougiouka et al., 1999). The PCR described combines high specificity and sensitivity with speed. PCR was, therefore, shown to be the method of choice for SPV diagnosis directly from clinical specimens (Mangana-Vougiouka et al., 2000).

1.12.1 Differential diagnosis

Generally, the lesions of SP were characteristic to establish sufficient diagnosis, however, if violent forms of Orf and SP occurred at same time, the two diseases may be confused with each other and they may be mistaken (Merck's and Company, 1998).

1.13 Prevention and Control

The most likely manner for SP to enter a new area is by introduction of infected animals. Thus restrictions on the movement of animals and animal products (meat, hair, wool, and hides) are essential to prevent introduction of SP. Wool, hair, and hides must be subjected to suitable decontamination procedures before entry into nonendemic areas (Radostits et al., 2000).

Capripox free countries maintain their disease free status by the restriction of imports of livestock and animal products from affected areas. In the case of countries remote from enzootic areas the swift implementation of a radical slaughter policy and severe movement restrictions, coupled with a ring vaccination of a radius 25-50 km, should result in elimination of the disease (Carn, 1993).

If a new case 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. Vaccination of susceptible animals on premises surrounding the infected flock should be considered (Merck's and Company, 1998).
When the disease transmitted over a large area, the most effective means of controlling losses from SP is vaccination; however, consideration should be given to eliminating infected and exposed flocks by slaughter; properly disposing of the animals and contaminated material; and cleaning and disinfecting contaminated premises, equipment, and facilities. All diseased animals should be given antibiotic coverage to restrict secondary bacterial infections (Nandi et al., 1999).

### 1.14 Vaccination

Epidemiologically the diseases of SP, GP and LSD differ, but all three viruses may be mechanically transmitted by biting insects, and control without vaccination is extremely difficult in endemic areas (Carn, 1993). Two kinds of vaccines were currently used, attenuated live vaccine and formalin-inactivated vaccine adsorbent to aluminum hydroxide gel, to provide protection to sheep and goat against capripox virus (Losos, 1986).

Killed vaccines have not proven to be practical under field conditions because they do not provide solid lasting immunity and give only short-term immunity (Pal and Soman, 1992). Binary ethyleneimine (BEI) was used to inactivate the local Egyptian strain of sheep pox virus; the inactivated virus was adsorbed on aluminium hydroxide gel. This vaccine proved to be safe, sterile and inducing protection for the vaccinated lambs when challenged by the virulent SPV up to 6 months post vaccination (Awad et al., 2003).

The widespread use of live vaccines had the disadvantage of causing a high incidence of generalized reactions (Khan, 1961). In addition, live virus vaccines constituted a dangerous source of infection for unvaccinated animals (Rafyi and Mir Chamsy, 1956). In spite of that, live attenuated vaccine conferred longer lasting immunity dependable on cell culture system used for passaging the viruses which had effect on the protective value of the vaccine (Martin et al., 1973). Moreover, Soman and Singh. (1980), observed that the virus grow in lamb testicle which produced only intranuclear inclusion bodies was poorly protective compared to that grown in lamb kidney system which produced intracytoplasmic inclusions.
Researches on subunit vaccine were also conducted. Carn et al. (1994) have cloned a selected surface protein of capripox virus to *E. coli* and the complex was found reasonably protective. Subunit vaccine has advantage over live vaccine in that they were non-infectious and safe to produce. Moreover, a single vaccine prepared from a strain of capripox virus that infects sheep and goats equally was effective in controlling against both GP and SP for at least 12 months (Kitching et al., 1987; Carn, 1993). Second generation vaccines appeared with genetic engineering: recombinant vaccines, vector vaccines, nucleic acids vaccines, and markers vaccines, among others. These novel technologies can permit the development of new vaccines and improve the quality of the vaccines already existing (Bazin, 2003).

Animals recovered from infection with one strain of capripoxvirus were resistant to infection with any other strain. Consequently, it is possible to use a single strain of the virus, regardless of their origin from Asia or Africa (OIE manual 1996). Live, attenuated, LSDV also can be used as a vaccine against SP and GP (Merck's and Company, 1998). Because of the antigenic homology among all strains of capripoxviruses, there is a potential to use a single vaccine strain to protect cattle, sheep and goats (Kitching, 2003).

