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**A screening for Schmallerberg virus
among sheep, goats and cattle in
Zambezia province, Moçambique
and preparations for a metagenomic survey of virus
in mosquitoes**

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A screening for Schmallenberg virus among sheep, goats and cattle in Zambezia province, Moçambique

En serologisk screening för Schmallenbergvirus hos får, get och nötboskap i Zambeziaprovinsen, Moçambique

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Summary SBV

As the world's population increases and people's living conditions are improving, larger areas are needed for houses as well as food production. This means that new areas are exploited and that people, livestock, blood sucking insects and wild animals are forced to live close together. In combination with the global warming, leading to extreme weather such as floods and storms, this allows both new and old pathogens to emerge. The number of "emerging infectious diseases", EIDs, has increased in recent years and many of them have their origin in poor, tropical countries. Moçambique is therefore considered to be a high risk area for EIDs.

Schmallenberg virus, SBV, was discovered in 2011 as an "emerging infectious disease" in dairy cows in Germany. The virus causes fever, diarrhea and reduced milk production in dairy cows as well as abortions and malformations in newborn calves. From these cows could SBV be detected using metagenomic technique. When the virus was identified in cows it soon was found in other ruminants, both wild and domestic. Hence it is mainly in cattle, sheep and goat the virus has led to problems and financial losses.

Phylogenetic studies showed that Schmallenberg virus is an Orthobunyavirus from the Simbu-serogroup. The virus is transmitted by biting midges, *Culicoides spp.*, and thru its vector it has spread to large parts of Europe.

The study done in Moçambique includes two parts, a serological screening for Schmallenberg virus in cattle, goats and sheep and a metagenomic study on viruses in mosquitoes in the Zambezia province. The samples were prepared and analyzed at the lab at the University of Eduardo Mondlane (UEM), Maputo, Moçambique. The metagenomic study will be completed in Sweden at the State University of Agricultural Sciences in Uppsala.

When screening for Schmallenberg virus an ELISA kit "ID screen Schmallenberg virus Competition Multi-species" was used. In the study a majority of the tested animals were positive. Most animals were also positive when the serum was diluted 1:2, 1:4 and 1:8. The largest numbers of positive animals were found among the cattle where 100% tested positive. Among the sheep the average prevalence was 63% and among the goats 84%.

This is the first study on Schmallenberg virus made outside Europe. In Europe, no viruses cross-reacting with SBV are known and therefore this is not investigated. However, on the African continent, there are a large number of viruses that Schmallenberg virus potentially could cross-react with. Therefore, to confirm the positive- ELISA result further studies need to be done, isolating Schmallenberg virus.

Sammanfattning

I och med att jordens befolkning ökar och människors levnadsvillkor förbättras krävs större ytor för så väl bostäder som livsmedelsproduktion. Detta gör att nya områden exploateras och att människor, boskap, blodsugande insekter och vilda djur kommer att leva närmare allt varandra. I kombination med den globala uppvärmningen med mer extremväder, så som översvämningar och stormar, gör det att både nya och gamla patogener kan få stor spridning. Antalet ”emerging infectious diseases”, EIDs, har ökat de senaste åren och många av dem har sitt ursprung i fattiga, tropiska länder. Därför anses Moçambique vara ett högriskområde för EIDs.

Schmallenbergvirus, SBV, upptäcktes 2011 som en ”emerging infectious disease” hos i mjölkkor Tyskland. Viruset gav upphov till en sjukdom med feber, diarré och sänkt mjölkproduktion hos mjölkkor. Från dessa kor kunde SBV detekteras med hjälp av metagenomisk teknik. En liten tid senare upptäcktes att viruset inte bara gav upphov till sjukdom hos vuxna djur utan även ledde till både aborter och missbildningar hos nyfödda kalvar. När viruset väl identifierats hos kor hittades det snart hos andra idisslare, både vilda och tama. Men det är framför allt hos produktionsdjur som kor, får och getter som sjukdomen lett till problem och ekonomiska förluster.

Fylogenetiska studier visade att Schmallenbergvirus är ett orthobunyavirus tillhörande simbuserogruppen. Viruset sprids med svidknott, *Culicoides spp.*, och har med vektorns hjälp spridit sig till stora delar av Europa.

Studien som gjordes i Moçambique inkluderar två delar, en serologisk screening för Schmallenbergvirus hos nötkreatur, get och får samt en metagenomisk studie på virus i myggor i Zambeziaprovinsen. Proverna preparerades och analyserades på labbet vid Eduardo Mondlane University, Maputo, Moçambique. Den metagenomiska studien kommer att slutföras i Sverige på Statens lantbruksuniversitet i Uppsala.

