EPIDEMIOLOGICAL INVESTIGATION OF RIFT VALLEY FEVER IN SHEEP IN SINNAR STATE, SUDAN.

BY

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Dedication

To my father’s soul and my mother Hawa.

To my family my wife Fatima, my daughters Durra and Hiba, my sons Mohammed and Omer.

To my brothers.

For their patience, Constant encouragement and Support
Acknowledgment

Firstly, my heartfelt thanks to Almighty Allah for giving me strength, patience and will power to complete this challenging task of study.

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LIST OF ABBREVIATIONS

RVF: Rift valley fever
OIE: Organization des intentionnel de epizootic
FAO: Food and Agriculture Organization
WHO: World Health Organization
CPE: Cytopathic effect
ELISA: EnzymeLinked-Immuno Sorbent Assay
AGID: Agar Gel Immuno Diffusion test
CFT: Complement fixation test
PBS: Phosphate Buffer saline
SNT: Serum neutralization test
HAI: Haemagglutination
PCR: Polymerase Chain Reaction
RT-PCR: Reverse transcriptase PCR
MBGV: Marburg virus
EBOV: Ebola virus
LASV: Lassa virus
CCHFV: Crimean Congo hemorrhagic fever virus.
DENV: Dengue virus
YFV: Yellow fever virus
Mab: Monoclonal antibodies
IgG: Immunoglobulin G
IgM: Immunoglobulin M
SW: Sennar West locality
SE: Sennar East locality
SU: Suki locality
DI: Dindir Locality
DA: Dallay locality
Abu: Abuhugar locality
PACE: Pan African Program for control of Epizootics.
OD: optical density
HRPO: Horse Radish Peroxides
IQC: internal quality control
SDS: Sodium dodecyl sulphate
EDTA: EthyleneDiamineTetraAcetic acie
BHK: Baby Hamster Kidney
E.M: Electro Microscope
NS: None structural protein
The aim of the present study was to conduct an epidemiological investigation and determine the prevalence of Rift Valley fever (RVF) in Sinnar state, during the year 2008 (March-June). Three methods were adopted to achieve this goal:

1. Questionnaire Survey among sheep owners in the study area.
2. Four years retrospective screening for the important diseases in the area.
3. Serosurveillance to detect antibodies against RVF virus.

The questionnaire survey outcomes showed that heavy rains with dense mosquitoes and insects populations were experienced during the season of the disease outbreaks (rainy season of 2007) as confirmed by all sheep owners interviewed (100%). All animal owners had also informed that no slaughterhouses were available in their area (100%). Vaccination against RVF, the main line of defense against disease spread, was not ever practiced in the state (100%). Moreover, most of the owners assisted their animal during parturition (55%), few of them drank raw milk (15%) while many observed abortion in their herds (47%). The questionnaire survey outcome revealed that, heavy rain fall, abundant mosquito populations and death among young animals and abortion in adults, are indications for occurrence of RVF.

The retrospective study showed that RVF was not reported except in the
year 2007 in which the disease was described as a hemorrhagic fever and unconfirmed RVF. Shortage of veterinary services in Sinnar state and deterioration of veterinary infrastructures together with lack or poor reporting system may not reflect the real situations of livestock status and may play the basic role for failure in the control program of the diseases.

A serological survey was carried out in Sinnar state during year 2008, after about six months of the suspected outbreak of RVF in three Sudanese States (Sinnar, White Nile and Gazeera) in order to determine presence of IgG antibodies against (RVF) virus in sheep sera. Out of 176 serum samples collected from sheep species, more than one year old and tested using sandwich Enzyme–linked–Immuno-Sorbent–Assay (ELISA) technique, to detect IgG antibodies to RVFvirus, 17(9.7%) serum samples were positive whereas 159(90.3%) were negative.

High level of positive serum samples was recorded in Sinnar East locality (20.8%) while no positive cases were recorded in Sinnar West locality (0.0%). The findings indicated wide and uniform distribution of RVF virus in Sinnar state. Considering the results of the questionnaire survey which revealed favorable condition for vectors breeding, and the poor system for reporting clinical cases, together with asero-prevalence of 9.7%, there is good indication of the presence of RVF in Sinnar state at the time of conduction of this study.
Abstract in Arabic :

لا يمكنني قراءة النص العربي بشكل طبيعي.
الولاية في الإيرادات السيرولوجية المسح في عام 2008 م. مارس – يونيو (الالتهاب عام بعد ولايات في المرضى في الستار، الجزيرة، الأبيض النيل (للفيروس المضادة الأجسام لتحديد الضاء الفصيلة في وجوده أو المتدفق الدموي.

وجمتي تم 176 عينة دم.

 السيطرة بالكامل خلال العام أكثر العمر الضاء الفصيلة حمي الفيروس 17 (9.7%) ومرتبطة 159 (90.3%) 17 وفاة في ضاء ت في كيس 40.0% في 1 17.9%0.8 17.9% 17.9%. ان تعداد الفيروس في كيس 05 يثير في ضاء 4 دم في كيس 40.0% في 1 17.9%0.8 17.9% 17.9%}

عند فإن الفيروس في كيس 05 يثير في ضاء 4 دم في كيس 40.0% في 1 17.9%0.8 17.9% 17.9%. ان تعداد الفيروس في كيس 05 يثير في ضاء 4 دم في كيس 40.0% في 1 17.9%0.8 17.9% 17.9%.
Introduction

Rift valley fever (RVF) is a peracute or acute insect borne disease of man and animals caused by member of phlebovirus genus of the *Bunyaviridae* (Swanepoel, 1994). RVF is an important zoonotic disease and of significantly acute hemorrhagic fever. Until recently, it had only being recognized in the African continent. But in 2000 it occurred in Arab peninsula in Kingdom of Saudi Arabia and Yemen republic (Shoemaker *et al.*, 2002).

As well as Socio–economic and public health consequences (FAO. 2002); RVF is the major constraint to international trade of livestock and livestock products (OIE 1996).

The Office International des, Epizootics, (OIE) listed RVF in list (A) diseases and it was defined as a communicable disease, which has the potential for serious and rapid spread irrespective of national borders (FAO, 2002).

Rift Valley fever is a mosquito-borne viral disease. Aedes mosquitoes serve as a major vector and reservoir, Transovarian transmission occurs within Aedes mosquitoes, infected eggs lie dormant for years until flooding occurs, allowing them to hatch and spread virus to the livestock on which they feed (Le Duc 1989).

The disease causes storm of abortion in sheep and other domestic animals, with heavy losses among the young animals; hence it is economically destructive disease, other than its zoonotic character (FAO 2002).
The disease is strongly related to heavy rainfall together with abundant population of mosquitoes especially Aedes type (OIE 1996). With presence of cases in humans, and symptoms in livestock like abortion among pregnant females, RVF is usually suspected.

Rift Valley fever is considered as one of the major constraints of sheep species production due to its impacts in losses of reproduction function and high morbidity and mortality in young lambs, and abortion among pregnant ewes.

**Objectives**

The present study was carried out to determine the prevalence of RVF in Sinnar state during 2007 outbreak of the hemorrhagic fever in the area. This can also help to fulfill the following goals:

1- To participate in future control program, if outbreaks have to take place.

2- To gather scientific information, accompanied by implementation of ideal control measures to protect national livestock for economic purposes.
CHAPTER ONE

LITERATURE REVIEW

1.1 Definition:

Rift Valley fever is a peracute or acute zoonotic disease of domestic ruminants in Africa; it is caused by a single serotype of a mosquito-borne bunyavirus of the genus Phlebovirus. The disease occurs in climatic conditions favoring the breeding of mosquito-vectors and it is characterized by liver damage. The disease is most severe in sheep, goat and cattle in which it produces abortion in pregnant animals and a high mortality rate in the newborn. Older none pregnant animals although susceptible to infection, are more resistant to clinical disease (OIE, 1996). Humans are susceptible to infection by handling infected material or by mosquito-vector bite (OIE, 1996).

1.2 Causative Agent:

Rift Valley fever is caused by hemorrhagic fever virus (RVF virus) that causes disease in human and animals, the virus belongs to the family Bunyaviridae, genus Phlebovirus. Members of this family cause hemorrhagic fever in both livestock and humans; and transmitted by insects, hence they called arboviruses, except genus Hanta which is transmitted by rodents.
1.3. Morphology:

Virion is enveloped, pleomorphic, 80-120 nm in diameter. Genome contains single stranded negative-sense RNA with three segments named S (small), M (medium) L, (large). Each segment is enclosed in a separate nucleocapsid within the virion (Peters & Meegan 1981).

- Major sites of viral replications, liver, and, spleen. The brain also is a common site, specially in fetuses neonates, The virus is inactivated by disinfectants (e.g. Na & Ca hydroxide) or PH less than 6.2 (e.g. acetic acid) (OIE, 2002).
- Viability: The virus can be maintained for 4 months at 4°C when stored in neutral or alkaline solution in the presence of protein (such as those found in serum), or for 8 years when stored below 0°C. (OIE, 2002)

1.4. History and distribution:

Rift Valley fever appears to be restricted to Africa; it was recognized first in the Rift Valley of Kenya at the turn of 20th century but the agent is not isolated until 1930. The disease was firstly observed in Southern Africa in 1950. A major epidemic of RVF occurred in Egypt 1979 (Meagan 1979) Additional outbreaks in Egypt have been reported, probably representing repeated introduction of the virus. Another large outbreak involving thousands of cases occurred in Somalia and Kenya 1997-1998 (Woods 2002).
In the fall of 2000, outbreaks occurred simultaneously in Yemen, and Saudi Arabia, the virus was thought to have been introduced from Africa, through the sheep trade (Shoemaker, 2002) (Jup et al., 2002).

This virgin–soil epidemic in the Arabian Peninsula raises the threat of expansion into other parts of Asia and Europe (WHO – 2000 Web. site.)

