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Institutionen för biomedicin och veterinär folkhälsovetenskap

Sektionen för virologi

# Seroprevalence of Rift Valley Fever in domestic sheep and goats of Gaza province, Mozambique

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## SAMMANFATTNING

Rift Valley Fever är en sjukdom orsakad av ett *Phlebovirus* som kan infektera både människor och djur. Genom tiden har viruset skapat utbrott framförallt i Afrika, ibland med allvarliga konsekvenser. I början på 1930-talet upptäcktes viruset för första gången, i Rift Valley i Kenya. Sjukdomen var då beskriven som utbrott av plötsliga dödsfall hos lamm och tackor på en kenyansk farm. Sedan dess har viruset orsakat dyra (både med tanke på ekonomi och omkomna människor och djur) epizootier refererade som abortstormar och hög mortalitet hos idisslare. Hos människor ses influensaliknande sjukdom, med ibland allvarliga komplikationer.

Målet med den här studien var att samla in data om seroprevalensen av Rift Valley Fever hos domesticerade idisslare (får och getter) i Gaza-provinsen, användbara för framtida studier. Studien bestod av fältstudier, laboratorieanalyser och en litteraturstudie. För att undersöka seroprevalensen analyserades serum i en ELISA för detektion av specifika IgG antikroppar, baserad på en studie av Paweska et al (2003a).

Det här projektet var en förlängning av en redan pågående studie i Maputo-provinsen, med det generella målet att få reda på den aktuella situationen gällande Rift Valley Fever virus (RVFV), med hjälp av analys av prov från relevanta jordbruk. Just den här studien var fokuserad på Gaza-provinsen.

Fyra distrikt i den valda provinsen valdes för insamling av material: Xai Xai, Chibuto, Chókwè och Guija. Blodprov för serologi samlades in från en studiepopulation bestående av små hjordar av får och getter med mellan två till tjugo djur, med undantag för två kommersiella farmar. Djuren valdes ut slumpvis; oberoende av symptom eller ålder.

Det finns få studier gjorda om situationen av Rift Valley Fever i Mozambique men den information som finns indikerar att sjukdomen finns i landet. I den här studien var 126 prov av 800 (400 från får och 400 från getter) positiva för IgG antikroppar mot Rift Valley Fever virus, vilket ger en total seroprevalens hos får med 11 % (42 prov) och hos getter med 21 % (84 prov). Jämfört med studier historiskt sett hos domesticerade idisslare i Mozambique är det här generellt högre än på 1960-talet (2,8 % hos nötkreatur i Gaza-provinsen), lägre i jämförelse med får år 2007 (35,8 % hos får i Zambézia-provinsen) och lika gällande getter (21,2 % hos getter Zambézia-provinsen).

I den här studien, var distriktet/staden med högst seroprevalens Chibuto, med 21 % hos får och 42 % hos getter, vilket ger en total seroprevalens av 31 % (63 av 203 djur). Proven var insamlade på en kommersiell farm. Det här resultatet, med funna antikroppar och inga uppenbara symptom hos djuren, indikerar en enzootisk cykel av Rift Valley Fever i Gaza-provinsen.

Allvarligheten i utbrotten, avsaknaden av uppdaterade studier av Rift Valley Fever-situationen i Mozambique, den stora betydelsen av animalieproduktion och det faktum att det här utgjorde en avgränsad studie, gör det viktigt att fortsätta sjukdomsbevakningen och utvecklingen av metoder för identifiering av sjukdom, vad som redan är på gång vid University Eduardo Mondlane.

## ABSTRACT

Rift Valley Fever is a disease caused by a *Phlebovirus* affecting both humans and animals. During history the virus has caused outbreaks mainly in Africa, sometimes of severe nature. In the early 1930s, the virus was first discovered, in the Rift Valley in Kenya. The disease was initially described by sudden deaths of lambs and ewes on a Kenyan farm. Since then the virus has caused expensive (both considering economics and lives taken) epizootics referred to as abortion storms and high mortality rate among ruminants. In humans the virus causes a flulike disease, which can be associated with severe complications.

The aim of this study was to obtain data on seroprevalence of Rift Valley Fever in domestic ruminants (sheep and goat) of Gaza province, useful for future projects. The study included field studies, laboratory studies and a literature study. To obtain data on seroprevalence the sampled sera was tested in an ELISA for IgG detection of specific antibodies, based on the assay described by Paweska et al (2003a).

This project was an extension of a study ongoing in Maputo province, with the overall goal to assess the current situation of Rift Valley Fever virus (RVFV) by analyzing samples from relevant livestock rearing areas. This particular study was focused on Gaza province.

Four districts in this province were chosen for the sampling of material: Xai Xai, Chibuto, Chókwè and Guija. Blood samples were collected for serology from a study population consisting of smaller herds of sheep and goats of two to twenty animals with the exception of two commercial farms. The animals were randomly chosen; without regarding neither symptoms nor age.

There are few studies about the situation of Rift Valley Fever in Mozambique but available information indicates that the disease is present in the country. In this study, from the 800 samples (400 of sheep and 400 of goat), 126 samples were positive for IgG antibodies against Rift Valley Fever virus, what gives a total seroprevalence in sheep of 11 % (42 samples) and in goats of 21 % (84 samples). Compared to the seroprevalence seen historically in domesticated ruminants in Mozambique this is generally higher than in the 1960's (2,8 % in cattle in Gaza province), lower concerning sheep in 2007 (35.8% in sheep in Zambézia province) and similar concerning goats (21.2% in goats in Zambézia province).

As seen in this study, the district/city with the highest prevalence was Chibuto with 21 % in sheep and 42 % in goats, what gives a total seroprevalence of 31 % (63 of 203 animals). The samples were all collected at a commercial farm. This result, with antibodies found and no obvious symptoms seen, indicates an enzootic cycle of Rift Valley Fever in Gaza province.

The emergency of the outbreaks, the lack of updated studies of the situation with RVF in Mozambique, the importance of livestock production and the fact that this was a delimited study makes it highly significant to continue the surveillance studies and setting methodologies for identification of infections, what is already ongoing at University Eduardo Mondlane.

## **INTRODUCTION**

### **Aims**

The aim of this thesis was to obtain data on seroprevalence of Rift Valley Fever in domestic ruminants (sheep and goat) of Gaza province, useful for future projects.

On a personal level, the main aim was to learn more about the disease, especially from the viewpoint of livestock production, zoonosis, emerging disease and how it impacts on animals and humans in developing countries.

The study included field studies, laboratory studies and a literature study. To obtain data on seroprevalence the sampled sera was tested in an ELISA for IgG detection of specific antibodies.

### **Background**

This project was an extension of a study ongoing in Maputo province, with the overall goal to assess the current situation of RVF by analyzing samples from relevant livestock rearing areas. This particular study was focused on Gaza province.

This study was a Minor Field Study (MFS) that was mostly financed by Swedish International Development Cooperation Agency (Sida), Gulli Strålfeldts fond and the Swedish University of Agricultural Sciences (SLU). The study was performed in collaboration between SLU, the National Veterinary Institute (SVA) in Uppsala and the Veterinary Faculty of University Eduardo Mondlane (UEM).

Rift Valley fever (RVF) virus is a mosquito-borne virus related with epidemics in livestock and humans (Bird et al, 2009). The disease and the factors behind its emergence is something many African countries are working for to get a better understanding of. Epidemiological and surveillance studies have been performed in some of the exposed countries, with the help of collected samples from ruminants (Evans et al, 2008; Jeanmarie et al, 2011), humans (Niklasson et al, 1987; Munyua et al, 2010) and mosquitoes (Smithburn et al, 1948, Nabeth et al, 2001). The studies have also evaluated risks (Anyamba et al, 2010; Abdo-Salem et al, 2011), developed methods (Paweska et al, 2003a; Fafetine et al, 2007; van Vuren et al, 2007; van Vuren and Paweska, 2010) and considered transmission to new areas (Balkhy and Memish, 2003). It has been shown that a variety of virus strains can cause outbreaks (Nderitu et al, 2010).