Single, safe and stable vaccine against capripox infection which provides substantial protection in sheep and goats was developed from the 0240 Kenyan strain of SP by Kitching et al. (1987). The same vaccine extensively used as lyophilized vaccine in the Sudan, and the duration of the immunity is one year.
CHAPTER TWO
MATERIALS AND METHODS

2.1 Study area

The study was conducted in Kassala State which located in the eastern region of the Sudan between the longitudes 40.34 -37 (east) and the latitude 45.14 -17 (north). The state covers an estimated area of 4282 Km² (Annual report of General directorate of animal resources. Kassala State, 2005). The state is divided into five localities, Kassala, Algash, Nahr Atbara, Setiet, and Hamshkoreib. Kassala state borders Algedaref state to the south, Khartoum state to the west, River Nile state to the northwest, Red sea state to the north and Eritrea country to the east. Also bordering the state are the Taka and Red sea mountains to the east and northeast respectively (Figure1).

The dominant climate is desert and semi desert in the northern parts, and poor savannah in the southern parts of the state. The minimum and maximum annual temperatures are 33°C and 47°C respectively. The annual rainfall ranges from 100 to 150 mm (Annual report of General directorate of animal resources. Kassala state, 2005).

The human population in Kassala state is estimated about one million and half. The population comprises of complex ethnic groups, mainly Hadandawa, Bani Amer, Amarar, Bisharein, Rashida and the Halanga tribes which co-exist with the northern tribes and Fellata tribe originating from West Africa. Over the last thirty years Kassala state has acted as a catchment area for cross border migrants from Eritrea and Ethiopia for the internally displaced, refugees and seasonal migrants. The major activities of the people in the state depend mainly on the trade, livestock ownership and agriculture.

2.1.1 Animal population

Kassala state is well considered as a rich state of animal resources and the different components of the livestock distributed all over the state according to the climate and the common tribes in the area. Since there was
no national or regional livestock census recently done, the general directorate of the animal resources in Kassala state estimated the animal population of about 3,585,309 animal head with following details.

1,383,840 head of sheep
1,068,258 head of goats
567,437 head of cattle
565,774 head of camels
About 300 tons of fish annually.

Nomadism is a natural phenomenon for the animal owners, they adopt a cyclic seasonal movement of their herds depending on the rainfalls to face their animal’s demands mainly the water and pasture. The regular pattern of the animal movement is usually between the north and south, but sometimes it may extend to the grazing land in the neighbouring states or to the border of the state with Eritrea mainly in the dry season.

The geographical distribution of the sheep population in the different localities of Kassala was estimated in 2003 by the General directorate of the animal resources as follows.

400,585 head in Nahr Atbara locality.
360,527 head in Setiet locality.
267,057 head in Algash locality.
200,293 head in Kassala locality.
155,378 head in Hamshkoreib locality.
Figure 1: Study area
2.2 Study design

To achieve the objectives of this study, the direction has been oriented towards three disciplines to come up with a conclusion on the epidemiological situation of the SP in the area of the study. The three methods which used were the data collection based on veterinary service reports, questionnaire and sero-surveillance using Agar gel immuno-diffusion test.

2.2.1 Questionnaire survey

Data on pastoralist's knowledge related to SP symptoms, impacts on their herds, their attitude to vaccination and the effect of animal's movement on the spread of the disease were obtained by means of a questionnaire distributed among owners of the sheep during each visit (Appendix 1).

The questionnaire survey was done in the four localities of the state (Kassala, Algash, Setite, and Nahr Atbara locality) based on the willingness of sheep herd owners to respond. The questionnaire was distributed to 50 pastoralists among different localities to come up with information related to the points mentioned above and to cover the pastoralist understanding of the disease in the state.

2.2.2 Veterinary service reports

Depending on the monthly and annual available reports of the General directorate of animal resources of Kassala state, all the data which were necessary for this study had been collected. The data which collected explained the situation of the SP in the area of the study, and the ability of the General directorate of animal resources for controlling the disease. Detailed information was collected on the personnel, infrastructures, previous outbreaks of the disease and its control by means of the vaccination.

2.2.3 Study population and sampling method

The study animals that were sampled are traditionally managed sheep regardless of its health status from different herds with different sites of
the state. Data on age, sex, and location of sampled sheep were recorded to study the association of the sex, age, and location with the prevalence of the disease (Appendix 2).

A total number of 502 serum samples were randomly collected from herds in four localities of the Kassala state, the fifth locality (Hamshkoreib) was out of reach due to the unrest constrains in the state. The number of herds tested was 25 in 18 different sites represent the most popular place for sheep to drinking water within the localities, which comprising of 4500 head of sheep. All the sera collected from different areas with no history of vaccination against SP were recorded for the three years that preceded the study. The aim of this step was to determine the presence of SP antibodies and the prevalence of the disease in the state.