Vi serologisk undersökning med ELISA-kitet ”ID screen Schmallenberg virus Competition Multi-species” hade majoriteten av djuren SBV-neutraliserande antikroppar. De flesta djur var även positiva när serumet späddes 1:2, 1:4 och 1:8. Störst antal positiva djur fanns bland nötkreaturen där 100% testades positivt. Bland fåren var den genomsnittliga prevalensen 63% och bland getterna 84%.

Detta är den första studie på Schmallenbergvirus som gjorts utanför Europa. I Europa finns inga andra virus tillhörande simbuserogruppen varför inga korsreaktionstester är gjorda. På den Afrikanska kontinenten finns dock ett stort antal virus som Schmallenbergvirus kanske skulle kunna korsreagera med. För att fastställa ELISA-resultaten bör vidare studier göras där Schmallenbergvirus kan detekteras samt korsreaktionsstudier göras.

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INTRODUCTION

There are two different focus points of this project:

- To thru a serology screening examine if the Schmallerberg virus is present among sheep, goat and cattle in the Zambezia province, Moçambique.
- To survey the viral fauna in mosquitoes in the Zambezia province, Moçambique, using metagenomic techniques.

Schmallerberg virus was first detected in 2011 as an emerging infectious disease among ruminants in Europe. This is the first screening for Schmallerberg virus outside Europe.

Moçambique is one of the world's assumed hotspots for emerging infectious disease and to survey the viriom in the area is therefore of great interest.

LITERATURE REVIEW

Schmallerberg virus

History

During the summer and autumn of 2011 a new disease was seen among cattle in Germany (HOFFMANN *et al.*, 2012). The disease was spreading across Europe from areas close to the town Schmallerberg, the very same area where the Bluetongue virus first was found in 2008 (CONRATHS FJ *et al.*, 2013). The disease was at first presented with fever and milk drop among the cows and later on the virus was also linked to cases presented with diarrhea, abortion and malformations among neonatals. When all formerly known pathogens causing these symptoms had been tested and dismissed, blood samples from affected cows were analyzed using metagenomical techniques. A previously unknown Bunyavirus of the genus Orthobunyavirus was found in the samples. The virus was very similar to viruses from the Simbu sero-group and was named Schmallerberg virus (SBV) after the location where it first was found (HOFFMANN *et al.*, 2012). The discovery of the SBV was the first detection of a virus from the Simbu sero-group in Europe. Viruses from the Simbu group had previously been found in Africa, North and South America, Asia, Australia and Oceania (SAEED *et al.*, 2001)

The majority of the viruses in the Simbu sero-group have been isolated from vertebrate hosts as well as arthropod vectors such as midges and mosquitoes (SAEED *et al.*, 2001)

Many of these Simbu group-viruses are known to cause congenital malformations in ruminants which are referred to as "arthrogryposis hydranencephaly syndrome". Depending on the time of infection during the gestation the virus will result in abortions, stillbirths and/or congenital defects in the neonatal animals. An increased mortality in the dams may be seen due to difficulties during partus.

After the first finding of SBV in cattle, SBV-anti bodies have been found in ruminants (cattle, bison, sheep, goats, cervids), alpaca and dogs (SVA 2013).

Genetic properties

In the first phylogenetic analysis of SBV, many similarities between SBV and viruses in the Simbu-group of *Orthobunyaviridae* were seen (HOFFMANN *et al.*, 2013). When using electron microscope SBV also looked like a typical *Bunyaviridae* - it is a spherical, enveloped RNA-virus with a diameter of approximately 100 nm. Like other *Orthobunyaviridae* the genome is presented as a negative single stranded RNA divided in to three molecules, the small (S), medium (M) and large (L) segment (GOLLER *et al.*, 2012, NAKOUNE' YANDOKO *et al.*, 2007)

On the surface of the SBV as well as on other *Bunyaviridae* there are two glycoproteins, Gc and Gn, encoded by the M-segment (NAKOUNE' YANDOKO *et al.*, 2007). The M-segment also encodes a non structural protein (SAEED *et al.*, 2001). Since the M segment is coding for the viral glycoproteins that neutralizing antibodies in the infected host are directed against, this is the segment with the most pressure of selection. This leads to frequent re-assortment procedures concerning this segment. Every member from a group of *Orthobunyavirus* is able to exchange segments between one another thru re-assortment (CONRATHS *et al.*, 2013). This may lead to rapid genetic change in existing viruses and to the emergence of new Orthobunyaviruses (YANASE *et al.*, 2010, TARLINTON *et al.*, 2012).