RVF is known to exist in Mozambique since the 60's (Valadão, 1969) and is believed to be endemic and to cause periodic outbreaks in different areas of the country (FAO, 2003a). The emergency of outbreaks in East Africa and the lack of updated studies of the situation with RVF (prevalence, vectors, strains etc.) in Mozambique, gives an interest in setting methodologies for identification of infections and to perform surveillance studies. The Veterinary Faculty at University Eduardo Mondlane has been working to establish some of these methodologies, where RVF is used a good model to study a reemerging disease of ruminants with zoonotic potential. The studies comprise:

- a) Development of serological and molecular assays for use in epidemiological studies and for virus detection.



- b) Epidemiological surveys in cattle, sheep and goats in different production systems in Mozambique.
- c) Evaluation of the effect of current vaccines.

### ***The livestock revolution***

Because of the growing population and urbanization in developing countries, the demand for food of animal origin is increasing, referred to as “The Livestock Revolution”. By improving the agricultural productivity the opportunities for economic growth in rural areas can come; it can lead to a reduction of rural poverty and the opportunity to feed a growing population (Delgado et al, 1999).

### ***Mozambique and livestock***

Mozambique lies along the southeastern coast of Africa (see Figure 1) and is divided into 10 provinces and 128 districts (FAO, 2007). The country is one of the poorest countries in the world, but has after being a Portuguese colony during 500 years and having survived a bloody civil war (ended 1992), experienced a process of economic recovery. Of a population of 23 million people, around 75 % works in agriculture, which comprises 21 % of the country’s GDP (FAO, 2011).



*Figure 1. Mozambique (The World Bank, 2011).*

Even if the people living in absolute poverty have decreased since 1997 the poverty is a big fact (from 70 % to 54 %). Poverty is most common in the rural areas where 70 % of the poor household is found. In the rural areas there are lack of clean water, infrastructure, access to health facilities and schools. The agricultural productivity is very low, mostly depending on traditional farming methods, and since

the alternatives to agriculture as an income for poor rural households are very few, threats of food security have a big importance (Rural Poverty Portal, 2007).

According to FAO (2005), in year 2002, the population year of cattle was (in thousands) 1320, and of sheep and goats 517, in comparison to pigs (180) and poultry (28670), with the biggest product to be meat. In the rural areas animals and humans live and work close together, and livestock production consist mostly of local breeds and just a few commercial farms can be seen in the country, in the south (my own observations). The situation is different in the north, and it has to do with difficulty to raise cattle in tsetse-infected areas. Only commercial farms can afford the required treatments for trypanosomiasis, which means that these cattle farms are more common in the north, where this disease is prevalent.

The climate in Mozambique is tropical, with an average temperature of 28 degrees Celsius. The summer season occurs from October to April (World Weather and Climate Information, 2010-2011).

## **Rift Valley Fever**

Rift Valley Fever virus, a member of the *Phlebovirus* genus in the *Bunyaviridae* family, causes this disease (Nichol et al, 2005). The infection is affecting both humans and animals. During history, the virus has caused outbreaks mainly in Africa, sometimes of severe nature (Bird et al, 2009). In the early 1930s, the virus was first discovered, in the Rift Valley in Kenya (Daubney and Hudson, 1931). The disease was initially described by sudden deaths of lambs and ewes on a Kenyan farm. Since then the virus has caused expensive (both considering economics and deaths caused by the disease) epizootics referred to as abortion storms and high mortality rate among ruminants (Daubney and Hudson, 1931). In humans the virus causes a flulike disease, which can be associated with severe complications.

### ***History of Rift Valley Fever epidemics worldwide***

Since the 1970s, there have been epidemics in periods, reported from an increasing number of countries of eastern and southern Africa. The epidemics have also happened in new countries (where there has been no earlier virus activity) such as Egypt, Mauritania, Saudi Arabia and Yemen (Nderitu et al, 2011) (see Figure 2). Described here are some of the bigger outbreaks seen, concerning both animals and humans.

The first, of Daubney and Hudson (1931), described outbreak of Rift Valley Fever, also called “Enzootic Hepatitis” took place in Kenya in 1930. The outbreak took place at a sheep farm in the Rift Valley, near Lake Naivasha. The lambing period of this farm took place at another season than usual; a season with rare rainfall this time. The animals had not before been sick of major diseases. Before the lambing period, ewes aborted, what at first were thought to be due to Bluetongue. The mortality came up to approximately 3500 lambs and 1200 ewes. The symptoms were not very certain. The majority of the lambs died at an age of three days, and often within 24 after the symptoms was seen (loss of appetite, depression and weakness). The ewes either were ill for a short time before the death or were found dead. The cause of the deaths was proved to be a filterable virus.

At the post-mortem examination the lambs had a widespread necrosis of the liver. The ewes did not have this acute liver lesion; their liver changes were less spread. The spleen was not enlarged, what was known to be an important diagnostic to distinguish this disease from Nairobi sheep disease, bluetongue and heart water. Of experimentally affected newly born lambs, the death rate came up to 90 per cent. For pregnant ewes the deaths because of complications to abortions were 20 per cent. At this time, no goats had been reported in outbreaks. However, pregnant goats had been reported with abortions, in infected areas (ewes aborting). Cattle had also been seen to be able to get affected; one outbreak in a dairy herd had been observed.

During the investigation of this outbreak, the virus infected four Europeans. They got fever, felt sick, got headache and pain in joints. After that it came to notice that almost every human engaged in herding sheep during the outbreak had suffered from what seemed to be influenza for some days. It was proposed that

Rift Valley Fever could fall into a group with dengue and yellow fever, because of the similarities (Daubney and Hudson, 1931).

Mundel and Gear (1951) described an outbreak of Rift Valley Fever in humans at a farm outside Johannesburg. The five men that got ill had all taken part of a post mortem examination of a dead bull that had died acutely ill (fever, anorexia, abdominal pain). Two of the men held the animal's legs (did not handle the intestines) and the other performed the examination, without gloves. Post mortem examination of the animal exposed a necrosis of the liver. From the liver it was possible to isolate Rift Valley Fever virus. The men had symptoms like pain in the muscles, nausea, headache and fever. The incubation time was four days.

In August of 1977 in Egypt an epizootic identified as RVF occurred (Meegan et al, 1979). Domesticated animals, especially sheep, became ill, died and aborted and humans were also involved widely (between 20 000 and 200 000 cases) with different forms of the disease (acute febrile, encephalitic, ocular or fatal hemorrhagic). Animals affected were lambs, sheep, calves, cattle, domestic buffalo, goats and camels. When the winter came and the mosquitoes decreased in numbers, also the reports of the disease in humans and animals decreased. Abortions were seen mainly in sheep, cattle and camels. Antibodies were found in sera from many domestic animals but the total animal loss was not estimated but widespread. Goats appeared to be quite resistant. The source of the outbreak was suspected to be from the south (Sudan) by infected camels and other livestock.

Later, in 1979, Hoogstraal et al examined the trekking camels (from Sudan to Egypt) for antibodies for RVF virus and found that 30 % was serologically positive. This meant that the camels could have had a possible role of introducing the virus into Egypt.

The severe outbreak in Saudi Arabia year 2000 (Al-Hazmi et al, 2003) showed that the virus had spread outside the African continent to the Middle Eastern. It is unknown how many people got infected during this outbreak, but it is estimated to 20 000, with the most common feature being subclinical or mild. It was found that, of 165 with moderate to severe RVF, 151 patients suffered from vomiting or nausea and 122 of fever. 31 of 122 fever patients had associated rigors or chills. Abdominal pain was unusually seen. More uncommon features were hepatomegaly and splenomegaly. 124 of 165 patients were diagnosed with hepatic failure, which, in 74 patients, was associated with hepatic encephalopathy. Acute renal failure was the fact for 68 of 165 patients and hemorrhagic manifestations were seen in 32. Retinitis (5 patients) and encephalitis (7 patients) were more rarely seen, of which 6 patients died and one stayed in a wakeful unconscious state. 56 of the hospitalized 165 patients died. Associated complications were hepatic- and renal failure. The overall case-fatality rate was reported to be 17 %.