2.2.3.1 Sample collection

The puncture area of the jugular vein was cleaned by 70% ethanol. A plain glass vacutainer, with a tube-holder and two way needle was used. Then 5 ml of blood was withdrawn. The vacutainer tubes were labelled indicating location, age and sex of the animal, put on a rack away from direct sun light for at least one hour to allow blood to clot and then placed in the refrigerator of the car and transferred to the laboratory. The vacutainer were kept overnight in the refrigerator (4°C), then centrifuged for 5 minutes at 1500 r.p.m. Each serum samples were collected using sterile Pasteur pipettes in eppendorf tubes, labelled indicating location, date, age and sex of the animal then stored at -20°C until used.

2.2.3.2 Laboratory test

The serological test which conducted in the study was AGID diffusion test to test all the serum samples for the detection of antibodies against sheep pox virus.
2.2.3.3 Hyper immune serum (HIS)

Sheeppox hyper immune serum against strain 0240 of the virus was kindly provided by Dr. Tag Eldin Abdalla of the Central Veterinary Research Laboratory –Khartoum-Sudan. The hyper immune serum was used as a positive control.

2.2.3.4 Preparation of the antigen

Sheeppox virus vaccine strain 0240 was used as antigen (1 vial of 100 doses). One vial was diluted in 1 ml normal saline (NS) and kept at -20°C till used. 2% of sodium deoxycholate (SDC) freshly prepared was mixed with equal volume of diluted antigen prior to the test to enhance antigen / antibody reaction.

2.2.3.5 Preparation of the agar

One gm of purified agar powder, 8 gm of sodium chloride were added to 100 ml of distilled deionised water (DDW), boiled for 20 – 30 minutes till the powder dissolved, 0.5 gram phenol was added. Prior to the test, 15 –17 ml from the dissolved agar was poured into each Petri dish, left to solidify and kept at 4°C overnight . In a 5 mm thick agar gel, 6 peripheral and one central well of 4 mm well diameter, and 2 mm apart were cut in the Petri dish. Every well was then labelled.

2.2.3.6 Procedure of the test

15 microliter of the reference antigen was put in the central well of each group of wells. The hyper immune serum in a determined well for each group of wells as positive control was also added, and the tested sera were put in the remaining wells. The dishes then were incubated in a humid incubator at room temperature for 24 – 48 hours before reading the result using illuminated chamber.

2.2.3.7 Data management and analysis

Microsoft Excel (Windows 2000) and Stata 6.0 for Windows 98/95 /NT were used for data analysis. In order, to find out the relationship between some factors and the occurrence of sheeppox, chi-square test was employed.
CHAPTER THREE
RESULTS

3.1 Questionnaire survey outcomes

The results showed that the production system of sheep owners were nomads (52%, n=26) and settled (48%, n=24). Nineteen owners (38%, n=19) selected SP as the most important disease in their areas, were (48%, n=24) selected another diseases, and (14%, n=7) have no ideas about diseases. Forty (80%) and 10 (20%) owners confirmed presence of SP in flocks and absence of it, respectively. Forty five (90%) know the signs of SP and described it as fever, skin lesion, anorexia, emaciation, respiratory disturbance, abortion, and death. However, five (10%) didn’t know the signs. Most of the owners, 17 (34%), selected both ages adult and young are susceptible to the disease, whereas 14 (28%), 9 (18%), and 10 (20%) selected adult, young, and have no idea, respectively. Forty (80%) recorded the morbidity is higher than mortality; while no one (0%) confirmed high mortality, and 10 (20%) have no ideas. Twenty two (44%), 18 (36%), and 10 (20%) owners confirmed, not confirmed, and have no answer about abortion in ewes, respectively. Twenty eight (56%) owners explained economic impacts of SP due to loss of production, 2 (4%) due to death, where as 10 (20%) for both reasons, and 10 (20%) have no comments. Seven (14%) owners vaccinated against sheeppox while 43 (86%), had not vaccinated their herds against SP. The responses to the questionnaire survey among the sheep herd’s owners are summarized in Table (1).