The following table (table 1) is showing countries in which outbreak occurred during the last 100 years (Meagan, (1979); Woods (2002); FAO (2003); Shoemaker (2002); WHO Web. site (2007); (Swanepoel, 1994).
<table>
<thead>
<tr>
<th>Years</th>
<th>Countries affected</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1910</td>
<td>Kenya</td>
<td>Confirm by retrospective study.</td>
</tr>
<tr>
<td>1930-1931</td>
<td>Kenya</td>
<td>• Isolation of the virus, confirmed as zoonotic &amp; spread by Mosquito (FAO 2003). (Daubency et al. 1931)</td>
</tr>
<tr>
<td>1950-1951</td>
<td>Kenya</td>
<td>• More than 100,000 sheep died (FAO 2003)</td>
</tr>
<tr>
<td>1950-1975</td>
<td>South Africa</td>
<td>• Infection of ruminants &amp; humans</td>
</tr>
<tr>
<td>1977-1978</td>
<td>Egypt</td>
<td>• Ruminants and human (Meaga 1979)</td>
</tr>
<tr>
<td>-1987</td>
<td>Senegal, Mauritania</td>
<td>• First time appear in West Africa</td>
</tr>
<tr>
<td>1989-1991</td>
<td>Cameroon, Togo, Benin, Cotedeavour, Nigeria, Senegal, Borkinafaso</td>
<td>• (Senegal River basin) IgM were found (Lederberg et al, 1992)</td>
</tr>
<tr>
<td>2000</td>
<td>Saudi Arabia / Yemen</td>
<td>• First time outside Africa in Arab peninsula (shoemaker, 2002)</td>
</tr>
<tr>
<td>2007</td>
<td>Sudan</td>
<td>• More than 500 people were affected in Sinnar, Gazeera and White Nile state (PHAC. 2007 Web. site.).</td>
</tr>
</tbody>
</table>
1.5. Epidemiological feature:

Outbreaks of RVF occur generally when particularly heavy, prolonged and, often heavy rainfall favors the breeding of mosquito vector. Epidemic mostly occurs in (5-10) year’s cycle in Eastern and South Africa, But in dry semi-arid zone periodicity are (15-30) years (Swanepoel, 1994).

1.5.1. Susceptible species:

All ruminants are susceptible to RVF infection, birds are not. Of the livestock species sheep are the most susceptible, followed by goats, cattle & camel and water buffaloes, (FAO, 2002). In Africa exotic livestock breed are more susceptible to the clinical disease than indigenous breed (FAO 2002).

Humans contract infection from affected animals. Wild antelopes susceptibility to disease has not been established fully but it is believed that at least some species suffer mortality and abortion. Some breeds of sheep and goats appear to be relatively resistant to the disease (Swanepoel, 1994; Elfadil et al; 2005).

Other susceptible species are cape buffaloes, monkeys' cats, dogs and rodents (FAO 2002). Exotic breeds which have been recently introduced in to an endemic area are more susceptible than breeds long adapted to local conditions. Animals of different ages also differ in their susceptibility to severe illness; over 90% of lambs infected with RVF die whereas mortality among adult sheep can be as low as 10 % (FAO; 2002). Abortion rate amongst pregnant ewes may reach about 100%. An epizootic of RVF is first manifested
as wave of unexplained abortion amongst livestock; this may signal the start of an epidemic (Swanepoel, 1994).

1.5.2 Transmission:

Rift Valley fever is a mosquito–borne viral disease in which, Aedes mosquitoes serve as major reservoir and vector. Transovarian transmission occurs within Aedes mosquitoes; infected eggs lie dormant for years until flooding occurs, allowing them to hatch and spread the virus to the livestock on which they feed. (Le Duc. 1989). Other species of mosquitoes such as Culex, Anopheles and other biting insects may transmit the virus mechanically (FAO, 2002). Infected mosquitoes may be transported for long distance in low level winds or air current which may lead to the rapid spread of the virus. The virus circulates between vertebrate hosts and mosquitoes. It does not require continuous vector–host–vector feeding cycle for maintenance. (FAO, 2002).

Unlike in humans’ non-vector transmission of RVF virus is not considered to be important in livestock. However in addition to mosquito transmission, humans are easily infected by contact with the body fluids of infected animals through contact with abraded skin, wounds or mucous membranes or by inhalation of aerosols. (FAO, 2002). Thus the slaughtering of infected animals, necropsy procedure and laboratory manipulation of tissues and isolating viruses are activities carrying a high risk of disease transmission (Swanepoel, 1994.).
1.5.3. Epidemic RVF disease pattern:

In Africa, major epidemics occur irregularly in eastern and Southern Africa (5 – 10) year cycles, but in dry semi-arid zones of eastern Africa the periodicity is (15-30)years (Swanepoel ,1994) ( FAO ,2002). For epidemic to occur three factors must be present:

- The pre–existence or introduction of the virus in the area.
- Presence of large population of susceptible ruminants.
- Climatic or environmental conditions that encourage a massive buildup in vector mosquito population; this occur when there is warm conditions and heavy persistent rainfall which causes surface flooding and leads to hatching of Aedes mosquitoes (FAO, 2002).

1.5.4. Interepidemic virus survival:

Recurrent viral activity occurs in localized areas in southern and eastern Africa where transmission of RVF virus to ruminants occurs during most years (Swanepoel, 1994). Virus activity may be revealed by random isolations from mosquitoes or by occasional human diseases. Small local RVF outbreak may occur when and where the micro-environmental conditions are favorable and susceptible livestock are present (FAO, 2002). Transovarian transmission of RVF virus occurs in some species of Aedes mosquitoes of the Neomelaniconium group. The egg of these mosquitoes, and the virus that they carry, may remain viable for very long period in the mud of dried-up surface
pools or shallow depressions or in floodplains. Infected mosquitoes hatch from these eggs when they are again flooded. This is the reason why the virus persists during prolonged inter-epidemic period in the grasslands and semi-arid regions of eastern, western and southern Africa, (FAO 2002), (Swonepoel 1994).

1.5.5. Cryptic (or Sylvatic) RVF:

In Africa the infection cycle among indigenous domestic and wild vertebrate animals and mosquitoes is sub-clinical both in livestock and humans. In the rain forests and wetter wooded areas of the country, the virus has been circulated silently between wild and domestic species and insect vector. This is referred to as cryptic or Sylvatic RVF (FAO, 2002).

1.6. Clinical Signs

1.6.1. Sheep and goats:

Incubation period for lambs is (12-36hrs), and for adults is (1-6 days). Clinical signs in lambs include fever 40°C- 41°C (104-105°F), mucopurulent nasal discharge, vomiting, anorexia diarrhea, and ictrus (Borio et al., 2002). Complications include: abortion rate of 100%, peracute hepatic disease in lambs and kids <1week old, hepatitis, cerebral infection and ocular infection. Case–fatality rate in Lambs <1 week of age may be as high as 100 % while in lambs.<1 week of age as high as 20% and in adults 20%- 30 %.( Borio et al., 2002)
1.6.2. Cattle:

Incubation period is about (1-6 days). In calves the clinical signs are fever of (40°-42°) C (104°-106) °F depression, anorexia, weakness, listlessness and abdominal pain. In cows the clinical signs are the fever 40°-42°C, excessive salivation, anorexia weakness fetid diarrhea, nasal discharge and fall in milk yield (FAO, 2002).

1.6.3. Camels:

Although infection is generally sub-clinical (FAO, 2002) pregnant she camel may abort at any stage of pregnancy and neonatal death can occur; abortion rate may reach 70% (FAO; 2002).

1.6.4. Humans:

During Epizootic of RVF in animal population, infection can spread to humans and result in concurrent outbreak of human disease. Humans can become infected through several different mechanisms; which include, bite of infected mosquitoes and direct contact with infected animal, and aerosols generated during slaughter (Borio et al., 2002). Aerosol generated in the laboratory setting was also reported as mechanism of transmission (smith burn et al. 1994). Sub-clinical infection in humans is common; in addition, there are four clinical patterns (Borio et al., 2002):

1/Undifferentiated fever, lasting 2-7 days, with or without nausea, vomiting or abdominal pain >90% of clinical cases.
2/ Hemorrhagic fever with hepatitis and bleeding >1% of cases.

3/ Encephalitis, <1% of cases 2-3 wks after onset of fever characterized by neurological symptoms such as convulsion.

4/ Retinitis, up to 10% of the cases occurs 1-3 weeks after onset of the fever which may results in blindness (Borio et al. 2002). Overall case fatality rate is <1%, however, for complicated hemorrhagic fever case fatality rate may be as high as 50% (Borio et al., 2002).

1.7. Pathology:

1.7.1. Gross pathology:

The pathogenesis of RVF results from spread of virus from the site of introduction to the body and initial replication sites to critical organs such as the lymph nodes and blood stream causing viraemia, subsequent replication occurred in reticulo-endothelial system (Aradaib, et al., 1997). The characteristic features include severe hepatic lesions and an enlarged liver that is friable, soft and reddish to yellowish-brown in colors with petichial hemorrhage (Mebus et al., 1998). (Eisa et al., 1980) The contents of the abomasums and small intestine of newborn lambs are chocolate brown (Mebus et al., 1998). In most animals edema and hemorrhages in the wall of gall bladder, as well as enlarged peripheral and visceral lymph nodes were observed all over the body (FAO, 2003).
Extensive subcutaneous and serosal hemorrhages ranging from petichial to ecchymotic on all serous surfaces, lymph nodes, sub-cutis and kidneys were reported (Eisa et al; 1980). Accumulation of blood-stained fluids in body cavities and hemorrhagic enteritis were associated with bloody diarrhea (Abdelhakeem et al., 1998). The body cavity fluid was blood stained and carcases were affected with jaundice, (FAO, 2002).

1.7.2. Histopathology:

In young animals necrosis was severe and characterized by dense aggregation of cellular and nuclear debris, fibrin and inflammatory cells. (Mebus, 1998); (FAO, 2002); (Swanepoel, and Coetzer; 1994).