Balkhy and Memish (2003) documented the outbreak of RVF in year 2000 in Saudi Arabia and Yemen. According to this study, among a total of 882 confirmed human cases, 124 died. The severity of the disease and the high fatality rate (14 %) was suspected to be a consequence of underreporting of mild disease.

Wildlife has also been investigated further. A study report prevalence performed by Evans et al (2007) during the inter-epidemic period (IEP, the period when there

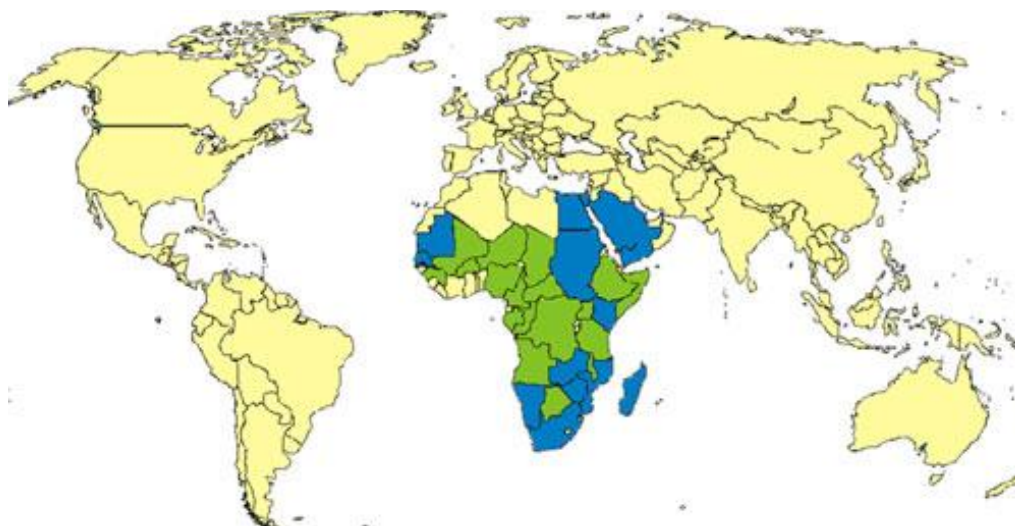
is low or none activity of the disease seen in livestock or humans) of 1999-2006, showed i.e. that >15 % of black rhinos and ruminants (kudu, impala, buffalo and waterbuck) had antibodies against RVFV. The highest titers were observed in buffalo and that included animals that were born during the period. The lions, giraffes, warthogs and zebras tested were either negative or had low titers of antibodies against RVFV. The sera, totally 1008 samples, were collected from 16 different species of wildlife in Kenya.

During October and December 2006, in the northeastern Province of Kenya, eastern Africa, heavy rainfall lead to perfect conditions for Rift Valley Fever to spread; the flood-water *Aedes* mosquitoes could increase in quantities because of the water. The outbreak of Rift Valley Fever was recognized by human deaths associated with high fever, headache, illness and hemorrhage. In the same region, illness and death could also be seen among animals. Suspected cases were taken care of at a hospital and it came to notice that most of them were young herdsmen, who had been in contact with ill ruminants. The symptoms accompanied with flooding, increased mosquito populations and the fact that sick animals lived in the same area, gave the suspicion of Rift Valley Fever. From November 2006 to March 2007, 684 human cases were reported, with a fatality rate of 23 %. Contact with blood and body fluids from viraemic ruminants was shown the most important way to contract the infection (WHO, 2007).



The outbreak spread to Somalia (114 human cases, fatality rate 45 %) and Tanzania (290 human cases, fatality rate 40 %). In all of these outbreaks an association with a suspicion of Rift Valley Fever among animals (abortions and deaths) could be seen (WHO, 2007).

Nderitu et al (2011) looked into these outbreaks in Kenya, Somalia and Tanzania to investigate reasons why the localized outbreaks occurred where they did. They suggested heavy rain, plane topology, water-absorbent soil types (higher risk of flooding) solid bush cover, many *Aedes* mosquitoes and high livestock populations to likely be responsible.

An investigation of the outbreak in Madagascar (2008) showed a suspicion of areas (the southern and northwestern part of the country) to be endemic for RVFV and the virus' amplification to more easily be performed in advantageous ecological conditions. Ruminants (both small and large) in Madagascar were sampled to find out how many animals had got infected during the Rift Valley fever outbreak. Sera were tested for both IgM and IgG against RVFV and the diagnostic used commercial ELISA. Past infections (presence of IgG and no IgM) were found in 25,8 % of cattle and in 24,7 % of small ruminants. Recent infections were less common with a seroprevalence in cattle of 0,3 % and in small ruminants of 3.3 %. In some areas no symptoms, neither in humans nor in animals, had been reported even if the virus was believed to have spread all over the country. In cattle it was showed an increased prevalence with the animals ages (Jeanmarie et al, 2011).



*Figure 2. Geographical distribution of RVF (CDC, 2011).*

	<b>Countries with endemic disease and substantial outbreaks of RVF:</b>	Gambia, Senegal, Mauritania, Namibia, South Africa, <b>Mozambique</b> , Zimbabwe, Zambia, Kenya, Sudan, Egypt, Madagascar, Saudi Arabia, Yemen
	Countries known to have some cases, periodic isolation of virus, or serologic evidence of RVF:	Botswana, Angola, Democratic Republic of the Congo, Congo, Gabon, Cameroon, Nigeria, Central African Republic, Chad, Niger, Burkina Faso, Mali, Guinea, Tanzania, Malawi, Uganda, Ethiopia, Somalia

### ***RVF in Mozambique***

According to reports from the 1960's, RVF existed with a seroprevalence of 2,8 % in cattle. During an epidemic in Gaza province in 1969, an amount of totally 134 bovines died. Of these 134 animals, 106 of them were found in the capital Xai Xai (Valadão, 1969).

Further on, in 1981-83, a serosurvey was carried out in pregnant women in Mozambique, from 8 of the 10 provinces. It was found that 28 (2%) out of 1163 women were sera positive for RVF virus specific antibodies (Niklasson et al, 1987).

Also in Mozambique, in 1999, in Zambézia Province, RVF was responsible for abortion in a herd of water buffaloes (Instituto Nacional de Investigação Veterinária, 1999).



*Figure 3. Herd of cattle in Xai Xai (personal photo).*

Later in Mozambique, in 2007, a serosurvey carried out in Zambézia Province in the central part of Mozambique showed that 35.8% of sheep sera and 21.2% of

goat sera contained neutralizing RVFV antibodies (Fafetine et al., 2012, unpublished results).

### **Clinical picture**

Pepin et al (2010) describes how to recognize RVF epizootics: “the large number of near simultaneous abortions among pregnant ruminants, regardless of the stage of pregnancy”. These “abortion storms” makes it possible to distinguish the disease from differential diagnosis like Q fever, salmonellosis, chlamydiosis, toxoplasmosis or listeriosis.

The virus can infect many species, including humans (i.e. zoonosis). Susceptible animals are sheep, goats, cattle, camels, water buffalos and other wild animals. Cats, dogs and rodents have also been seen to be susceptible (FAO, 2003b).