3.2 Veterinary service reports outcomes
3.2.1 Veterinary service structure

The General directorate of animal resources works under the umbrella of State ministry of agriculture and animal resources and employed as the director general of the ministry, which consist of many departments including animal health, extension and training, animal production, and directorate of animal resources in the localities.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Kassala</th>
<th>Algash</th>
<th>Setiet</th>
<th>Nahr Atbara</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal owners respond</td>
<td>21 (42.00)</td>
<td>11 (22.00)</td>
<td>9 (18.00)</td>
<td>9 (18.00)</td>
<td>50(100.00)</td>
</tr>
<tr>
<td>Herd composition</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. sheep</td>
<td>16 (76.19)</td>
<td>6 (54.55)</td>
<td>7 (77.78)</td>
<td>5 (55.56)</td>
<td>34 (68.00)</td>
</tr>
<tr>
<td>b. others</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>c. mixed</td>
<td>5 (23.81)</td>
<td>5 (45.45)</td>
<td>2 (22.22)</td>
<td>4 (44.44)</td>
<td>16 (32.00)</td>
</tr>
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<td>Production system</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>a. nomads</td>
<td>13 (61.90)</td>
<td>2 (18.18)</td>
<td>5 (55.56)</td>
<td>6 (66.67)</td>
<td>26 (52.00)</td>
</tr>
<tr>
<td>b. settled</td>
<td>8 (38.10)</td>
<td>9 (81.82)</td>
<td>4 (44.44)</td>
<td>3 (33.33)</td>
<td>24 (48.00)</td>
</tr>
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<td>Migratory route</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. east, middle, west</td>
<td>9 (42.85)</td>
<td>0.00</td>
<td>5 (55.56)</td>
<td>6 (66.67)</td>
<td>20 (40.00)</td>
</tr>
<tr>
<td>b. north, middle, south</td>
<td>4 (19.05)</td>
<td>2 (18.18)</td>
<td>0.00</td>
<td>0.00</td>
<td>6 (12.00)</td>
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<td>Sheep herd mixed with others</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>a. yes</td>
<td>11 (52.38)</td>
<td>11 (100.00)</td>
<td>8 (88.89)</td>
<td>7 (77.78)</td>
<td>37 (74.00)</td>
</tr>
<tr>
<td>b. no</td>
<td>10 (47.62)</td>
<td>0.00</td>
<td>1 (11.11)</td>
<td>2 (22.22)</td>
<td>13 (26.00)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. sheeppox</td>
<td>7 (33.33)</td>
<td>5 (45.45)</td>
<td>5 (55.56)</td>
<td>2 (22.22)</td>
<td>19 (38.00)</td>
</tr>
<tr>
<td>b. other diseases</td>
<td>9 (42.86)</td>
<td>5 (45.45)</td>
<td>4 (44.44)</td>
<td>6 (66.67)</td>
<td>24 (48.00)</td>
</tr>
<tr>
<td>c. no idea</td>
<td>5 (23.81)</td>
<td>1 (09.09)</td>
<td>0.00</td>
<td>1 (11.11)</td>
<td>7 (14.00)</td>
</tr>
<tr>
<td>Presence of sheeppox in herd</td>
<td></td>
<td></td>
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<td>a. yes</td>
<td>15 (71.43)</td>
<td>10 (90.91)</td>
<td>6 (66.67)</td>
<td>9 (100.00)</td>
<td>40 (80.00)</td>
</tr>
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<td>6 (28.57)</td>
<td>1 (09.09)</td>
<td>3 (33.33)</td>
<td>0.00</td>
<td>10 (20.00)</td>
</tr>
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<td>Knowledge of sheeppox signs</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>a. yes</td>
<td>18 (85.71)</td>
<td>10 (90.91)</td>
<td>8 (88.89)</td>
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</tr>
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<td>b. no</td>
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<td>1 (09.09)</td>
<td>1 (11.11)</td>
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<td>Most age effected</td>
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</tr>
<tr>
<td>a. adult</td>
<td>4 (19.05)</td>
<td>4 (36.36)</td>
<td>3 (33.33)</td>
<td>3 (33.33)</td>
<td>14 (28.00)</td>
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<tr>
<td>b. young</td>
<td>5 (23.81)</td>
<td>2 (18.18)</td>
<td>1 (11.11)</td>
<td>1 (11.11)</td>
<td>9 (18.00)</td>
</tr>
<tr>
<td>c. both</td>
<td>6 (28.57)</td>
<td>4 (36.36)</td>
<td>2 (22.22)</td>
<td>5 (55.56)</td>
<td>17 (34.00)</td>
</tr>
<tr>
<td>d. no idea</td>
<td>6 (28.57)</td>
<td>1 (09.09)</td>
<td>3 (33.33)</td>
<td>0.00</td>
<td>10 (20.00)</td>
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<tr>
<td>Morbidity and mortality</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>a. morbidity is high</td>
<td>15 (71.43)</td>
<td>10 (90.91)</td>
<td>6 (66.67)</td>
<td>9 (100.00)</td>
<td>40 (80.00)</td>
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<td>Presence of abortion</td>
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<td>6 (28.57)</td>
<td>5 (45.45)</td>
<td>3 (33.33)</td>
<td>8 (88.89)</td>
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</tr>
<tr>
<td>b. no</td>
<td>9 (42.86)</td>
<td>5 (45.45)</td>
<td>3 (33.33)</td>
<td>1 (11.11)</td>
<td>18 (36.00)</td>
</tr>
<tr>
<td>c. no answer</td>
<td>6 (28.57)</td>
<td>1 (09.09)</td>
<td>3 (33.33)</td>
<td>0.00</td>
<td>10 (20.00)</td>
</tr>
<tr>
<td>Economic impact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. death</td>
<td>2 (09.52)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>2 (04.00)</td>
</tr>
<tr>
<td>b. loss of production</td>
<td>9 (42.86)</td>
<td>7 (63.64)</td>
<td>5 (55.56)</td>
<td>7 (77.78)</td>
<td>28 (56.00)</td>
</tr>
<tr>
<td>c. both</td>
<td>4 (19.05)</td>
<td>3 (27.27)</td>
<td>1 (11.11)</td>
<td>2 (22.22)</td>
<td>10 (20.00)</td>
</tr>
<tr>
<td>d. no comment</td>
<td>6 (28.57)</td>
<td>1 (09.09)</td>
<td>3 (33.33)</td>
<td>0.00</td>
<td>10 (20.00)</td>
</tr>
<tr>
<td>Vaccination against sheeppox</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. yes</td>
<td>6 (28.57)</td>
<td>0.00</td>
<td>1 (11.11)</td>
<td>0.00</td>
<td>7 (14.00)</td>
</tr>
<tr>
<td>b. no</td>
<td>15 (71.43)</td>
<td>11 (100.00)</td>
<td>8 (88.89)</td>
<td>9 (100.00)</td>
<td>43 (86.00)</td>
</tr>
</tbody>
</table>