Hepatic samples showed, eosinophilic, rod-shape intra-nuclear inclusion bodies (FAO, 2002).

1.8. Immunity:

1.8.1 Natural immunity:

This can be measured by detection of IgG antibodies in serum of animals (Elfadil, A, A. and Ali S.S; 2006). As animals mature their susceptibility to RVF disease decreases (Swanepoel et al., 1994). Innate immunity varies between breeds. Some breeds of sheep and goats appear to be relatively resistant (Elfadil.A, A.and Ali S, S; 2006).
Herd immunity levels are high after epidemics, the immunity appear to be life-long (Elfadil A., A. and Ali S, S. 2006). Sheep are more susceptible to RVF than goats and other species (Elfadil A.A. and Ali S, S. 2006).

1.8.2. Vaccines:

Vaccination of animals against RVF disease has been used to prevent disease in endemic area and to control epizootics. The most commonly used vaccine is a live-virus vaccine derived from Smithburn-Strain, which was attenuated vaccine causes abortion in pregnant ewes and pathogenic for human. Inactivated vaccines also has been developed for use in both the humans and animals, the vaccine is safe and effective but required two dose and limited to outbreak control. Duration of immunity induced by vaccination of sheep with live attenuated vaccine may last for three years (Losos G.J. 1986), the vaccine developed IgG antibodies by the fourth week following inoculation ((Elfadil A.A. and Ali S.S. 2006), the IgG antibodies induced by vaccination disappear from the blood by the elapse of time (Elfadil A.A. and Ali S.S. 2006). This could be also true for IgG antibodies induced by natural infection (Elfadil A.A. and Ali S, S. 2006).

1.9. Diagnosis:

1.9.1 Field diagnosis:

Rift Valley fever epizootics should always be strongly suspected when there was sudden onset of large numbers of abortion in sheep, goats, cattle and
death in lambs, kids or calves (FAO 2002, Tamadur 2006). This is always the cases if there is flooding in Savannah or Semi-arid area following prolonged rainfall or in irrigated areas; if the mosquito population is high, and if there is concurrent illness in human population (Tamadur, 2006).

The disease in domestic animals may only be noticed after illness of people has been identified as RVF. Sporadic cases or small outbreak in non endemic circumstances, are more difficult to diagnose and could be missed (FAO, 2002; Tamadur, 2006).

1.9.2 Histopathology:

The finding of characteristic histopathological lesions with liver necrosis of young animals or fetuses is suggestive of RVF (FAO, 2002).

1.9.3. Laboratory diagnosis:

1.9.3.1. Collection and transport of specimens:

Whole blood, liver, spleen and lymph nodes are the tissue of choice for the isolation of the virus (FAO, 2002). Blood samples should be collected from febrile animals into Ethylene–diamine tetra–acetic acid (EDTA) or heparin to which antibiotics have been added as preservatives (penicillin 200units and streptomycin 200µg/ml, final concentration) (FAO, 2002). Samples of liver and spleen should be collected aseptically both from freshly dead animals.

Blood sample about 20 ml each, should be collected from animals in acute and convalescent phases of the disease for serum. (FAO, 2002)
1.9.3.2. Virus isolation and identification:

Rift Valley fever virus can be isolated from whole blood or homogenous of fresh tissues by intracerebral injection of suckling mice or intraperitoneal injection of adult mice or hamsters (FAO, 2002).

It can also be readily isolated in various primary cell cultures (e.g. primary lamb and calves kidney or testis) or cell lines (e.g. BHK-21) (FAO, 2002). It is recommended that work with this agent should be conducted only in biosafety level three facilities which provided for High Efficient Particulate Air (HEPA) filtration of all exhaust air prior to be discharged from the laboratory. (Sall et al., 1999) stated that RVF represents a hazard to all laboratory workers engaged in its study.

1.9.4. Organism detection test:

1.9.4.1. Electron Microscope and Immunofluorecent:

Rift Valley fever virus attained intracellular titer of at least 3,6 log/ml 4 hours post infection in CV1, Vero and BHK21 cells. At 22 hours post infection, a peak titer of 7,7logs/ml was reached in CV1 cells, where 50% of the cells showed cytopathic effects. The same degree of (CPE) was only observed 45 hours post infection (Tamadur, 2006) in the other cell lined tested.

Virus particles were detected by EM, 22 hours post infection in CV1 cell, but in Vero & BHK21 cell the virus could be detected only at 45 hours post infection (Tamadur 2006).
1.9.4.2 Animal Inoculation:

Morrill, et al; (1989) Tamadur; (2006) Stated that Rhesus monkeys inoculated with RVF virus provided a model in which serial observations of serum viral antigen and antibodies can be made. In 9 non fatal and 3 fatal injections either antigen or IgM, (Enzyme Linked–Immuno-Sorbent Assay) (ELISA) antibodies were detected in every serum sample during the acute phase.

1.9.5. Serological Techniques:

Serology may not identify an active infection and cross reaction at the serogroup level which is likely to occur with other members of the phlebovirus genus. However, it is useful to determine past infection in a sero epidemiologic survey, (Aradaib and Abbas, 1985; work et al., 2002, Tamadur 2006).

Several serodiagnostic techniques have been validated for the diagnosis of RVF virus, the Agar Gel Immuno-Diffusion test (AGID); Complement Fixation test (CFT), Enzyme–Linked–Immuno–Sorbents–Assay (ELISA) Hem agglutination (HA); and Hemagglutination Inhibition (HAI) test are routinely used. (Aradaib and Abbas 1985).

The use of monoclonal antibodies (Mab) in competitive ELISA (CELISA) technique has improved sensitivity and specificity at the Sero-group level (Aradaib et al., 1994). This technique is applicable only to blood (serum) and requires at least 14 days post infection. The ELISA test has now replaced
the older inhibition of haemagglutination (IHA); Immuno fluorescence assay (IFA); and serum neutralization test (SNT); as the test of choice, it is highly specific with little or no cross neutralization with other phleboviruses. It can be used to detect antibodies in all animal species (Sall et al., 1999).

1.9.5.1. Sandwich and Capture ELISA:

Paweska et al., (2003) reported development and validation of sandwich and capture ELISA for detection of IgG and IgM antibody to RVF virus in bovine, ovine and caprine sera. The IgG ELISA was more sensitive in detection of the earliest immunological responses to infection or vaccination with RVF virus. Its sensitivity and specificity derived from field data sets ranged in different ruminant species from 99.05% to 100% and from 99.1% to 99.9%, respectively.

1.9.5.2 Antigen detection:

Rift Valley Fever (RVF) virus antigen may be detected by direct or indirect immuno fluorescence tests on impression smear or Cryostat section of liver, spleen and brain. A rapid diagnosis can sometimes be made by Agar gel immunodiffusion (AGID) test on fresh tissues (FAO, 2003, Tamadur, 2006).

Immuno capture ELISA and histochemical staining of cryostat section or formalin fixed tissues and PCR are now much more widely used for RVF. (FAO, 2003)
1.9.6. Nucleic Acid Detection tests:

1.9.6.1 Detection of viral genetic material:

A reverse Transcriptase PCR test is now available for detection of viral genetic material. Sequencing of the NS (S) protein – coding region of the genome may be used for phylogenetic analysis (genetic fingerprinting) of virus isolates (FAO, 2003), (Sall et al., 2001).

19.6.2 Reverse Transcriptase Polymerase chain reaction (RT-PCR):

RT-PCR is important diagnostic tool for rapid detection and differentiation of RVF infection during endemicity of the disease, it has been successfully applied for detection of RNA viruses by addition of complementary DNA (c DNA) synthesis step using reverse transcriptase enzyme caution in light of presence of viral nucleic acid and absence of infections virus (Aradaib et al., 1995). Viral hemorrhagic fevers (VHF) are acute infections agents with high case fatality rates. Important VHF agents are Ebola and Marburg viruses (MBGV/EBOV). Lassa virus (LASV), Crimean Congo hemorrhagic fever virus (CCHFV), Rift valley fever virus (RVFV), dengue virus (DENV) and yellow fever virus (YFV). VHFs are clinically difficult to diagnose and to distinguish. A rapid and reliable laboratory diagnosis is required in suspected cases, six one–step of real- time RT–PCR assay for these pathogen have been established (Drosten et al., 2002).
A reverse transcriptase polymerase chain reaction (RT-PCR) assay to detect RVF virus in experimentally infected mosquitoes was developed (Ibrahim et al., 1997).

1.9.7. Differential diagnosis:

There are a number of disease that may be confused clinically with RVF, it should also be remembered that conditions favorable for RVF outbreak may also be favorable for other insect borne disease such as blue tongue. Nairobi sheep disease, and wesselsbron disease, (FAO, 2002)

Other livestock diseases and transboundary diseases such as Peste de Petites Ruminants (PPR), Rinder Pest (RP), and Foot and Mouth Disease (FMD), Contagious Caprine Pleuropneumonia (CCPP) may also occur through farming communities and movement of animals as a result of flooding.

The simultaneous occurrence of other disease may compound diagnostic difficulties (FAO 2002).

Together with all causes of abortion in ruminant animals, diseases to be taken in consideration in the differential diagnosis of RVF include:

- Wesselsbron disease under same condition of RVF.
- Orbivirus infection e.g. blue tongue and epizootic heamorrhagic disease.
- Nairobi sheep disease (Transmitted by Rhipociphalus and amblyoma sp tick) causes abortion in sheep.
• Intoxication by poisonous plant associated with hepatic lesions; hemorrhage & Jaundice.

• Bacterial septicemia diseases e.g. *Anthrax*, *pasteurlosis*, *Salmonellosis*.

• Leptospirosis which mimic RVF clinical signs.

• Ephemeral fever & lumpy skin disease.