In summary, as seen from the outbreaks described in this thesis, animals' symptoms are fever, loss of appetite, depression and weakness. Other symptoms seen in animals are vomiting, nasal discharge, colic, diarrhea or hemorrhages. The symptoms depend on age (most severe in young animals) and animal species (sheep are most susceptible). There are different scenarios of the disease: peracute (found dead or suddenly weakens and dies fast), acute (short incubation period followed by the symptoms mentioned above, death within 1-3 days) and subacute (more likely in adult animals, the symptoms are fever, anorexia and weakness) (FAO, 2003b). As has been shown in studies, of experimentally affected newly born lambs, the death rate can be as high as 90 per cent. For pregnant ewes the deaths because of complications to abortions can be 20 per cent (Daubney and Hudson, 1931) and the risk of abortion when pregnant and infected can approach 100 % (FAO, 2003b).

In humans, the disease is most often asymptomatic (WHO, 2008). In case of clinical signs, they are most often influenza-like (fever, nausea, joint pain, headache etc.) (Daubney and Hudson, 1931). The overall case fatality rate for humans is estimated at <1 % and the fatal cases are associated with severe complications, especially hemorrhagic disease (WHO, 2008). The case fatality rate for humans has nevertheless been higher during the outbreaks described in this thesis and the clinical signs seen in severe disease have been retinitis, hepatic- and renal failure, encephalitis and hemorrhagic disease.

Human children, pregnant women and neonatal infants have not got affected in the same way as the ruminants, thinking of abortion storms and high fatality etc. (Madani et al, 2003).

### **Vectors and transmission**

During the first described outbreak, in Kenya 1930, the disease were suspected to transmit indirectly, because of the fact that the disease was not able to spread naturally between sheep in a laboratory. The suspected vector later was identified as *Taeniorhynchus brevipalpis* (Daubney and Hudson, 1931).

In 1944, the virus for the first time was isolated from a wild-caught mosquito (Smithburn et al, 1948). The longest period of demonstrated holding of the virus was 13 days. In the experiments it came to notice that the virus could be

transmitted (experimentally) from animal to animal (mouse and lamb) by the bites of mosquitoes.

The female mosquito of *Aedes lineatopennis* has been seen to be able to transmit the virus to her offspring via eggs. The virus can survive in the egg during dry periods and when flooding of dambo formations enables the eggs to hatch, the risk of outbreak of disease in RVF epizootic area increases. A theory is that the virus can maintain during the inter-epizootic periods by this transovarian transmission (Linthicum et al, 1985).

An infected mosquito can infect animals, so as direct contact with infected tissue (body parts or –liquids). The virus is present in large amounts especially in aborted material (Pepin et al, 2010). For humans, the most common way to get infected is exposure to organs or body fluids from infected animals, via inoculation (for example via broken skin) or through inhalation of aerosols (WHO, 2008). Also humans can get infected by mosquitoes, but less commonly.

RVFV can spread by a lot of different vectors, including mosquitoes, ticks and different flies (Daubney and Hudson, 1931; Labuda and Nuttall, 2004; Linthicum et al, 1985). During the inter-epizootic period, the ruminants' main transmission mechanism is most likely to be a bite by infected mosquitoes (Chevalier et al, 2010). The most competent vectors are considered to be genera *Aedes* and *Culex*, but up to 30 different species (7 genera) have been shown infected.

A connection is suggested between RVF infection in humans and consumption of raw milk. 21 % of the patients described in Saudi Arabia had consumed raw milk as a habit. It has been found that both milk and body fluids of domestic animals contained RVF virus in low concentrations. In the Mauritania epidemic this was considered as a connection (Al-Hazmi et al, 2003).

## Aetiology

The virus belongs to the genus *Phlebovirus*, family *Bunyaviridae*. As all viruses of *Bunyaviridae*, the RVFV is composed as enveloped single-stranded RNA (negative or ambisense) with a three-segmented genome titled L (large), M (medium) and S (small) (Nichol et al, 2005) (see Figure 4).

The genome segments encode four structural proteins (the viral polymerase on the L segment, two glycoproteins (G<sub>N</sub> and G<sub>C</sub>) on the M segment and the viral nucleocapsid protein (N) on the S segment) (Nichol et al, 2005).

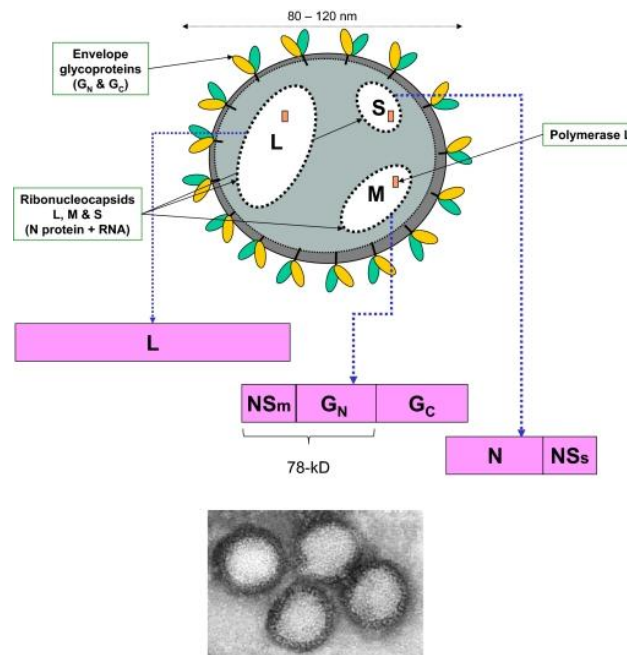


Figure 4. Schematic diagram of Rift Valley Fever virus (electron micrograph from Linda Stannard) (Pepin et al, 2010).

RVFV also expresses two nonstructural proteins (Bouloy and Weber, 2010).

Nderitu et al (2010) investigated the epidemic of 2006-2007 in eastern Africa (Kenya, Somalia and Tanzania) and found 3 different lineages of the RVF virus. According to Bird et al (2008), there are 7 main genetic lineages within the virus. These 7 lineages all contain distant originated virus strains.

RVFV has a genetic diversity that is relatively low (Shoemaker et al, 2002), what is not seen in some other viruses of *Bunyaviridae* (Carroll et al, 2010). The possible reasons why are suggested to be that mutations within the genome are very little tolerated by the virus or that the RVFV have a quite current common ancestor (Pepin et al, 2010).

## Virus detection and diagnosis of RVF

RVF can be diagnosed using virus isolation (Digoutte et al, 1989), detection of viral antigen (Meegan et al, 1989), nucleic acid techniques (different PCR techniques) (Garcia et al, 2001) and detection of specific antibodies (Paweska et al, 2003a). Here will focus on methods of antibody detection; the method used in the project.

Haemagglutination inhibition, complement fixation, virus neutralization and indirect immunofluorescence are referred to being classical methods of detection of RVFV antibodies (Swanepoel et al, 1986).

The ELISA used in this study was based on the assay described by Paweska et al (2003a). In the study they validated (in-house) “an indirect enzyme-linked immunosorbent assay for the detection of specific immunoglobulin G against Rift



Valley fever virus". 3055 sera from sheep, goats, cattle, African buffalo and some other wild ruminants were used in the study. Sera tested with VN were regarded as reference controls. After the optimization of the I-ELISA the diagnostic sensitivity (in %) was determined to 98,91 (sheep), 99,18 (goats), 84,31 (cattle), and 94,44 (buffalo). The diagnostic specificity was determined to 99,16 (sheep), 99,23 (goats), 99,34 (cattle) and 98,28 (buffalo). Virus neutralization was considered "extremely sensitive" and referred to as the best candidate for being a golden standard method. It was also described that cross-reactivity in serological assays has been seen under African settings.

Apart from the validation of the diagnostic, a study of kinetics of production of RVFV antibodies was made. In the kinetics study, sera from sheep, experimentally infected, were sampled 28 days post infection. Antibodies could be detected by all tests used (virus neutralization (VN) test, haemagglutination-inhibition (HI) test and indirect ELISA). The initial, short period of low antibody level was first detected by the VN. The following, high level of antibodies was easily detectable by the two other tests.