Orthobunyaviridae also have a viral RNA-polymerase complex, encoded by the L-segment, responsible for the viral replication and transcription that occur exclusively in the cytoplasm of the host cell (NAKOUNE' YANDOKO *et al.*, 2007). The nucleocapsid protein, N, as well as a non-structural protein are encoded by the S-segment (SAEED *et al.*, 2001, NAKOUNE' YANDOKO *et al.*, 2007, GOLLER *et al.*, 2012).

The viral RNA-polymerase lacks a proof-reading function that is present in DNA-viruses. Due to that, point mutations will occur more frequent in RNA-viruses than in DNA-viruses. RNA-viruses also lack uracil-glucosylase, a repair enzyme; consequently the deamination of cytidine to uracil will often occur as a point mutation. The absence of these "security systems" leads to a great antigenic variation among Bunyaviruses and drive the formation of "escape mutants". Bunyaviruses are also able to do re-assort the gene segment, called antigenic shift, by exchanging parts of their genomes during a mixed infection. This makes these viruses more able to avoid the immune system of the host than the common virus (MOUTAILLER *et al.*, 2011, STEUKERS *et al.*, 2012).

Hoffmann et al (2012) suggested that SBV was formed due to re-assortment of viruses from the Simbu sero-group. In a phylogenetic analysis the most similar segment to the SBV S-segment was the S-segment from a Shamonda virus isolated from cattle in Japan. The sequence showed 97% identity (HOFFMANN *et al.*, 2012). Later phylogenetic analyses conducted by YANASE *et al.*, (2012) also indicated that the SBV is a re-assortant, with the M segment originating from Satupheri virus and the L and S segments originating from Shamonda virus

(YANASE *et al.*, 2012). Due to the lack of published sequences of Shamonda virus segment L and M, the phylogenetics of Schmallenberg is still uncertain (YANASE *et al.*, 2012, HOFFMANN *et al.*, 2012).

Transmission

The SBV is assumed to be an arbovirus, a vector borne virus, and has been found in *Culicoides* midges such as; *Culicoides obsoletus*-complex, *Culicoides dewulfi*, *Culicoides chiopterus* and *Culicoides pulicaris*-complex (DE REGGE *et al.*, 2012). Linden *et al.*, (2012) found that midges from *Culicoides spp.* had been able to transfer virus between and cause infection in sheep, goats, cattle, and red deer. Since the active vector period in Europe are spring- and summertime, the prevalence of Schmallenberg cases among newborns are highest in this area during late summer and autumn.

The virus can only be transmitted between animals thru the vector or vertically transferred via the placenta in a pregnant animal. The fetus is most likely to develop clinical symptoms if the mother is infected with SBV after the formation of the placenta (GARIGLANY *et al.*, 2013). However no clinical signs of infection are present in the fetus if the infection occurs when the immune system of the fetus has developed enough to control the infection (DOUCEUL V *et al.*, 2013).

Clinical signs will be seen in the neonatal animal if the mother is infected during the first part of the gestation, cattle: 0 – 5 months and goat/sheep: 0 – 3 months. If the mother is infected at the time for the neurological differentiation and development in the fetus the damage will be severe (STEUKERS *et al.*, 2012).

Clinical signs

The clinical signs differ between different species. Adult goats and sheep get very mild or clinical signs when infected by SBV, but the fetuses can get severe congenital malformations. In adult cows an infection with SBV is presented with more apparent symptoms; hyperthermia, mucous diarrhea, loss of appetite and milk drop – up to 50 % of the production. The viraemia of SBV is short, it only lasts for 2 to 6 days in cattle (HOFFMAN *et al.*, 2012) and an adult animal usually recovers within a few days (STEUKERS *et al.*, 2012).

If the dam is infected during the first trimester of the gestation the virus will transmit to the fetus which will develop malformations. These malformations most frequently lead to intrauterine death and abortion or death immediately after birth. Common malformations due to infection with SVB are neuro-musculo-skeletal disorder called arthrogryposis, severe torticollis, ankylosis, kyphosis, lordosis, scoliosis, brachygnathia inferior and neurological disorders such as amaurosis, ataxia and/or behavioral abnormalities – so called “dummies” (GARIGLANY *et al.*, 2012).