• Others include *Brucellosis*, *Vibriosis*, Trichominiasis, Heart water disease & Ovine enzootic abortion (*Borio, et al; 2002*).

**1.10. Prevention and Control:**

Rift Valley Fever (RVF) is very serious and an important zoonosis; countries should take whatever steps they can to prevent the entry and/or occurrence of the disease (*FAO 2002*). Quarantine measures should be regarded as the first line of defense. However, no records show to date that RVF has been transported by animal movement from one country or area to another (*FAO, 2002*).

**1.10.1. Prevention:**

Vaccination of animals against RVF has been used to prevent disease in endemic area and to control epizootics, (*Pittman et al., 1999*). Currently the most commonly used vaccine is a live–virus vaccine derived from Smithburn strain which was attenuated through serial intracerebral inoculation of mice.

One inoculation confers immunity for 3 years and produce protection in 6-7days (*Elfadil, A, A. and Ali S, S. 2006*). The vaccine causes abortion in
pregnant, the vaccine also pathogenic for human. Inactivated vaccine also has been developed for use in both animal and human (Pittman et al., 1999).

The animal’s vaccine has been shown to be safe and effective but required booster dose 2-4 weeks after first injections (Pittman et al., 1999).

Human vaccine is still under investigation, although initial studies suggest that it is safe and provides good long term immunity (Pittman, et al., 1999). Additional attenuated live-virus vaccine (MVP12) is also under development. It appears to be safer for pregnant animals (Baskerville 1992; Morrill 1997).

People infected with the RVF virus may have a high enough viraemia to re-infect mosquitoes so that it is theoretically possible for incoming airline passenger to introduce the disease to a new country (FAO, 2002).

Consequently cooperation is required with health ministries to insure that the correct human quarantine procedures are implemented for incoming passengers at airports (FAO 2002).

1.10.2. Control of RVF in Vertebrate hosts:

Immunization of susceptible animals is the most effective means for control of RVF (Meegan and Bailey 1989). Two types of vaccine are currently used to immunize sheep and cattle in Africa. The attenuated live virus vaccine (Smithburn strain) is highly effective, but it causes a small number of sheep to abort after immunization. It is not recommended for non endemic area or for
animal being exported from endemic area (Meegan and Bailey 1989, Tamadur 2006). Formalin inactivated vaccines has been used for many years (Tamadur 2006) these are safe and effective but require multiple injections.

Recently an innovate subunit vaccine was evaluated in mosquito cells (Tamadur, (2006). In general, the subunit vaccine requires boosting and adjuvant to provide long lasting protective immunization (Aradaib et al., 1995, Tamadur, (2006). The vaccine is not yet available.

When RVF virus activity has been confirmed in a country where the disease is enzootic and which is exporting livestock; the veterinary authorities should, (FAO, 2002):-

- Define the extent of infected area and target population.
- Define a buffer zone.
- Monitor physical indictors of flooding; persistence of floodwater and rainfall to predict a time.
- Scale for epidemic virus activity.
- Monitor mosquito population in RVF affected area.
- Carryout surveillance for clinical disease and sero-conversions to RVF (IgM and IgG).
- Determine the date of last evidence of RVF virus activity.

Vaccination in outbreak area is not recommended at this time, when
there is evidence of high levels of RVF transmission by mosquito

(FAO, 2002).

1.10.2.1. Quarantine measures:

Since enforcement quarantine of animals is difficult in Africa; it is not generally an effective control measure. However, to whatever extent possible, movement of animals from epizootic situation should be restricted to prevent the further spread of RVF (Meegan and Bailey 1989; Tamadur 2006).

1.10.3. Mosquitoes Avoidance:

A person chance of becoming infected can be reduced by taking measures to decrease contact with mosquitoes and other bloodsucking insects through the use of mosquito repellents and bed nets (Tamadur 2006).

1.10.4. Avoidance of exposure to blood or Tissue:

Avoiding exposure to blood or tissue of infected animals is an important protective measure for persons working with animals in RVF- endemic area (FAO, 2002).

1.10.5. Treatment:

Treatment is supportive and may require intensive care for humans (Anon; 1988). There is no specific therapy for infected animals, however, Ribavirin may be efficacious in humans (Borio, et al; 2002).
CHAPTER TWO
MATERIALS AND METHODS

2.1 Study Area:

The study was conducted in Sinnar state, Sudan which is located in the southern–east part of the Sudan, between longitude 32-42° east and the latitudes 12.5° and 14.7° North. (Figure 1).

The state covers an estimated area of 40680km2. The state is divided into seven localities, named Sinnar West (SW), Sinnar East (SE), Singa (SI), Suki (SU), Dindir (DI), Dalley (D), and Abuhugar (Ab), (Figure 2).

Sinnar state is bordered by Gazeera state to the north, White Nile and Upper Nile state to the west, Blue Nile state to the south, Gadarif state to the northern- east, and long borders with Ethiopia to the east. The dominant climate in the state is poor savannah in the north with an annual rainfall of 400-600mm and rich savannah in the Southern regions of the state with an annual rainfall of 800mm. The minimum and maximum annual temperatures are 20°-25°C and 35°-40°C respectively. The human population in Sinnar state is about 1,200,000. Most of them work in agriculture and livestock management, with few traders and employees. The human population comprises many complex tribes divided between civil and rural area and few nomads, the most common tribes in the state are kennana, Rufaa Taaish, Erigat, Funj, Howsa, Jaalin, Kawahla, Gawasma and some other movable tribes like falata, (Ambararo), Lahaween,
Nifidea as nomadic tribes. (Ministry of Agriculture and Animal Resources Sinnar state)

2.2. Animal population:

Sinnar state is one of the richest states of animal resources, distributed all over the state localities; in addition there is migratory livestock from other states towards Sinnar state during the dry season searching for water and pasture. Moreover the state is rich with wildlife in the Dindir National Park. There is no, national or regional livestock census carried out recently, but the Ministry of Agriculture and Animal Resources estimated the animal population in Sinnar state of about 6,383,134 heads as shown in table(2) in year (2003) last estimation by General Directorate of Animal Resources in Sinnar state.

Table (2) Animals population in Sinnar state

<table>
<thead>
<tr>
<th>Species</th>
<th>Approx. numbers</th>
<th>Note.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>2,351,593 heads</td>
<td>Fishes: 1500Ton/year</td>
</tr>
<tr>
<td>Goats</td>
<td>1,348,529</td>
<td>Wildlife: uncountable</td>
</tr>
<tr>
<td>Cattle</td>
<td>2,284,356</td>
<td></td>
</tr>
<tr>
<td>Camel</td>
<td>398,656</td>
<td></td>
</tr>
</tbody>
</table>
Figure: 1  Sudan map showing Sinnar state location
Figure: 2  *Sinnar state localities map*
Most of the animal owners are nomads, while few are residents with seasonal movement searching for water and food, mainly in west-Butana and East-Butana- in Gadarif state.

The study concentrates on sheep species in Sinnar state. The breed of Sinnar state’s sheep is watish, with a weight of about 25-30 kg, length 80-100 cm without horn, with long fatty tail, and shiny eyes. The ewes mostly deliver twins. The population of sheep according to state localities is given in table (3)

Table (3) Distribution of sheep population by locality:

<table>
<thead>
<tr>
<th>Locality</th>
<th>No of sheep</th>
<th>New localities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singa</td>
<td>1,133,280</td>
<td>Dally and Abuhugar</td>
</tr>
<tr>
<td>Sinnar</td>
<td>642,160</td>
<td>Sinnar east</td>
</tr>
<tr>
<td>Aldindir</td>
<td>576,153</td>
<td>Alsuky</td>
</tr>
<tr>
<td>Total</td>
<td>2,351,593</td>
<td></td>
</tr>
</tbody>
</table>

2.3. Study design:

This is prospective cross sectional study that targeted sheep population in Sinnar state. To achieve the objectives of this study the direction has been oriented towards three methods for data collection. These three methods included retrospective study, questionnaire survey and sero-surveillance using the Enzyme Linked Immuno Sorbent Assay. (ELISA)

2.3.1. Questionnaire Survey:

Questionnaire forms were distributed among owners of sheep during each visit when blood samples were collected from chosen herds. The questionnaire survey was conducted in seven localities of the state (SI, SE, SW, SU, DA, DI and Ab). In each locality fifteen samples of questionnaire had been taken, except SU locality from which ten questionnaire samples had been taken. A Total of 100 forms were distributed among state localities, in order to reach information pertinent to the aim of the study.

2.3.2 Retrospective Study:

The retrospective study depended on the monthly or annually available reports of the General Directorate of Animal Resources in Sinner state. Because Sinnar state has no, reports about veterinary hospitals, centers, or even veterinary clinics all over the state; it was so difficult to collect data for certain disease for 4 or 5 years ago; (poor reporting system). Also most of the diseases were tentatively diagnosed except when there is an outbreak, at that time samples were taken to confirm a diagnosis.
Accordingly, information regarding disease occurrence were obtained from the monthly and annual reports of PACE (Pan African program for Control of Epizootic) which is centered in Nairobi city and monthly reports were sent to it during the last four years from the Federal General Directorate of Animal Health and Epizootics. The diseases reported in the PACE reports were confirmed by a regional laboratory, central or even reference laboratory.

2.3.3. Serology:

2.3.3.1 Species Selection and Sampling Method:

The study animals that were sampled were sheep which were traditionally managed, from different sites in Sinnar state. Ages of study animals that were sampled are of value; here only ewes more than one year old were sampled. In addition, sampled herds should be resident in the locality that was chosen for sampling not less than six months. Also the number of animals per herd should be ranged between 200-300 heads, to take 10% sample. Moreover, herd or herds in the area selected should not be subjected to RVF vaccination during its/ their life. Accordingly, a total of 176 serum samples were randomly collected from seven herds in seven localities of Sinnar state (SI, SE, SW, SU, DA, DI, and Ab) as shown in table (4)

Here the state represents target population, and locality represents cluster from each cluster; one herd was selected randomly to represent target population.
Table (4): Summary of Samples Collection in Sinnar state.