In the study performed by Fafetine et al (2007) the result showed that the N-protein based I-ELISA can be as good as VN and HI tests, concerning sensitivity and specificity in detecting RVFV specific IgM and IgG antibodies. The sera used were collected from experimentally infected, vaccinated and field-collected sera from ruminants (sheep, goats and cattle). The study also showed the N-protein to be safe, stable and cheap to use and to produce.

The N protein for detection of IgG and IgM antibodies was prepared and evaluated in another study too. Sera from humans (immunized with an inactivated RVF vaccine) and sheep (experimentally infected or vaccinated with the live attenuated Smithburn RVFV strain) were used. In experimentally infected (wildtype RVFV) sheep, it was seen that the IgG I-ELISA could, faster than virus neutralization and haemagglutination-inhibition test, detect the immune response earlier seen. In sheep, IgM antibodies were detected from day 3-4 post infection and IgG antibodies were detected from day 4-5. It was seen that the antibody response was slower in using the Smithburn vaccine strain than to wild type RVFV infection (van Vuren et al, 2007).

Van Vuren and Paweska (2010) compared different ELISA methods for the detection of RVFV antibodies. The sera used (from sheep) were preinactivated by a thermochemical treatment. The techniques compared were indirect-, IgG sandwich-, IgM capture- and inhibition ELISAs. The difference seen was associated with detection of the early humoral responses to an infection, which can be of importance considering recognition of an outbreak. IgM capture ELISA could detect humoral response 4 days p.i. (post infection), compared with 5 days for the IgG sandwich. The indirect IgG ELISA was less sensitive than IgG sandwich, a problem solved by using another conjugate. The high concentration of viral antigen in sheep p.i. gave false positives when using inhibition ELISA, but the ability to detect humoral response was the same as for IgM-capture ELISA.

### ***Prevention and vaccines***

According to Ikegami and Makino (2010) vaccinations effectively of ruminants and humans is the best way of controlling Rift Valley fever. Although, there are no licensed RVFV vaccines to immunize people generally in the affected countries. According to studies made, the ideal vaccine would be one that can safely be used, that quickly gives humoral immune responses, which neutralizes known RVFV strains and protects for a long time.

The immune response seen following infection, are antibodies mostly directed to the viral glycoproteins. IgM and IgG can also rise against the nucleoprotein and the nonstructural protein (McElroy et al, 2009).

After three to five days after the onset of RVF infection, IgM antibodies appear. IgG antibodies appear later, in 10-14 days, and can stay as long as lifetime or at least for one to two years (FAO, 2003b). In the majority of cases, IgM cannot be seen after 50 days post infection (Niklasson et al, 1984; Paweska et al, 2003b).

Formalin-inactivated RVFV vaccine requires repeated vaccinations to be able to induce and carry maximum protection (Pittman et al, 1999). As an alternative to this vaccine, virus-like particles (VLP) may be developed (Näslund et al, 2009). In mice, there is evidence that a protective immunity can be induced by administration of RVF VLPs.

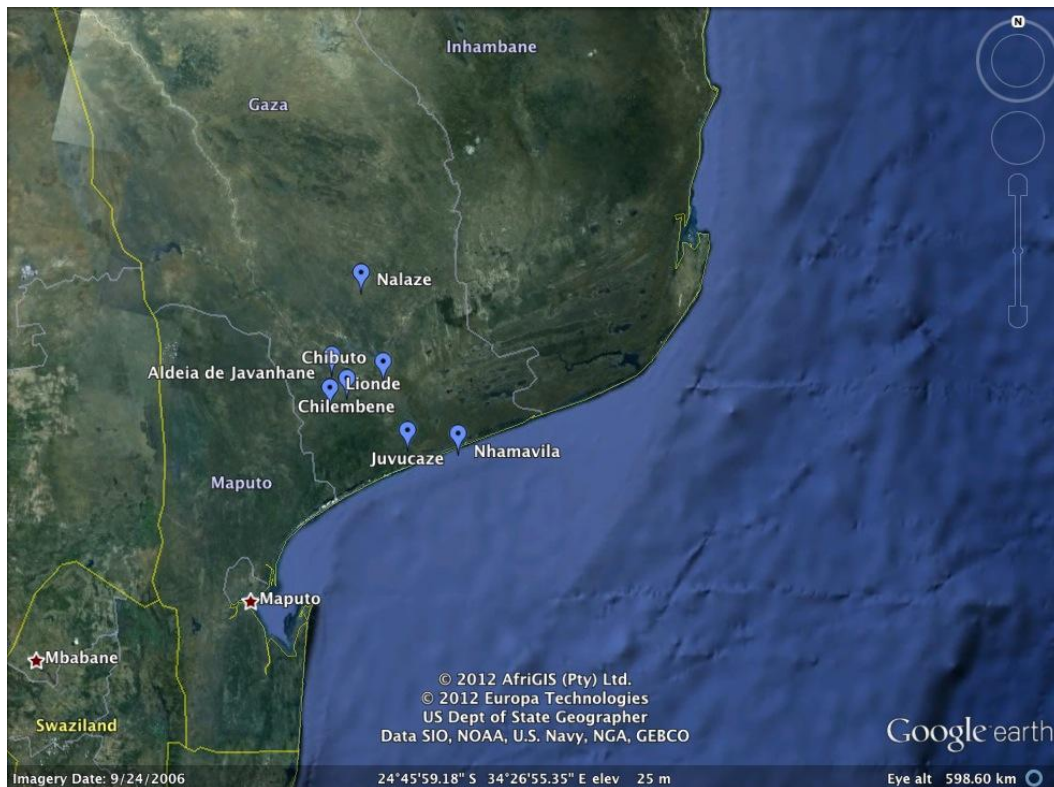
Live attenuated RVFV vaccines have shown a protective immunity without booster administrations, but an induction of abortion and teratogenic effects in newborn lambs, have been seen in early pregnancy in ewes (Hunter et al, 2002).

For human use, an inactivated vaccine has been developed. The vaccine has only been used experimentally, to protect persons at high risk for exposure to the virus (WHO, 2008).

## METHODS AND MATERIALS

### Study region and study population

The study region was Gaza province and four districts in this province were chosen for the sampling of material: Xai Xai, Chibuto, Chókwè and Guija. In each district the local supervisor had asked the farmers for permission and prepared an approximated trip for blood sampling.



*Figure 5. Map showing the locations of the sites for sampling of material.*

The study population consisted of smaller herds of two to twenty animals with the exception of two commercial farms for meat production where there were hundreds of sheep and goats. Some farmers held both goat and sheep and some held one of the species. The animals were randomly chosen; without regarding neither symptoms nor age.

Officially, the only Mozambican province that the National Veterinary Services vaccinate against RVF is Zambézia. However, in some coastal provinces where it is believed that RVF occurs, veterinarians do vaccinate against RVF in cattle (the owners and private veterinarians buy the vaccine in South Africa and vaccinate the animals). That happens in Maputo, Gaza (the study region) and other provinces; the reason why cattle were not included in the study population.

Samples were collected from both young and old animals, categorized as “baby” (<3 months), “young” (between 3 months and 1 year) and “adult” (>1 year), what was approximated. “Female” or “male” were also noted.



*Figure 6. Blood sampling in Chókwè (personal photo).*



*Figure 7. Bleeding a goat from the jugular vein (personal photo).*

The total number of samples was calculated to 800; 400 of sheep and 400 of goat; 100 of each from each place chosen (see Table 1). The sample size was calculated based on an estimated seroprevalence of 50%, confidence level of 95% and a maximum allowable error of 10%, giving a required sample size of 97. For each species: 100 animals per district (4 districts).