n = number of owners  (% = percentage of owner
3.2.2 Manpower

Animal health, extension and training, and animal production are responsibility of the personnel who work in the general directorate of animal resources. Table (2) shows the total personnel of general directorate working all over Kassala state.

**Table 2: Manpower engaged in animal health services in Kassala state**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Vets</th>
<th>Tech</th>
<th>Assistance</th>
<th>CAHWS</th>
<th>Support staff</th>
<th>Drives</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>State Head Quarter</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>_</td>
<td>13</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Kassala</td>
<td>11</td>
<td>2</td>
<td>18</td>
<td>58</td>
<td>1</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>Algash</td>
<td>1</td>
<td>_</td>
<td>9</td>
<td>34</td>
<td>1</td>
<td>_</td>
<td>45</td>
</tr>
<tr>
<td>Setiet</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>38</td>
<td>_</td>
<td>_</td>
<td>45</td>
</tr>
<tr>
<td>Nahr Atbara</td>
<td>3</td>
<td>_</td>
<td>_</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>24</td>
<td>6</td>
<td>33</td>
<td>146</td>
<td>17</td>
<td>8</td>
<td>226</td>
</tr>
</tbody>
</table>

Vets = Veterinarians, Tec = Technicians, CAHWS = Community Animal Health Workers

3.2.3 Transportation

The transportation means which are especially important for vaccination regimes in the veterinary services of the state are described in Table (3). Where as most of the vehicles need maintenance.

**Table 3: Vehicles involved in animal health activities**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Lorries</th>
<th>Cars</th>
<th>Mobile units</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>State Head Quarter</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Kassala</td>
<td>_</td>
<td>1</td>
<td>_</td>
<td>1</td>
</tr>
<tr>
<td>Algash</td>
<td>_</td>
<td>1</td>
<td>_</td>
<td>1</td>
</tr>
<tr>
<td>Setiet</td>
<td>_</td>
<td>1</td>
<td>_</td>
<td>1</td>
</tr>
<tr>
<td>Nahr Atbara</td>
<td>_</td>
<td>1</td>
<td>_</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>
3.2.4 Previously reported sheeppox outbreaks

Most of the data about SP outbreaks in the state were obtained from the records of Federal general directorate of animal health and epizootic disease control. Table (4) represent SP outbreaks during the last five years (2000 - 2004). Figure (2) shows the nodular form of SP observed in the state during serum collection.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of reports</th>
<th>No. of AAR</th>
<th>Morbidity</th>
<th>Mortality</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>2</td>
<td>unknown</td>
<td>49</td>
<td>zero</td>
<td>treatment</td>
</tr>
<tr>
<td>2001</td>
<td>3</td>
<td>unknown</td>
<td>24</td>
<td>3</td>
<td>treatment &amp; isolation</td>
</tr>
<tr>
<td>2002</td>
<td>7</td>
<td>936</td>
<td>149</td>
<td>24</td>
<td>treatment &amp; isolation</td>
</tr>
<tr>
<td>2003</td>
<td>2</td>
<td>unknown</td>
<td>3</td>
<td>1</td>
<td>isolation</td>
</tr>
<tr>
<td>2004</td>
<td>1</td>
<td>1100</td>
<td>5</td>
<td>zero</td>
<td>vaccination</td>
</tr>
</tbody>
</table>