<table>
<thead>
<tr>
<th>No</th>
<th>Locality</th>
<th>No. of samples</th>
<th>Date of samples collection</th>
<th>Location</th>
<th>No. of questionnaires</th>
<th>Animal Species</th>
<th>Age</th>
<th>Sex</th>
<th>Date of lab. diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Singa (SI)</td>
<td>25</td>
<td>1.3. 2008</td>
<td>Serage</td>
<td>15</td>
<td>Sheep</td>
<td>≥ 1year</td>
<td>Females</td>
<td>24.6.2008</td>
</tr>
<tr>
<td>2</td>
<td>Abuhugar (Ab)</td>
<td>28</td>
<td>8.3. 2008</td>
<td>Azaza</td>
<td>15</td>
<td>Sheep</td>
<td>≥ 1year</td>
<td>Females</td>
<td>24.6.2008</td>
</tr>
<tr>
<td>3</td>
<td>Sinnar East (SE)</td>
<td>24</td>
<td>15.3.2008</td>
<td>Kareema</td>
<td>15</td>
<td>Sheep</td>
<td>≥ 1year</td>
<td>Females</td>
<td>24.6.2008</td>
</tr>
<tr>
<td>4</td>
<td>Sinnar West (SW)</td>
<td>24</td>
<td>25.3.2008</td>
<td>Sugar area</td>
<td>15</td>
<td>Sheep</td>
<td>≥ 1year</td>
<td>Females</td>
<td>24.6.2008</td>
</tr>
<tr>
<td>5</td>
<td>Aldindir (D)</td>
<td>23</td>
<td>2.4. 2008</td>
<td>Azaza damose</td>
<td>15</td>
<td>Sheep</td>
<td>≥ 1year</td>
<td>Females</td>
<td>24.6.2008</td>
</tr>
<tr>
<td>6</td>
<td>Alsuki (SU)</td>
<td>25</td>
<td>8.4. 2008</td>
<td>Alguba</td>
<td>10</td>
<td>Sheep</td>
<td>≥ 1year</td>
<td>Females</td>
<td>24.6.2008</td>
</tr>
<tr>
<td>7</td>
<td>Alldaly (DA)</td>
<td>27</td>
<td>15.4.2008</td>
<td>Alsahba</td>
<td>15</td>
<td>Sheep</td>
<td>≥ 1year</td>
<td>Females</td>
<td>24.6.2008</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>176</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.3.3.2 Sample Collection:

Because (RVF) is one of the transmissible disease between animals and humans “Zoonotic disease “hygienic measure should be of high standard to prevent the researcher from infection. Hence, over-all coat, plastic Glass; gloves, mask and boots had been used during sample collection.

The apparatus used during sample collection are: A plain glass vacutainer without anticoagulant, tube holder, two ways needle with one side covered with plastic cover, Eppendorf tube for serum collection, centrifuge, ethanol 70% and sterile 3ml syringes.

To collect blood sample, the puncture area of the jugular vein was cleaned by 70% alcohol, vacutainer tube with tube holder and two ways needle was used; and then 5-10 ml of blood was withdrawn. The vacutainer tubes were labeled with number, and location e.g. SI 1, 2, 3…..etc SI indicate location which represent locality.

The tubes were put on a rack away from direct sunlight and racks were handled with care, especially during transportation from far distance places. After one hour the blood in vacutainer tube clotted and serum started to separate from clotted blood. If no clot occurred the vacutainer was kept in refrigerator over night at (4 C) then centrifuge for 3 minutes at 5000 r.p.m. Each serum sample was collected using sterile disposable 3 ml syringes in Eppendorf tubes, then stored at -20C till used.
2.4. Laboratory test:

The serological test conducted in the study was the Enzyme Linked Immuno Sorbent Assay (ELISA). It was used to test all the serum samples for the detection of antibodies, especially Immunoglobulin G (IgG). The ELISA diagnostic test adopted for detecting IgG antibodies in the serum of sheep in this study is based on the technique developed and validated in a previous study. ([Paweska et al., 2003](#)).

For detection of IgG antibodies, the Sandwich ELISA technique was adopted. As a control, high positive and low positive as well as negative control sera were used. The optical density was read at 405nm.

2.4.1. Technique:

The ELISA is based on a sandwich format in which the plates are coated with mouse anti- RVFV serum and then reacted with antigen. Test sera are applied and specific anti- RVFV IgG antibodies is detected with an anti-species IgG HRPO “Horse Radish per Oxidase Conjugate and ABTS substrate Anti-sheep HRPO can be used for detection of IgG in sheep and Goats. The reagents have been irradiated to inactivate RVF virus within the limit of sensitivity of the methods used to detect viable virus, hence, the products are safe.
2.4.2. The RVF ELISA kits:

The kit contains the following:

- Mouse anti RVFV serum “Coating antibody “1x100µl
- RVF antigen “RVFV Ag Freeze- dried 2x300µl
- Control antigen (control Ag) Freeze- dried 2x300µl
- Rabbit Anti-sheep1gG horseradish peroxides (HRPO) conjugate 1x100µl
- Control sera high positive (C)^++, low positive (C)^+ and negative (C)^-
- Control serum, freezed dried 1x200 µl each.
- Phosphate- buffered- saline (PBS) powder 20x Sackets
- Skim milk powder 2x50gms
- Tween 20 1x100ml
- Immuno plates 25x

ABTS –substrate, 3x100 ml
SDS- stops solution, 10x concentrated1x100 ml.

Note: For each day test the required volume / working dilutions of reagents should be freshly prepared from undiluted stock.

2.4.4: plate layout:

- C^^+ High positive control serum
- C^+ Low positive control serum
- C^- Negative control serum
1-40: Test sera

Rows A –D 1-12 RVF Antigen.

Rows E-H 1-12 control Antigens.

See diagram bellow
2.4.3. Preparation of Reagents and Working Dilutions:

PBS 0.01M PH 7.4 dissolves the required number of Sackets of PBS powder in sterile Distilled water, 1 sachet /1 litre of water.

- Wash Buffer: Dilute Tween 20 in PBS to final concentration of 0.1%.
- Diluents Buffer: prepare 2% skimmed milk in PBS.
- Blocking Buffer: prepare 10% skimmed milk in PBS.
- Coating antibody: Rehydrate in 100% µL of sterile distilled water.
- Antigens: Rehydrate each in 300µL of sterile distilled water.
- Control sera: Rehydrate each in 200µL of sterile distilled water.
* Working dilution of coating antibody (1:5000): prepare in PBS.

* Working dilution of antigen (1:400). Control and test sera (1:400) and conjugate (1:3000): prepare in diluents buffer.

* Substrate used as supplied.

* Stop solution: dilute 1:10 in distilled water.

2.4.5. Test procedure:

Unless otherwise stated, volumes used are 100µl /well, and all washes are performed 3 times for 15 s using 300 µl of wash buffer / well.

Steps:

1/ Coat plates with 100µl mouse anti-RVF serum diluted 1:5000 in PBS and incubate plates covered with lids at 4°C Overnight wash plates (3 times).

2/ Add 200µl /well blocking buffer and incubate for one hour in moist chamber at 37°C, wash plates (3 times).

3/ Add 100µl of RVFV Ag and control Ag diluted 1:400 in diluted buffer to rows A-D1-12 and rows E-H 1-12 respectively: incubate for one hour in moist chamber at 37°C wash plates (3 times).

4/ Add 100µl of test and control sera diluted 1:400 in diluted buffer into wells as shown in plate layout and incubate for one hour in moist chamber at 37°C wash plates (3 times).
5/ Add 100µl/well Anti-sheep IgG HRRO conjugate diluted 1:3000 in diluents buffer and incubate for one hour at 37°C in moist chamber. Wash plate (6 times).

6/ Add 100µl of ABTS/well. Leave plates for 30 min. at room tem. (22°-25°C) in dark. Add 100µL of concentrated SDS Step solution and read optical density at 405nm. (Diagram procedure) Fig. (3).

2.4.6. Results, Data expression, Acceptance Criteria, and diagnostic interpretation:

- The amount of color developed is proportional to the amount of anti – RVFV IgG antibody that has bound to RVF Ag and is available to react with the detection system.

- Net optical density (OD) values are first recorded for each serum as the values determined with RVFV Ag minus the value determined with control Ag. Three levels of micro plate’s acceptance are applied. The results on a test plate fulfill the first level of internal quality control (1QC) acceptance of at least three of the net (OD) values recorded for C++ fall within the range 0.8 (lower control limit) to 1.8 (upper control limit) if the results of two or more of the four replicates of C++ fall outside IQC limits then the plate must be rejected and repeated if the plate accepted, then the two intermediate net OD values of C++ are used for the calculation of the net
mean OD value of \( C^{++} \). This value is then used in subsequent calculation of percentage positivity (PP) of \( C^+ \), \( C^- \) and test sera as follows:

\[
PP = \frac{\text{Net OD (\(C^+, C^-\) or test serum)}}{\text{Net mean OD \( C^{++} \)}} \times 100
\]

(the results obtained on a test plates fulfill the second level of 1GC acceptance if the coefficient of variation (CV= \{SD\times100\} for pp value of two replicates of \( C^{++} \) (calculated from intermediate net OD values) and two replicates of \( C^+ \) are less than 15%) using the threshold pp values provided below. Both replicate of \( C^+ \) and \( C^- \) control Sera must fall within the same interpretive, group i.e. positives or negative (third level of 1GC acceptance). The same principals applied for the acceptance of individual test sera if they are assayed in duplicate. Threshold pp value for sheep and Goat sera \( \geq 11.1 \) pp and \( \geq 18 \) pp, respectively are considered to be positive and less than these values are considered to be negative. (Paweska, J.T. et al. 2003).
**Fig. (3): Flowchart for sheep and goats RVF Sandwich IgG-ELISA**