*Table 1. Number of serum samples from sheep and goats from each district.*

District	Total	Sheep	Goats
Xai Xai	205	105	100
Chibuto	203	102	101
Chókwè	202	100	102
Guija	190	93	97
All districts	800	400	400

From the district Xai Xai samples were collected in Juvuaze and Nhamavila, from Chibuto district Chibuto, from Chókwè district Lionde, Aldeia de Chilembene and Chilembene and from Guija district Aldeia de Javanhane and Nalaze (see Figure 5).

### **Blood sampling**

Blood samples for serology were sampled. The sampling material (syringes, needles, plastic holders, plastic tubes etc.) was mostly brought from Sweden. A plastic table, bag for carrying sampling material, disinfectant, soap etc. were bought or borrowed in Mozambique.

The blood was collected to serum tubes with vacutainer from the jugular vein, without shaving of the fur before the sampling. Each tube was marked with sex, age, G for goat and the date. For each place the district, owner of the farm and the geographic coordinates (GPS location) were noted in the lab book.

The serum tubes were kept in a cooling box while sampling, and then stored in the fridge at the accommodation to be separated (without being centrifuged before) one or two days later depending on how much time was present and how well the

serum was separated. Some of the blood had got haemolysed, supposedly because of the car transport (bumpy roads).

Back in Maputo, at the Veterinary Faculty of UEM, the serum was transferred to two new tubes (1,5 ml Eppendorf); one consisting of 200 microliters (working sera) and one consisting of the remaining sera (to store). The tubes were marked with numbers and G for goat sera, and then stored in the freezer (-20 degrees Celsius).

### Laboratory analyses

All laboratory analyses were performed at the serology lab at the Veterinary Faculty of UEM. The ELISA kit chosen was “Rift Valley Fever recN IgG Indirect ELISA” for domestic ruminants; a recombinant antigen based indirect ELISA for the detection of anti-RVFPV IgG antibody. The ELISA is based on the assay described by Paweska et al (2003a).

Some of the lab material (pipette tips of different sizes with stoppers, paper for the washing step, Eppendorf tubes for diluting sera, gloves etc.) was brought from Sweden.

The test procedure followed, with only one exception, the test procedure provided in the kit. The first 160 samples of sheep were tested in duplicates. The remaining samples were not tested in duplicates because of the too small (for that occasion) amount of RVFPV recN antigen.

#### Plate layout:

C++ High positive control  
C- Negative control serum  
CC Conjugate control  
1-40 Test sera

	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	C++	1	2	3	4	5	6	7	8	9	10
B	C++	C++	1	2	3	4	5	6	7	8	9	10
C	C-	C-	11	12	13	14	15	16	17	18	19	20
D	C-	C-	11	12	13	14	15	16	17	18	19	20
E	CC	CC	21	22	23	24	25	26	27	28	29	30
F	CC	CC	21	22	23	24	25	26	27	28	29	30
G	CC	CC	31	32	33	34	35	36	37	38	39	40
H	CC	CC	31	32	33	34	35	36	37	38	39	40

Figure 8. Layout of the ELISA plate.

The plates were coated with RVFV recN antigen (bacterially expressed recombinant RVFV antigen) diluted in Carbonate-bicarbonate buffer. The samples were diluted 1:400 in diluent buffer (2% skimmed milk in PBS).

The specific anti-RVFV IgG antibodies were detected with a recombinant protein G HPRO conjugate (binding the antibodies) and ABTS substrate (hydrolyzed by HPRO causing green color development in positive wells).

The washing of the plates was performed manually by turning the plate upside down and by washing of the wells by using approximately 300 microliters of wash buffer per well, three times.

The optical density was read at 405 nm. The darker the color, the greater the optical density, proportional to the amount of anti-RVFV IgG antibody bound to recN.

The results were analyzed in Excel. For the plate to be accepted, at least three of the C++ OD values had to be within the range of 0,9 (lower control limit) to 1,8 (upper control limit). The two intermediates of the OD values were used for the calculation of the mean OD value of C++, to be used in the formula to calculate the PP (percentage positivity) of C-, CC (conjugate control) and test sera:

$$PP = \frac{\text{meanODserum}(C-, CC \text{ test serum})}{\text{meanODC++}} \times 100$$

To be considered positive, the PP values of sheep- and goat sera had to be  $\geq 25$ . All values below this were considered to be negative.

## RESULTS

From a total of 800 samples collected from four different districts, 126 samples were positive for IgG antibodies against Rift Valley Fever, what gives a total seroprevalence of 16 %. The seroprevalence of sheep verses goats differed; of sheep 11 % (42 samples) and of goats 21 % (84 samples) were seropositive (see Table 2).

*Table 2. Seroprevalence of Rift Valley Fever in unvaccinated sheep and goats from each district.*

District	Total	Sheep	Goats
Xai Xai	9,3 % (19)	8,6 % (9)	10 % (10)
Chibuto	31 % (63)	21 % (21)	42 % (42)
Chókwè	12 % (25)	9,0 % (9)	16 % (16)
Guija	10 % (19)	3,2 % (3)	16 % (16)
All districts	16 % (126)	11 % (42)	21 % (84)

*Table 3. Seroprevalence of Rift Valley Fever in unvaccinated sheep and goats from each city of the districts.*

District	Total	Sheep	Goats
Juvuaze	9,5 % (18)	9,0 % (8)	10 % (10)
Nhamavila	6,3 % (1)	6,3 % (1)	-
Chibuto	31 % (63)	21 % (21)	42 % (42)
Lionde	16 % (12)	14 % (4)	17 % (8)
Aldeia de Chilembene	13 % (12)	9,8 % (4)	15 % (8)
Chilembene	3,2 % (1)	3,2 % (1)	-
Aldeia de Javanhane	18 % (8)	0	29 % (8)
Nalaze	7,6 % (11)	3,9 % (3)	12 % (8)
All districts	16 % (126)	11 % (42)	21 % (84)

*Table 4. Distribution of the different categories of sheep.*

District	Total	Female	Male	Adult	Young	Baby
Xai Xai	105	82	23	77	19	9
Chibuto	102	97	5	93	9	0
Chókwè	100	74	26	62	27	11
Guija	93	88	5	88	4	1
All districts	400	341	59	320	59	21

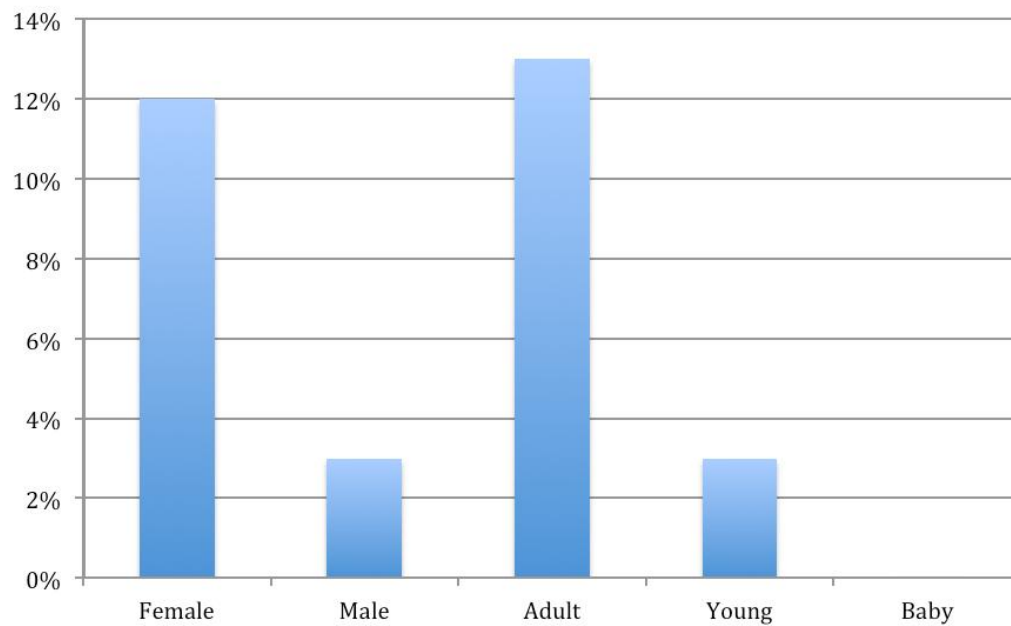


Figure 9. Percentage of seropositive sheep, of each category.