Diagnostics

An infection with SBV can be detected in two ways, via direct or indirect detection of antigen.

Direct detection – detection of the pathogen: The virus is detected in blood or tissue by real-time PCR or virus isolation by inoculating virus in special cell types. Blood samples for virus detection must be taken during the acute infection, while there is a viremia, when clinical signs are shown. Virus can be detected in EDTA blood or serum. The pathogen can also be found in abortions, stillbirths, fetuses and in material from malformed ruminants: brain, amniotic fluid and placenta (EFSA 2013).

Indirect detection – detection of antibodies: SBV-antibody detection by ELISA, indirect immuno-fluorescens or virus neutralization test. Antibodies can be detected in serum or EDTA blood. Samples for indirect detection can be taken from fetuses, stillbirths, abortions and in material from malformed ruminants: pericardial fluid or blood (pre-colostral), placenta and amniotic fluid (EFSA 2013).

Immunity

It is known that animals infected with SBV will be immune to infection after recovery, although at this time it is not known for how long the immunity will last (EFSA 2013). In areas where the prevalence of SBV is high, animals probably will be infected on pasture before they are old enough to breed. This will lead to an instant increase of cases of malformed fetuses and abortions if the SBV is introduced to a naïve population, which will return to normal when the area becomes endemic.

Profylaxis and treatment

Today there is one vaccine protecting against SBV available on the market, Bovilis SBV, which is an inactivated vaccine developed for sheep and cattle. The onset of immunity is three weeks after immunization but the duration of the immunity is at present unknown (Mereck animal health 2013).

An alternative to vaccinations is to make sure that susceptible animals are exposed to the virus and have developed immunity before they first are bread. This can be done by moving young naïve animals to SBV endemic areas. Other alternatives are alteration of sites and time for breeding, so that the dam is not infected during the first trimester of the gestation. A reduced numbers of vectors thru use of pesticides, drainage of wet lands, using repellents on the animals etc are also a way to protect animals from infection (STEUKERS *et al.*, 2012)..

At present, there are no available therapies for bunyavirus-induced diseases and the treatment is limited to supportive treatment of the clinical disease (STEUKERS *et al.*, 2012). Although an infection with SBV is quite mild, particularly in sheep and goats, the result can be not, only loss of offspring but difficult deliveries resulting in death of the dams.

Schmallenberg virus in Europe

Since the detection of SBV in 2011 a multiple of serology screenings have been conducted in Europe among ruminants. Results from these surveys indicate that the virus epidemiology on a herd basis is that most animals in a heard, from the same geographic area, either will be seronegative or seropositive (ELBERS *et al.*,

2012, MEROC *et al.*, 2013). A number of 1082 sheep from 82 herds were sampled in Belgium from November 2011 to April 2012, 98% of the sampled flocks were positive. The sero-prevalence within a positive sheep herd, ranged from 36,7% to 96,7% with a median of 89,3% (MEROC *et al.*, 2013). Results from this study indicate that the sero-prevalence of SBV-antibodies among sheep in Belgium is corresponding to areas where the densities of sheep are high and that the sero-prevalence among sheep is low in areas with low flock density (MEROC *et al.*, 2013).

EID – emerging infectious diseases

Background

The expression “Emerging infectious diseases” (EIDs) was defined by Lederberg *et al.*, (1992) as “*the infections that newly appear in a population, or which have existed but are increasing in incidence or geographic range*”. Most of the EIDs are of minimal global impact, but some of them have had a huge impact on both people and animals (e.g. the avian flu – H1N1, Nipha virus and HIV/AIDS) (GRACE *et al.*, 2012). When research is done on EIDs it is mainly the threat to the human society that is considered.

More than two thirds of the EIDs from the last thirty years originate from animals (GARGANO *et al.*, 2010), approximately 70% of these, from wildlife (JONES *et al.*, 2008). The interface of wildlife in an EID, e.g. wild animals being the natural host or reservoir of the pathogen, makes the control and prediction of the disease hard (GRACE *et al.*, 2012). Since most of the EIDs, 60, 3%, are zoonoses they are a threat to both human and animal health as well as to the economy. Therefore it is of great importance to have global surveillance and alarm systems to increase the possibility to react to outbreaks of an EID in an early stage (JONES *et al.*, 2008).

Globally over 25% of the annual deaths are directly related to infectious diseases. The victims are mostly people in developing countries, children in particular (MORENS *et al.*, 2004). In relation, less than 10% of the annual deaths in the EU are caused by primary infectious diseases (QUAGLIO *et al.*, 2012).