AAR = animal at risk

Table 4: Reported outbreaks of sheeppox in the state during the last five years

3.2.5 Vaccination programme

The demand for vaccination against SP increased in recent years in the state. The remained vaccines from each year used in the next one and no vaccines return back to the Central veterinary research laboratory. Table (5) shows the vaccination figures five years ago.
### Table 5: Vaccination figures in the state 2000 – 2004

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccine distributed (doses)</th>
<th>Vaccine used (doses)</th>
<th>Remain (doses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>20800</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>2001</td>
<td>5600</td>
<td>11100</td>
<td>unknown</td>
</tr>
<tr>
<td>2002</td>
<td>5000</td>
<td>5700</td>
<td>6500</td>
</tr>
<tr>
<td>2003</td>
<td>58000</td>
<td>unknown</td>
<td>5000</td>
</tr>
<tr>
<td>2004</td>
<td>100000</td>
<td>100000</td>
<td>00</td>
</tr>
</tbody>
</table>

### 3.3.1 Sero-prevalence of sheeppox in the state

The 502 serum samples (151, 115, 120, and 116 from Kassala, Algash, Setiet, and Nahr Atbara, respectively) were tested for detection of antibodies against SP antigen using AGID test. Generally 319 samples were positive with prevalence of 63.55%, while 183 samples were negative (36.45%). Prevalence of SP in Kassala state as detected by AGID test is shown in Figure (3). High prevalence of sheeppox was recorded from serum samples in Algash locality as 69.57% (n=115). However, other localities Kassala, Nahr Atbara, and Setiet were recorded as 66.89% (n=151), 63.79% (n=116), and 53.33% (n=120), respectively. A significant correlation (Chi-square = 7.9324, \( P \)-value = 0.047) was found between sheeppox prevalence and the localities. Figure (4) shows the result of AGID test.

![Figure 3: The Sero-prevalence of sheeppox in Kassala state](image-url)
Figure 4: Agar gel immunodiffusion test (AGID) for detection of sheep pox antibodies: reference sheep pox antigen in the central well and test, determined well for hyper immune serum and test sera in peripheral wells.

3.3.2 Relationship between the age and SP

Chi-square test was used to determine the significance between AGID test and the age factor. There was no significant association between different ages and the prevalence of the disease (Chi-square= 2.3751, P-value= 0.498) was noted. High prevalence of SP observed in the age less than one year (65.63, n= 233). The result is summarized in Table (6).

Table 6: The frequency of AGID results in relation to the age

<table>
<thead>
<tr>
<th>Test</th>
<th>1-12</th>
<th>13-24</th>
<th>25-36</th>
<th>37-48</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>n</td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Positive</td>
<td>233</td>
<td>51</td>
<td>30</td>
<td>5</td>
<td>319</td>
</tr>
<tr>
<td></td>
<td>(65.63)</td>
<td>(57.59)</td>
<td>(60.00)</td>
<td>(55.56)</td>
<td>(63.55)</td>
</tr>
<tr>
<td>Negative</td>
<td>122</td>
<td>37</td>
<td>20</td>
<td>4</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>(34.37)</td>
<td>(42.05)</td>
<td>(40.00)</td>
<td>(44.44)</td>
<td>(36.45)</td>
</tr>
<tr>
<td>Total</td>
<td>355</td>
<td>88</td>
<td>50</td>
<td>9</td>
<td>502</td>
</tr>
<tr>
<td></td>
<td>(100.00)</td>
<td>(100.00)</td>
<td>(100.00)</td>
<td>(100.00)</td>
<td>(100.00)</td>
</tr>
</tbody>
</table>

Chi square = 2.3751  P-value = 0.498  (P > 0.05)
### 3.3.3 Relationship between animal sex and SP

No statistical significance (Chi-square = 0.2039, \( P \)-value = 0.652) was found between the presence of antibodies in serum samples and sex of animals tested. The AGID positive cases were 66 (61.68%) for males, whereas 253 (64.05%) for females. However, 41 (38.32%) negative are among males, and 142 (35.95%) among females. Females show high prevalence than males. The correlation between sex of the sheep factor and AGID data is demonstrated in Figure (5).