**Flowchart for Sheep and Goats RVF Sandwich IgG-ELISA**

1. **Coating (overnight):**
   - 100 μl/well, 1/1000 in PBS
   - Mouse anti-RVFV antibody diluted to plate
   - Wash 3x with PBS-0.1% Tween

2. **Blocking**
   - 200 μl/well, 10% milk powder/PBS
   - Add blocking buffer
   - Incubate 1 hour at 37°C
   - Wash 3x with PBS-0.1% Tween

3. **Add RVFV antigen & control antigen**
   - 100 μl/well, 1/400 in 2% milk powder/PBS
   - Incubate 1 hour at 37°C
   - Anti-RVFV antibody present in control and test sera binds to RVFV antigen
   - Wash 6x with PBS-0.1% Tween

4. **Add rabbit anti-sheep IgG-HRP conjugate**
   - 100 μl/well, 1/4000 in 2% milk powder/PBS
   - Incubate 1 hour at 37°C
   - Specific rabbit anti-sheep IgG binds to this sheep anti-RVFV immune
   - Wash 6x with PBS-0.1% Tween

5. **Add ABTS substrate/developer**
   - 100 μl/well, as supplied
   - Incubate for 20 min in dark at 22-25°C
   - Hydrolysis of ABTS by H2O2 causes green colour development in positive wells, the darker the colour, the greater the optical density

6. **Stop reaction by adding TBS**
   - 100 μl/well, 1% solution in distilled water

   **Negative result**
   **Positive result**

   **Read plate at 405 nm**
## Plate (0)

### Plate lay out and results

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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RESULTS

3.1. Questionnaire Survey Outcomes:

The results of the questionnaire survey in Sinnar state localities showed that (100%) of the owners agreed with the absence of slaughter houses in their area, while (55%) helped their animals during parturition, most of the owner’s (85%) used to drink boiled milk, while (100%) used to eat cooked meat.

There was good pasture situation (63%) at the dry season during conduction of this study (March to June). This is indicative of heavy rainfall before the period of study, which represent (100%) of the answers.

Vaccination program against RVF was not performed allover the state in previous years; accordingly presence of serum positive for IgG indicates that there is a moving virus. FAO (2002) and, OIE (1996) recommended that vaccination is the first line of defense for outbreak control. Abortion among the herds represented (47%) of the answers, while (53%) their answer was No abortion observed recently. However, deaths in herds constituted (84%) in newborn kids. Open grazing in traditional situation subjected all types of animal species to mix with each others; this was represented by answer of (93%).

Heavy rainfall is usually associated with the presence of dense populations of all types of insects, specially mosquitoes, which was represented in the questionnaire answers by (100%), while animals contact
with wildlife represented about (15%) in the area adjacent to Dindir National Park at the Eastern direction of the state.

Accordingly, favorable circumstances for virus replication and spread, such as rainfall (100%), mosquitoes presence (100%), absence of slaughter houses (100%) absence of RVF vaccination (100%), owners assist their animals during parturition (55%) presence of some cases of abortion (47%) and death of new born kids (84%), all these are suggestive for the presence of RVF virus.

The responses to the questionnaire survey among the sheep herd’s owners are summarized in table (5).
Table (5): Summary the Questionnaire survey responses by herds owners

**Sinnar State localities n (%)**

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<td>15 (100)</td>
<td>15 (100)</td>
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<td>15 (100)</td>
<td>15 (100)</td>
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<td><strong>6/ Pasture situation</strong></td>
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<td>0 (00)</td>
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<td>0 (00)</td>
<td>15 (100)</td>
<td>0 (00)</td>
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</tr>
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<td><strong>7/ Rainfall Quantities:</strong></td>
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<td>00.00</td>
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<td>15 (100)</td>
<td>15 (100)</td>
<td>10 (100)</td>
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<td>15 (100)</td>
<td>15 (100)</td>
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<td>00.00</td>
<td>00.00</td>
<td>00.00</td>
<td>00.00</td>
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<td>-------</td>
<td>-------</td>
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<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
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<td></td>
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<td>15 (100)</td>
<td>15 (100)</td>
<td>10 (100)</td>
<td>15 (100)</td>
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</table>

<table>
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<th>6 (40)</th>
<th>6 (40)</th>
<th>6 (60)</th>
<th>3 (20)</th>
<th>7 (40.3)</th>
<th>10 (66.7)</th>
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<td></td>
</tr>
<tr>
<td>B/ No</td>
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<td>9 (60)</td>
<td>9 (60)</td>
<td>4 (40)</td>
<td>12 (80)</td>
<td>8 (53.7)</td>
<td>5 (33.4)</td>
<td>53 (53)</td>
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</table>

<table>
<thead>
<tr>
<th>10/ Age of animals died:</th>
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<th>2 (13.3)</th>
<th>1 (6.6)</th>
<th>0.00</th>
<th>0.00</th>
<th>4 (26.6)</th>
<th>3 (20)</th>
<th>16 (16)</th>
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</thead>
<tbody>
<tr>
<td>A/ Adult</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/ Kid</td>
<td>9 (60)</td>
<td>13 (86.7)</td>
<td>14 (93.4)</td>
<td>10 (100)</td>
<td>15 (100)</td>
<td>11 (73.4)</td>
<td>12 (80)</td>
<td>84 (84)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>11/ Contact with other Sp</th>
<th>10 (66.7)</th>
<th>15 (100)</th>
<th>15 (100)</th>
<th>9 (90)</th>
<th>14 (93.3)</th>
<th>15 (100)</th>
<th>15 (100)</th>
<th>93 (93)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/ No</td>
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<td>0 (00)</td>
<td>0 (00)</td>
<td>1 (10)</td>
<td>1 (6.7)</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>7 (7)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>12/ Presence of Mosquitoes:</th>
<th>15 (100)</th>
<th>15 (100)</th>
<th>15 (100)</th>
<th>10 (100)</th>
<th>15 (100)</th>
<th>15 (100)</th>
<th>15 (100)</th>
<th>15 (100)</th>
<th>100 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/ Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/ No</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>0 (00)</td>
<td>0 (00)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>13/ Contact with wildlife:</th>
<th>0 (00)</th>
<th>0 (00)</th>
<th>0 (00)</th>
<th>0 (00)</th>
<th>0 (00)</th>
<th>15 (100)</th>
<th>0 (00)</th>
<th>15 (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/ Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/ No</td>
<td>15 (100)</td>
<td>15 (100)</td>
<td>15 (100)</td>
<td>10 (100)</td>
<td>15 (100)</td>
<td>0 (00)</td>
<td>15 (100)</td>
<td>85 (85)</td>
</tr>
</tbody>
</table>

n: number of owners (%) Percentage of owner
3.2. Retrospective study about RVF:

3.2.1. Veterinary Services Structure:

The General Directorate of Animal Resources works under the umbrella of the State Ministry of Agriculture and Animal Resources division which consist of:

1. department of animal health and clinics
2. department of animal production
3. department of epizootic control
4. department of fisheries
5. department of pharmaceutical affair.

The veterinary infrastructure has badly deteriorated and accordingly the monthly and annual reporting system does not reflect the accurate health situation of livestock.

3.2.2. Manpower:

Animal heath extension and training, and animal production are responsibility of the personnel who work in the General Directorate of Animal Resources. Table (6) show manpower in Sinnar state, year 2008.
Table (6): Manpower engaged in animal health services in Sinnar state.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Vets</th>
<th>Tech</th>
<th>Ass.</th>
<th>CAHW</th>
<th>Support Staff</th>
<th>Drivers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>State head quarter. (SI)</td>
<td>10</td>
<td>15</td>
<td>3</td>
<td>-</td>
<td>5</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>Sinnar West (SW)</td>
<td>6</td>
<td>11</td>
<td>3</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Sinnar East (SE)</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Alsuki(SU)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Alldindir (DI)</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>20</td>
<td>4</td>
<td>2</td>
<td>38</td>
</tr>
<tr>
<td>Alldaly (DA)</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Abuhugar (Ab)</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>38</td>
<td>13</td>
<td>20</td>
<td>22</td>
<td>7</td>
<td>130</td>
</tr>
</tbody>
</table>


3.2.3. Transportation:

Vehicles and cars are important to use for animal vaccination, follow up programs of sero-surveillance for the epizootic diseases, and investigation when there are outbreaks in certain area of the locality or state, also it is important for quick contact with diagnostic laboratory, or fast submission of samples to the nearer laboratory. For quick diagnosis, transportation is very important in Veterinary services and all vehicles should be in a good status to fulfill their functions all right. Table (7) show vehicles involved in veterinary services in Sinnar state.
Table (7): Vehicles involved in veterinary services in Sinnar State:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Lorries</th>
<th>Cars</th>
<th>Mobile Clinic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>State head Quarter / SI</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Sinnar West</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Sinnar East</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aldindir</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Alsuki</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Abuhugar</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alddaly</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>12</td>
</tr>
</tbody>
</table>

Note: Most of the vehicles aggregate in the state head quarter, where as some localities without any vehicle, so transportation is the main problem during control of any disease.