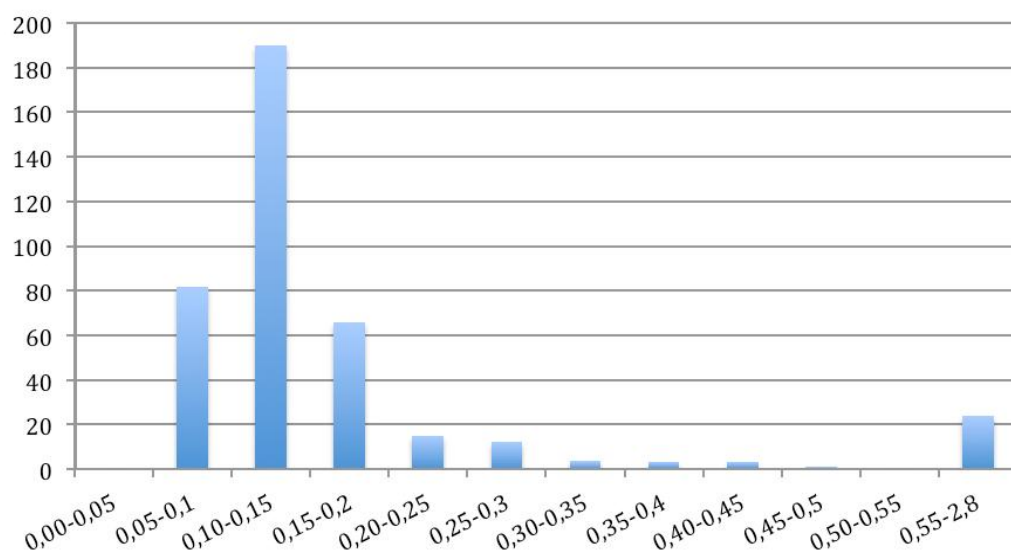
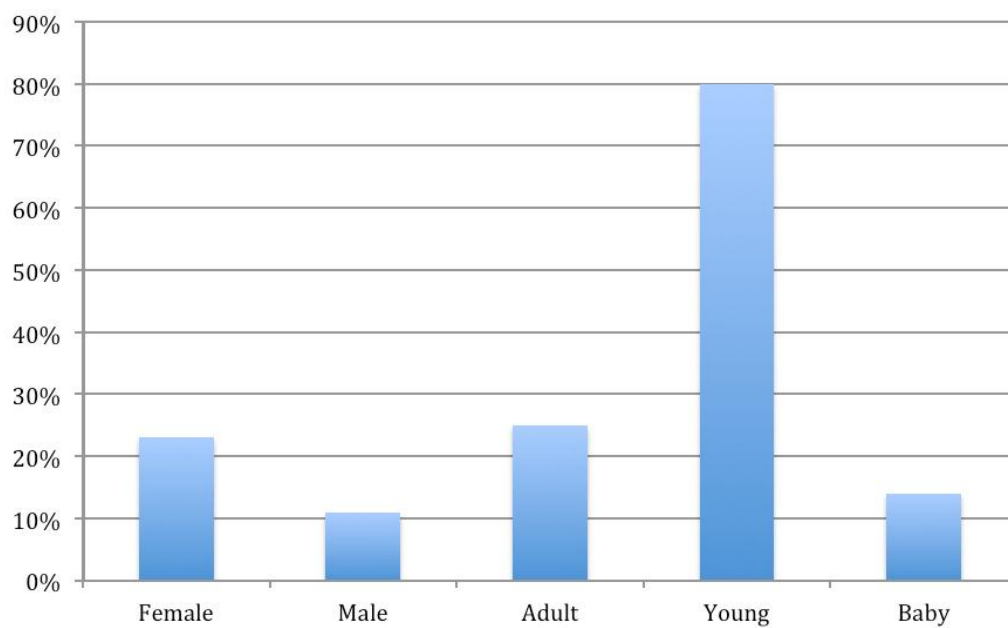


Figure 10. Histogram of OD values, sheep.

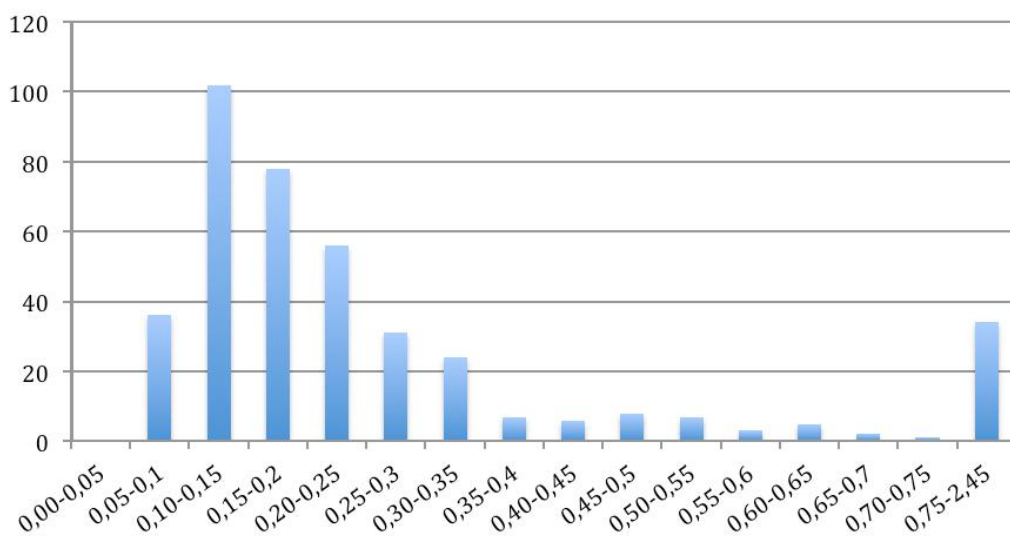
Table 5. Distribution of the different categories of goats.

District	Total	Female	Male	Adult	Young	Baby
Xai Xai	100	77	23	66	28	6
Chibuto	101	84	17	86	15	0
Chókwè	102	85	17	58	36	8
Guija	97	80	17	89	7	1
All districts	400	326	74	299	86	15





*Figure 11. Percentage of seropositive goats, of each category.*



*Figure 12. Histogram of OD values, goats.*

## DISCUSSION

### *Results and comparison with previous studies*

There are few studies about the situation of Rift Valley Fever in Mozambique but available information indicates that the disease is present in the country. In this study, from the 800 samples collected from the four different districts, 126 samples were positive for IgG antibodies against Rift Valley Fever, what gives a total seroprevalence in sheep of 11 % (42 samples) and in goats of 21 % (84 samples). Compared to the seroprevalence seen historically in domesticated ruminants in Mozambique this is generally higher than in the 1960's (2,8 % in cattle in Gaza province), lower concerning sheep in 2007 (35.8% in sheep in Zambézia Province) and similar concerning goats (21.2% in goats in Zambézia Province).

From the results it can be seen that female and adult has the highest percentage of being seropositive (see Table 4 and Table 5). The most of the animals were female and adult, maybe that's why they were most seropositive and maybe it is not possible to analyze the other categories because of the too small quantities? To be able to really compare, the number of different categories would have been the same.

As seen in this study, the district/city with the highest prevalence was Chibuto with 21 % in sheep and 42 % in goats, what gives a total seroprevalence of 31 % (63 of 203 animals) (see Table 3). The samples were all collected at a commercial farm.