![Bar chart showing sero-prevalence of sheeppox and sex](image)

**Figure 5**: The relationship between sero-prevalence of sheeppox and sex

Chi square = 0.2039 \hspace{1cm} P-value = 0.652 \hspace{1cm} (\( P > 0.05 \))
CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

Contagious viral diseases represent a major health problem for livestock in Sudan. Moreover, seroepidemiological studies on contagious diseases are highly recommended to determine the present situations of these diseases in the country, so as to pave the way to a precise control strategy. In Sudan the endemicity of sheeppox have been reported by many authors namely, Bennet et al. (1944) and continuous outbreaks reported by Sheikh Ali (1997), Elfatih (2002) and Ali et al. (2004), had provoke the need to carry out this study.

In the present study, the result of the questionnaire survey revealed that most (68%) of the animal owners interviewed in the Kassala state are owning sheep, as well as most (52%) of them are nomads. This finding may refer to quick build up of sheep flocks which lead to increase of income for nomads to face their family needs. In addition, the importance of sheep in exportation purpose was also evident. Keeping sheep in nomadic system is associated with the basic needs for pastures and watering, whereas the movement of animals depending on the tribe’s behaviour and rainfall season from east to west or north to south. As the virus is hardy and resistant to desiccation, the transmission of SP due to keeping sheep in nomadism phenomena leads to increase transmission of SP within sheep flocks in the state. However, quarantine measures and restriction of sheep movement where not well practiced in the borders station with other neighbouring states may also play a role in the transmission of the disease. This was already confirmed by Ali et al. (2004) who reported the high prevalence of SP in the Red Sea and River Nile states.

The results of this study also showed that 38% of the owners confirmed SP as most important disease in their sheep flocks, and most of them (80%) observed the presence of the disease in their flocks and most (90%) of the owners looked familiar with signs of SP. They were described the
clinical signs of the disease perfectly. 80% of the owners confirmed the morbidity is higher than the mortality rate, and no one confirmed reverse. These results are in agreement with Losos (1986) who estimated the morbidity rate of about 5 to 10% with a very low mortality in enzootic areas. The infection, morbidity and mortality rates, in addition to abortion (among infected ewes), as mentioned by the nomads in this study indicate that SP is a great problem for sheep owners in Kassala state. Moreover, most (66%) of the owners did not vaccinated their animals against sheeppox which may be due to inadequate vaccine doses available and veterinary services or may be attributed to some tribal traditions.

The data from the general directorate of animal resources were obtained with great difficulties due to the irregular reporting system in the state. The results of reports showed that the structure of the general directorate theoretically looked acceptable, but practically there were a lot of gaps like performing of regular investigation system of the disease and disease mapping in the state need to be fulfilled to come up with an established and organized work concerning animal health. Personnel who engaged in animal health services in Kassala state verified the ability of the state to conduct many projects at veterinary services specially the control program of infectious diseases. Accordingly, rearrangement of personnel distribution within the localities in the state are highly needed. In remote areas, special care should be made to provide the personnel with all needs to establish separate units from the Headquarter of directorate to facilitate the investigation and control strategies. The available vehicles and other facilities known from this study are suitable for running the control program for epidemic diseases in the state. In contrast, there are no clear plans or strategies for using these vehicles in vaccination, survey and other investigations. In addition, budgets are not enough to maintain the available vehicles.

The reports also elucidated that many outbreaks of SP had occurred during the previous five years (2000 - 2004) and the state has no clear reports to reflect any role for the investigation teams and collection of
samples to be sent to the central veterinary laboratory to confirm the clinical
diagnosis of SP in the state. No epidemiological studies had done in the state
to indicate the infection foci of the disease, trace back and quarantine
measures. Within some localities, like Algash, there are no reports for
sheeppox, although during serum collection of this study many clinical cases
of SP observed. These findings may agreed with Ali et al. (2004) who reported
the high prevalence of SP detected in the Red Sea state which located to the
north of Algash locality. The nodular form of SP which was prevalent in the
Sudan and reported by Sheikh-Ali et al. (2004) was noted during field
observation of this study. Ring vaccination practiced in the resent years
played major role in the blocking of the disease spread and the number of the
cases thus declined. It's worthy to mention that the outbreaks were occurred
in all localities of the state and hence most of sheep owners know the signs of
the disease.