3.2.4. Previously reported RVF:

Most data of the retrospective study of RVF were obtained from the records of PACE, which is connected directly to the Federal General Directorate of Animal Health and Epizootic Disease Control; because of poor reporting system in Sinnar State. Table (4) represents reports about the main diseases in the state during the last four years (2004 – 2007).
Table (8): Reported diseases in Sinnar state during the last four years (2004-2007):

<table>
<thead>
<tr>
<th>Year</th>
<th>PPR</th>
<th>Bab.</th>
<th>H.S</th>
<th>Th.</th>
<th>RVF</th>
<th>Bab.</th>
<th>L.D.S</th>
<th>B.T.</th>
<th>CBPP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>66</td>
<td>131</td>
<td>246</td>
<td>114</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>591</td>
</tr>
<tr>
<td>2005</td>
<td>723</td>
<td>46</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>819</td>
</tr>
<tr>
<td>2006</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>12</td>
<td>52</td>
</tr>
<tr>
<td>2007</td>
<td>-</td>
<td>8</td>
<td>97</td>
<td>-</td>
<td>69</td>
<td>-</td>
<td>-</td>
<td>139</td>
<td>21</td>
<td>334</td>
</tr>
<tr>
<td>Total</td>
<td>789</td>
<td>185</td>
<td>392</td>
<td>114</td>
<td>69</td>
<td>24</td>
<td>16</td>
<td>149</td>
<td>73</td>
<td>1796</td>
</tr>
</tbody>
</table>

PPR= Peste de Petits Ruminant. Bab= Babesiosis. H.S. = Heamorrhagic septicemia


L.D.S= Lumpy skin disease. B.T. Blue tongue.

CBPP. = Contagious bovine pleura pneumonia.

Note II:

1/ Base for diagnosis of above mentioned, Bacterial or ricketsial disease in regional laboratory in Sinnar state and tentative diagnosis as field diagnosis before submission of sample to laboratory, while viral disease samples were sent to central veterinary laboratory.

2/ RVF and other arthropod borne viral disease appear during last year (2007) (year of RVF epidemic) in Sinnar state and mentioned as heamorrhagic fever and not confirmed as RVF.
3.2.5. Vaccination program:

Inactivated vaccines were used for immunization of animals against RVF disease, when the disease was suspected after epidemiological investigation. Only 10,000 and 5000 doses of inactivated RVF vaccine had been used during the last epidemic for sheep and cattle, respectively; followed by booster dose after one month.

3.3. Sero-prevalence of RVF in Sinnar state:

The 176 blood serum samples (25, 28, 24, 24, 23, 25, 27) from Singa, Abuhugar, Sinnar East, Sinnar West, Alldinder, Alsuki and Alldaly localities, respectively, were tested for detection of antibodies against RVF specially IgG which last for years after outbreaks (Alfadil A.A. and Ali, S.S; 2006).

In the overall state, only 17 samples were tested positive with a percentage of (9.7%) while 159 samples were tested negative with a percentage of (90.3%).

In the localities, Singa recorded one positive out of 25 samples with a percentage of (4%). Sinnar East recorded 5 positives out of 24, with a percentage of (20.8%) Alldalley recorded 3 positives out of 27 with a percentage of (11.1%), Abuhugar recorded 4 positives out of 28 with a percentage of (14.3%) and Sinnar west recorded 0 positive (0%) (Sinnar west locality is said to be free from the virus). Alsuki recorded 3 positives out of 25 samples with a percentage of (12) and Alldindir locality recorded one positive
out of 23 samples with a percentage of (4.2%). Table (9) shows the distribution of IgG positive serum in each locality and table (10) show the percentage positivity evaluation by locality.

The ELISA kits used in the diagnosis of IgG antibodies to RVF virus were prepared in Biological Diagnostic Supplies limited (BDSL) Scotland-United Kingdom. The ELISA diagnostic test adopted for detecting IgG antibodies in serum of sheep and goats was the Sandwich ELISA technique. As control high +ve. Low +ve as well as –ve control Sera were used.

Figure. 5. Shows Histogram of Sandwich ELISA result.

Figure.6. shows the result of plate’s photo.
### Table 9: Distribution of IgG antibodies positive serum percentage:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>% of positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abuhugar</td>
<td>24</td>
<td>4</td>
<td>28</td>
<td>14.3</td>
</tr>
<tr>
<td>Sennar West</td>
<td>24</td>
<td>-</td>
<td>24</td>
<td>0.0</td>
</tr>
<tr>
<td>Dindir</td>
<td>22</td>
<td>1</td>
<td>23</td>
<td>4.3</td>
</tr>
<tr>
<td>Sennar East</td>
<td>19</td>
<td>5</td>
<td>24</td>
<td>20.8</td>
</tr>
<tr>
<td>Dally</td>
<td>24</td>
<td>3</td>
<td>27</td>
<td>11.1</td>
</tr>
<tr>
<td>Singa</td>
<td>24</td>
<td>1</td>
<td>25</td>
<td>4.0</td>
</tr>
<tr>
<td>Suki</td>
<td>22</td>
<td>3</td>
<td>25</td>
<td>12.0</td>
</tr>
<tr>
<td>Grand</td>
<td>159</td>
<td>17</td>
<td>176</td>
<td>9.7</td>
</tr>
</tbody>
</table>

### Table 10: Percentage positivity evaluation by state localities:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Mean pp</th>
<th>SD pp</th>
<th>Min pp</th>
<th>Max pp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abuhugar</td>
<td>39</td>
<td>13.0</td>
<td>-15.3</td>
<td>57.4</td>
</tr>
<tr>
<td>Sinnar West</td>
<td>0.5</td>
<td>2.9</td>
<td>-3.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Dindir</td>
<td>0.4</td>
<td>5.2</td>
<td>-10.4</td>
<td>11.9</td>
</tr>
<tr>
<td>Sinnar East</td>
<td>4.0</td>
<td>10.0</td>
<td>-18.3</td>
<td>31.1</td>
</tr>
<tr>
<td>Dally</td>
<td>5.2</td>
<td>12.4</td>
<td>-0.4</td>
<td>54.0</td>
</tr>
<tr>
<td>Singa</td>
<td>1.2</td>
<td>6.0</td>
<td>-8.0</td>
<td>25.4</td>
</tr>
<tr>
<td>Suki</td>
<td>-0.5</td>
<td>16.9</td>
<td>-69.6</td>
<td>26.5</td>
</tr>
</tbody>
</table>
Figure: (5) Histogram of sandwich ELISA results - Sinnar state
Plates( 0 ) serum positives test photograph
Plate (1) serum positive test photograph:
Plate (2) serum positives test photograph:
Plate (3) serum positive test photograph:
Plate (4) Serum positive test photograph:
CHAPTER FOUR

4.1. DISCUSSION

Rift Valley fever is an arboviral disease transmitted by mosquitoes; it affects primarily ruminants causing high mortality in off-spring and abortions in pregnant females. The disease occasionally affects humans, with clinical picture that ranges from a mild febrile case to hemorrhagic fever with complications such as hepatitis and retinitis (Laughlin et al., 1979).

Epidemics of RVF in Southern Africa occur in 5-10 years cycles, but in dry semiarid zones of Eastern Africa the periodicity is 15-30 years. During prolonged droughts RVF virus may entirely disappear from large areas due to death of reservoir drought-resistant mosquitoes (FAO, 1995).

The disease was previously reported in Sudan based on serological survey that indicated presence of antibodies to RVF virus in animals and human sera, (Findlay, 1936, Eisa, and Obeid;, 1977, Eisa et al; 1980, Eisa, M. 1984, Watts et al., 1994, Suhair Kambal, 1997 and Tamadur, 2006). From all the data collected by the aforementioned authors, it seems that RVF was prevalent in subclinical form, but the lack of reporting system of the clinical cases did not reflect the real situation, because indigenous animals may not show clinical signs due to innate resistance (FAO, 2002). The virus activity
may be revealed by random isolations from mosquitoes or by occasional human disease, (FAO, 2002).

Many serological tests were used for diagnosis of RVF such as hemagglutination inhibition, complement fixation; agar gel immunodiffusion and neutralization test (Meegan and Bailey, 1989). These methods were replaced by IgG antibody-capture or antigen-capture ELISA. ELISA test has been developed with an inactivated viral antigen to provide safe and highly accurate alternate so it has the advantages over serum neutralization test which is specific and sensitive but time consuming and constitutes hazard to the laboratory diagnosticians as it requires the use of live virus (Swanepool and Coetzer, 1994) found that ELISA was sensitive and specific in detecting RVF viral antibodies in comparison with plaque reduction neutralization test (PRNT). Similarly, Niklasson et al., (1984) found a close correlation between ELISA and PRNT in detecting RVF viral antibodies in serum samples from human RVF vaccines. Moreover, serum neutralization and PRNT were not recommended for use outside endemic countries unless a high level of biocontainment was available in laboratories. In addition the IgG and IgM ELISA were
sensitive tests. There was no evidence of serological cross reactivity of RVF with other African phlebo viruses which could obscure the diagnosis of RVF (Swanepool and Coetzer, 1994). Also ELISA can detect earliest immunological responses to infection with RVFV and this is in agreement with (Paweska et al., 2003) who found the sensitivity of both ELISA (IgG and IgM) from 99.05% to 100% and from 99.1% to 99.9% respectively. It was also stated that; detection of IgM by ELISA was a reliable marker of infection. Antibodies appear 5 to 14 days after onset and coincided with clinical improvement (Peters. 2005).

In the present study, the results of the questionnaire survey revealed that, heavy rainfall all over the state (100%), mosquitoes population (100%), absence of slaughter houses in most area (100%), assistance of animals during parturition (55%), and death of kids (84%), were favorable factors for presence and spread of the virus(Elfadil et al.2004) and Le Duc (1989) stated that heavy rainfall and irrigation of fields by flooding, were ideal environmental conditions for breeding of the mosquitoes Aedes vexans arabiensis and Culex, the biological vectors responsible for transmission of RVF in Saudi Arabia in 2000-2001.(FAO ,2002). And Elfadil A, A. and Ali S, S. (2006) stated
that heavy rain fall and irrigated area are favorable conditions for mosquitoes breeding which incriminates in RVF transmission

According to *Vaughan, et al.,* (1996) who stated that malaria sporozoites can disrupt salivary glands barriers and enhance mosquito transmission of arboviruses. Taking together with similar studies using microfilarial parasite, it is increasingly apparent that mosquito-borne parasites have the potential to enhance mosquito transmission of arboviruses (*Vaughan, et al., 1996*). Despite the fact that all Sudan is endemic by malaria and in some areas filariasis, the endimicity of different species of mosquito vectors, the presence of susceptible animal species and in some areas in contact with human, occurrence of flooding and high rainfall from time to time; outbreaks of RVF is not uncommon.