What does these results say, is Rift Valley fever enzootic in Gaza province, Mozambique? The animals sure carry antibodies, and the question is where they have got infected. The expected results were that the animals would have antibodies, but with fewer antibody positive animals than would have been likely to see later in the season. The results indicate that the animals have been exposed to the virus and that the exposure may not be limited to the rainy season (when increased vector activity is expected). As example in the complementary studies looking at infection in vectors (Björkman, 2012, unpublished results), RVFV was detected by PCR in mosquitoes collected at the end of dry season. This suggests that vectors harbouring virus may transmit it in between rainy seasons.

According to Pepin et al (2010), an active circulation (but without evident signs of disease) of the virus among animals likely to be affected is considered to be "an enzootic cycle", with the transmission by mosquitoes to make the virus circulate. This could be the case according to the results of this project; with antibodies found, no obvious symptoms seen and the tentative explanation above as potential source of infection. Nevertheless, without having looked for clinical signs nor asked the farmers more specific about the animals' situation, one cannot exclude that symptoms could be present. It would be important to evaluate the determinant factors for an enzootic situation in follow up studies.

What causes an outbreak; what decides if an outbreak or not? According to history, Rift Valley Fever seems to have outbreaks in cycles. In between the outbreaks, the virus seems to circulate without giving any symptoms. As mentioned above this could be the current situation in Gaza province. It would be

interesting to investigate what keeps the virus circulating in between the outbreaks, and how the situation (climate conditions, topology, vectors, animals' situation and other factors) differs from the one during an outbreak.

Goats have appeared to be quite resistant (Meegan et al, 1979). But in this thesis the goats were more affected than sheep. The fact that goats show higher seroprevalence than sheep can depend on that there, from hearsay, are more goats than sheep in the sampled area.

### ***Method, analyses and possible sources of errors***

In this study IgG was the antibody analysed. The reason why IgG was tested, instead of IgM and instead of both, was that because of the budget for this project one test had to be chosen. When choosing IgG it was in thought of having the biggest chance to catch animals with antibodies; IgG shows past infections and the antibodies stays longer than the IgM, which are seen in recent infections. Of course, the optimal study would have been to test for both.

During the collecting of blood samples, the samples were treated as much as possible in the same way, but still some of them contained erythrocytes. The time from sampling to separating of the serum differed a bit, as did the roads (bumpy roads lead to shaking of the samples). How much this can have affected the results is hard to say.

The ABTS substrate was heated in 37°C for 30 minutes before use (the one exception of the test procedure provided) for a quicker reaction. Before this step, the OD's took longer time to get within the limits. How the ELISA functioned also depended on the weather; with higher temperature the reactions was quicker and vice versa.

When reading the plates, in some plates it was necessary to wait 15 more minutes (45 minutes) for the optical density of C++ to reach the value of 0,9 (lowest limit accepted). It was said in the protocol that this could be necessary; to pre-read the substrate plate (before stopping the color development) and when necessary incubate the plate for 10-15 more minutes and read again.

When it was decided which values to use in the calculations those with the OD's most within the range, as less difference between the OD's as possible and the values that gave the most positives was chosen. The positive samples will be sent to another laboratory to be analysed again, why the values chosen where the ones which gave the most positives as possible.

In some of the plates, something went wrong when adding the stop solution. In some of the samples the OD's differed more after adding the solution than before. One reason could have been creation of bubbles when adding the solution. When deciding which OD to have in the calculations, some OD's with the value less than 0,9 (lowest limit accepted) had to be chosen because of the alternative to be that the OD's differed a lot.

Usually, it is accepted that samples with higher OD have more IgG than the others with lower OD in ELISA. But since this test was not "really" quantitative, the

values of OD's were used only to say if the animals were positive or not (PP >25 was positive).

### ***Transmission and prevention***

The reason for the spreading of the disease could be climate changes with heavy rainfalls, the fact that trading with livestock is getting more and more common and the lack of knowledge of the risks of handling and eating meat from infected animals. The poverty is one thing that makes it difficult; the humans need the meat or the money from selling the meat, and sometimes that is from a sick animal. In Egypt it was seen that livestock trading was suspected to be a cause of the disease entering the country.

The highest risk to get affected has exposed individuals; ruminant animals in humid areas and farmers, slaughterhouse employees and veterinarians working with affected animals and -meat. Children, pregnant women and neonatal infants have not got affected like the ruminants with abortion storms and high fatality etc. (Madani et al, 2003). Maybe this is a fact because these categories of humans normally are not exposed to the material (infected tissue and body fluids etc.), which have been shown to be the main way of infection for humans.

To prevent the disease there are different ways. One strategy is to vaccinate animals, but with the problem that both the vaccines available has its disadvantages. Animal vaccination should be implemented prior to the outbreak because of if vaccinating when the outbreak have occurred, there is a high risk of intensifying the outbreak. Another way could be vector control, which could be complicated and extensive in thinking of extinction. Another way of vector control could be to protect the animals with help of mosquito-proof stables or to chemically try to decrease the vector amounts. Maybe the best way to prevent spreading the disease would be to monitor the disease and to teach about the virus and how to protect the farmers and their animals from it.

As Daubney and Hudson (1931) suggested, it could be useful to plan the farming in areas where there are less risks of spreading of the disease. For example, herd the animals at a higher, well-drained altitude to get away from the vectors. A problem with this is probably that in the rural, poor areas there are no possibilities to move because of the economics of building a new home etc. Perhaps, if the areas with high density of the vectors were sited, the animals living there could be vaccinated.

In summary, the suggestions for preventing disease in animals could be:

- Vaccination of animals, especially in exposed areas
- Movement of livestock to areas consisting of inferior conditions concerning spreading of the disease
- Mosquito-proof stables
- Put to sleep and remove all infected livestock
- Control of livestock trading
- Chemical control of vectors

***Extension of the study***

One of the farms, in Lionde in Chókwè, the farmer told us that they had had problems with abortions during a period. It would have been interesting to, in general, ask questions about the animal's situation to have in the results to analyze with the seroprevalence, but that was not the aim for this project. Except for this farm, no information about animals having health problems came to our notice and as far as we could see the animals looked healthy and in good condition.

## **CONCLUSION**

### **Future aspects**

Rift Valley Fever is a severe disease affecting both animals and humans. The disease also results in economic losses because of the abortions and death among infected livestock. The disease has, since 1931, had epidemics more and more frequently, mostly in Africa but has also spread to Yemen and Saudi Arabia.

One way of improving the agricultural productivity and economic growth is to keep animals healthy. Apart from confirming (by virus neutralization) the positive samples to ensure the result of the seroprevalence of Rift Valley Fever in Gaza province, it would be desirable to continue the work on the RVF situation in Mozambique. Other places of course can have a different picture. When the situation is surveyed, a plan easier can be decided of how to prevent the disease.

It would also be interesting to investigate the animals' health to have in the results and analyze with the seroprevalence. At the same time it could be an opportunity to teach about the disease and how to protect the farmers and their animals from it.

The emergency of the outbreaks, the lack of updated studies of the situation with RVF in Mozambique, and the fact that this was a delimited study gives an interest in continuing the surveillance studies and setting methodologies for identification of infections, what is already ongoing at University Eduardo Mondlane.

What would also be desirable to develop are more efficient vaccines for animals and to develop vaccines for humans. There are no licensed RVFV vaccines to immunize people generally in the countries (Ikegami and Makino, 2010). It would be interesting to know the difference in the prevalence before and after vaccination.

The risk factors for the disease to spread to non-endemic countries could be infected animals, mosquitoes and travellers (Ikegami and Makino, 2010). According to Chevalier et al (2010) the risk of getting widespread RVF in Europe is very low, but within the right conditions (humid area, large amount of ruminants) it could locally occur, and be a risk for exposed humans. The best way of preventing disease is suggested to be developing an efficient and coordinated way of monitoring and controlling the disease.

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