The result of this study also revealed the higher figures for
vaccine distribution and vaccine doses used during the year 2004. 189400
doses were distributed and used during the last five years. The distributed and
used doses of SP vaccine indicated the great shortage of the vaccination
program as compared with total population of the sheep in the state. The
vaccination activates against sheeppox have been carried out in response to
outbreaks and not as a part of a regular control program. The vaccination
figures have been increased in the recent years in the state, this result reflects
the seriousness of the disease and its unavailable treatment, also the
understanding of sheep owners about the importance of vaccination in
protection of their flocks against the disease.

The result of cross sectional study showed the high sero-
prevalence of SP in the studied area where about 63.55% showed positive
result by AGID test. High prevalence of SP was detected in the samples
collected from Algash locality followed by Kassala, Nahr Atbara and Setiet
locality. The prevalence rates obtained by this study look to be low compared
with Elfatih (2002) who reported the prevalence of SP in West Kordufan state
as high as 83% based on the AGID test result. This may be due to the high
population of sheep in West Kordufan state than Kassala state. The two localities Algash and Kassala which located near the boarder with Eritrea and the free movement of sheep for pastures and watering may be involved in the spread of the disease in that areas.

Most of the reviewed reports on AGID test stated by researchers indicated the AGID test is not considered as specific test for detection of SP antibodies due to false positive results which may be due to other pox or pox like viruses especially Orf which known to be endemic in Sudan (Eisa and ElAmin, 1993). However, the use of AGID for detecting SP antibodies was previously reported by many authors and proved useful result (Kitching et al., 1986; Tiwari et al., 1996 and Kitching and Carn, 1996). In spite of the low sensitivity of the test, the high prevalence of SP antibodies in the collected serum which detected by the test must be considered a real problem in the state.

No statistical significance in correlation between SP with age and sex was observed during this study. Age and sex of the sheep are not proved to be factors in the occurrence of the disease. These findings are similar to the report of Woldemeskel and Ashenafi (2003) who explained that there is no statistical association ($P> 0.05$) of SP disease with age and sex of the sheep, but the disease spread is correlated with poor management, climatic factors, feed scarcity and inadequate veterinary services. However, the high prevalence obtained by the test, was in age less than one year, this result may associated with the severity of the disease in lambs which coincide with those obtained by Losos (1986) for SP infection. Moreover, females elucidate high percentage of prevalence compare with males, this may be due to the deficiency of the immune system of the female during pregnant and lactating time, which may play role in the high susceptibility of females to the disease.

In conclusion, the result of the present study revealed that SP is prevalent in Kassala state and the AGID test proved efficient in the detection of SP antigens and antibodies. Most of the sheep owners explained a great
degree of knowledge about the disease and its negative impacts on the sheep industry. Age and sex of sheep were not incriminated as risk factors for SP occurrence.

**Recommendations**

Based on the results of the present study, and due to the nature of the disease, any delay in the interference when outbreaks took place can result in a wide spread of the infection. This may complicate and greatly increase the cost of any control measures adopted. The following recommendations should be considered.

- An attempt should be made to increase awareness of sheep owners on the importance and the impacts of the disease in their sheep flocks.

- Movement of sheep should be restricted when come in contact in the grazing and watering areas.

- Restricted quarantine areas should be established surrounding the infected areas. In addition, check points must be done to secure the state borders especially in Kassala and Algash localities.

- Establishment of information system and plan unit to come up with a clear strategy for controlling epizootics.

- Seromonitoring studies should be conducted regularly to show the state disease mapping.

- Mass vaccination against sheeppox is of extreme importance and should be held seriously with systematic program and care to control sheeppox virus dissemination.
• Use of other more sensitive techniques namely enzyme linked immuno-sorbent assay and polymerase chain reaction for routine diagnosis of sheep pox were highly recommended.
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Appendix 1: General questionnaire

Date ……………. No……………………

Name of owner:………..
Locality:…………
Site:………………
Tribe:………..

Herd composition: cattle sheep goat mixed
Situation : nomad settled
Migratory route: east Middle West
North Middle South
Has the herd mixed with others? Yes no

Would you ranking the most five important disease in your area?
Yes no
If yes mention them 1…….. 2………… 3………..4……….5………

Do you know the signs of the sheep pox? Yes no
Can you mention these signs?
1……….. 2………… 3………..4…………… 5……………

Have you ever seen these signs within your herd? Yes no
Age category most affected? Adult…. young……
Morbidity rate %………
Mortality rate %:………
Abortion rate within affected pregnant ewes %:………………
The effect of the disease in the production?
Comment…………………..

Have you ever vaccinated against sheep pox? Yes no
If no why? comment………..
Appendix 2: Sample collection form

Date ............

No..................

Name of owner:...........

Locality:............

Site:...............  

Sex............

Age............  

Observation.........