The prevalence rate of IgG antibodies during 2008 in Sinnar state localities ranged from 4.2% to 20.8%. These percentages represent hazard all over the state when suitable environmental conditions occur. The results obtained in this study were in contrast with that reported by (Eisa 1984) who stated wide spread and high prevalence of RVF antibodies in Sudanese livestock, the results of this study show a wide and uniform distribution of
serum positive samples for IgG all over the state localities (table 9). This wide
distribution may be due to favorable environmental conditions occurred in the
rainy season of the year 2007.

The data from the State General Directorate of Animal Resources were
obtained with great difficulties due to the irregular reporting system in the
state. The result of retrospective study for previous four years (2004 – 2007),
according to the state general directorate, show lack or poor reporting systems
concerning epidemic disease in veterinary clinics, hospitals or centers, which
are not found all over the state, but the main source of information for reports
is PACE reports.

Manpower in the state who engaged in animal health services in Sinnar
State is very few in number with low qualifications status.

Accordingly, rearrangement of personnel distribution with the localities
in the state, increasing the number of manpower and employing qualified
personnel is highly needed. In remote areas, special care should be made to
provide personnel with all needs to do their work in investigation and control
strategies properly.

There are shortage in vehicles in the state, the available vehicles and
other facilities known from this study are unsuitable for running control
program for epidemic disease in the state, here after, many other problems facing control program of the epidemic disease, such as budget are not enough to mention the available vehicles, in addition owners refuse to vaccinate their livestock due to traditional opinions. The reports also show that, no, outbreak of RVF has been reported during the previous four year (2004 -2007) according to PACE reports, except during year 2007, when there were reports about a hemorrhagic fever but it was not confirmed as RVF at that time. The Federal Animal Health and Epizootic Control Department moved towards the state to investigate about the hemorrhagic fever with large investigation teams. The investigation covered all the state; they described the disease and its circumstances and its favorable conditions during that autumn clearly. However, the disease was not confirmed as RVF. As precaution the Federal Animal Health and Epizootic Control Department started vaccination program with the inactivated RVF smithburn vaccine in a line to separate the suspected area through a buffer zone of 50km from safe area. Ten thousands (10,000) and five thousands (5000) doses were used for small ruminants (sheep and goats) and cattle respectively, with 1 c.c. for sheep and goats and 2 c.c. for cattle. A booster dose had been given after one month. The distribution and used doses of RVF vaccine indicate the great shortage of the vaccination program as
compared to the total population of sheep in the state (approximately about (2.357.593 head).

Rift valley fevers outbreaks usually occur during heavy rainy season (OIE, 1996). Hundred percent of the respondents to the questionnaire agree with heavy rainfall and abundant mosquitoes population in the rainy season of the year 2007. The periods between outbreaks may extend to several decades during which it is difficult to diagnose cases of recent RVF infection except with special epidemiologic and laboratory techniques (OIE, 2002).

Herd immunity can be induced by natural infection or acquired through vaccination. It can be measured by detection of IgG antibodies in the animal sera (Elfadil A, A and Ali S, S. 2006).

In this Study, herd immunity was estimated by the percentage of IgG antibodies positive serum samples (9.7%). Since Sinnar state was affected by the 2007 outbreak of RVF and most animals in the state were not vaccinated with RVF vaccine neither attenuated nor inactivated vaccine, the level of IgG positive in this study indicate that there was natural immunity. The distribution of IgG positive samples by localities in sheep species is summarized in table (5).
The highest IgG positive percentage was reported in Sinnar east locality (20.8%), whereas the lowest positive percentage was reported in Sinnar west locality (0.0%) which is said to be free from the disease. The overall IgG positive percentage all over the state, according to this study was 9.7% which represents suggestive results for the presence of RVF in the state.

This study was conducted after suspected wave of outbreak in Sennar state (Nov. 2007), and the samples of the study were collected 3-4 months later i.e. March and April 2008 as it was summarized in table (4).

Duration of immunity to RVF induced by vaccination or natural infection was not thoroughly investigated. A previous report documented that immunity induced by vaccination of sheep with the live attenuated vaccine may last for three years (Loses G.J. 1986).

The results of a recent study indicated that 87% of sheep and goats vaccinated with the live attenuated RVF vaccine developed IgG antibodies by the fourth week following inoculation (Elfadil A, A; and Ali S, S; 2006).

The result of this study indicated that IgG antibodies were detected in 4.2 – 20.8% of animals all of them were not vaccinated with RVF vaccine in their life. These results indicated that these IgG antibodies are due to natural RVF infection.
Conclusion:

In conclusion, the result of the present study suggested the presence of RVF in Sinnar state, and the data collected by previous authors indicated that RVF in Sudan is prevalent in subclinical form, but the lack and poor reporting system together with deterioration of veterinary infrastructure did not reflect the real situations of livestock health status.

4.2 Recommendations

4.2.1. General Recommendations:

Based on the results of the present study, and due to the nature of the disease, any delay in the interference when an outbreak took place may result in wide spread of infection, human deaths and destruction of the national economy. Moreover, cost of control measures will increase, hence the following recommendations should be considered:

- Public awareness programmes are essential to keep the public fully and accurately informed.

- Surveillance involves examination of livestock at risk and serological monitoring of statistically significant samples at short intervals to determine if virus transmission is occurring.

- Vector studies may also be needed.
- Surveillance and vector control staff should be thoroughly trained in their roles, duties and responsibilities in RVF emergency.
- Application of FAO and OIE Recommendations which are:
  - Any necessary vaccination and/or insect vector abatements programmes are initiated.
  - Livestock movement restrictions are put in place.
  - Steps should be taken to ameliorate the spread and severity of the disease in people and animals in affected area.
  - Negotiations should be undertaken with importing countries with a view to minimizing international trade losses and to agree on measures to be taken before any bans can be lifted.
  - During an outbreak, restricted quarantine area should be established around infected area.
  - Sero monitoring studies should be conducted regularly to show the state disease mapping.
  - Lastly; mass vaccination against RVF especially in sheep species for exporting purposes.
4.2.2. Human Recommendations:

Given the current outbreak, humans are at increased risk of RVF. Hence, it should be recommended that humans take the following precautions:

- Use personal insect protective measures to avoid insect bites. 
  \((WHO)\)
- Avoid contact with domestic animals cows, goats and sheep and avoid contact with their products, \((WHO)\)
- Avoid handling raw meat. \((WHO)\)
- Avoid ingestion raw milk and milk products.
- Contact nearest physician when symptoms in chapter one (clinical signs) were observed

4.2.3 OIE Recommendations for importation:

When importing from infected countries, veterinary administration should require for domestic and wild ruminant, of a veterinary certificate attesting that: \((OIE\ 2001)\).

A. vaccinated animals:

* showed no clinical sings of RVF on the day of shipment.
* were vaccinated using a vaccine complying with the standards not less than 21 days and not more than 90 days prior to shipment.
* were kept in quarantine station in the country of origin for the 30 days prior to shipment and showing no clinical signs during that period.

B. **Unvaccinated Animals:**

* showed no clinical signs of RVF at the day of shipment.

* were subjected to diagnostic test for RVF with negative result 30 days before entry into quarantine.

* were kept in quarantine station in the country of origin for 30 days prior to shipment with no clinical signs.

* were subjected to diagnostic test for RVF with negative result not less than 14 days after entry into quarantine station.

* were protected from insect vector during quarantine and transportation to the place of shipment.
REFERENCES


Aradaib, I.E; Sawyer, M.M and Osburn B.l (1994). Experimental epizootic haemorrhagic disease virus infection in calves; Virologic and
serologic Studies Journal of Veterinary Diagnostic investigation, 6: 489-492.


*Food and Agriculture organization (FAO, 2002)* Preparations of RVF contingency plan chapter 1 and 4 Animal Health Manual Vol. (15) pp: 3-28

*Food and Agriculture organization (FAO, 2003)* News reports.


Ministry of Agriculture and Animal Resources-Sinnar state- Department of Information and Planning


PHAC, Public Health Agency of Canada – WHO 2007 Travel Health Advisory.


Web Site References: Web Site I Rift Valley fever manual. Empress livestock@fao.org.


- Web Site VI Outbreak of Rift Valley fever in Sudan www.phac.aspee.gc.ca/tmp-pmv/2
Appendix form 1

General Questionnaire

Rift valley fever outbreak investigations

1. State:__________________________ 2. Locality _____________________
3. Location: ____________________ Latitude_____________ Longitude_____
4. Animal owner_______________________ Village/Camp leader__________
who takes care of the animals at home?________________________________
5. Animal health problems__________________________________________
_______________________________________________________________
______________________________________________________________
6. Is there slaughter house/slab in your area? Yes (        ) No (        ).
7. Did you help your animals (any) during parturition? Yes (        ) No (        ).
10. Rainfall: poor (        ) normal (        ) heavy (        ) flood (        ).
11. Pasture situation________________________________________________
12. Age of affected animals: less than one year (        ) 1-2 year (        ) 2-3
year (        ) > 3 year (        ) All (        ).
Sex: Male (        ) Female (        ) All (        ).
14. Age of animals died: less than one year (       ) 1-2 year (       ) 2-3 year (       ) > 3 year (       ) All (       ).

15. Do your animals come in contact with other species No (       ) Yes (       ) Name them ____________________________________________________________

16. Are there any insects or rodents in your area? Yes (       ) No (       ). Name them ____________________________________________________________

17. Do your animals contact with wildlife? Yes (       ) No (       ).
Appendix II

Sample collection from locality

- State:-----------------------------------------------
- Animal owner:---------------------------------------
- Location:-------------------------------------------
- Animal species:-------------------------------------
- Date of collection:----------------------------------
- Total samples:--------------------------------------
- Observation:----------------------------------------