There is strong evidence of the association between EIDs and hunger, keeping of livestock and poverty (GRACE *et al.*, 2012). It is also well known that other factors increasing the risk for EIDs, such as tropical climate, vector-borne diseases and wildlife transmitting zoonotic pathogens, are concentrated in developing countries on lower latitude rather than in richer countries further from the equator (JONES *et al.*, 2008). Even so, the highest concentration of EIDs per area and the main hotspots for EIDs are found in the western of Europe, the northeastern parts of the United States, Australia and in Japan (JONES *et al.*, 2008). Jones *et al.*, (2008) assumed this to be the result of a reporting bias caused by financial and social factors.

Risk factors

To make it possible to control and prevent the emergence and re-emergence of diseases many researchers have tried to target the risk factors for these events. When trying to predict the emergence or re-emergence of a disease not only viral and microbial traits are of importance, but environmental and social factors should be considered as well (MORENS *et al.*, 2004).

Among the risk factors for EIDs in the human population, the ones below are ones most frequently listed. Most of these risk factors also apply on zoonotic pathogens as well as on EIDs strictly in the animal community, and should always be considered when the risk for an emerging disease is evaluated.

- The susceptibility to infection of the host (MORENS *et al.*, 2004).
- Weather and natural disasters e.g. flooding, earthquakes and hurricanes. (MORENS *et al.*, 2004, WEISS *et al.*, 2004).
- Climate – increasing global temperature, change in climate (MORENS *et al.*, 2004, WEISS *et al.*, 2004).
- Changing of ecosystems and use of land – destruction of natural habitat due to farming, deforestation, hunting, keeping per animals, pastures etc. (MORENS *et al.*, 2004, Desselberger *et al.*, 2000, SANJEEV *et al.*, 2012, CHUA *et al.*, 2000).
- Human behavior and demographics – unplanned migration from rural areas to urban areas and formation of peri-urban slums, change in personal behaviour (MORENS *et al.*, 2004, WEISS *et al.*, 2004, SANJEEV *et al.*, 2012).
- Rapid growth of population (SANJEEV *et al.*, 2012).
- Increased numbers of arthropod vectors (SANJEEV *et al.*, 2012).
- Poverty and famine (MORENS *et al.*, 2004).
- Social inequality (MORENS *et al.*, 2004).
- Intense global trading and travel (SANJEEV *et al.*, 2012).
- Poor or lack of sanitations (SANJEEV *et al.*, 2012).
- Political ignorance, lack of political will and lack of economical resources (MORENS *et al.*, 2004, WEISS *et al.*, 2004).
- Breakdown of public health measures or inadequate public health infrastructure (MORENS *et al.*, 2004, SANJEEV *et al.*, 2012).

Arboviruses as EID

Arboviruses are virus transmitted by arthropod vectors, e.g. mosquitoes, midgets and ticks. The vector borne viruses have great impact worldwide and are responsible for 22,8% of the events of emerging infectious diseases (JONES *et al.*, 2008). Zoonotic agents transmitted by arthropod vectors have repeatedly been ranked among the EIDs of greatest importance, e.g. hantavirus pulmonary syndrome and arenavirus haemorrhagic fevers (Argentine, Bolivian, Venezuelan and Lassa haemorrhagic fevers) (MORENS *et al.*, 2004). Many of the risk factors

listed above may lead to a change in numbers and exposure to arthropod vectors that can carry pathogens infecting humans and animals (SANJEEV *et al.*, 2012).

The factor of greatest importance to spread of arbovirus is the climate. The global warming leads to a higher average temperature and change in weather, e.g. causing flooding, which not only allows a wider spread of vectors, changes in vector ranges and hosts but an increase of the vector population (Hollidge *et al.*, 2010). For example outbreaks of Rift valley fever virus have been linked to the weather phenomena ENSO (El Niño–Southern Oscillation) (GRACE *et al.*, 2012).

GARGANO *et al.*, (2010) listed 7 vector-specific factors that determine whether a vector borne pathogen has the capacity to emerge or re-emerge in an area:

1. Presence of vector's preferred breeding habitat at the site of pathogen's origin
2. Vector's preferred host
3. Efficiency of vector
4. External incubation period
5. Possibility for vector of surviving to the second blood meal
6. Possibility of transovarial transmission of pathogen
7. Ability to remain infectious during time

Moçambique – a risk area for EIDs?

Since the middle of the 20th century the social and political situation in Moçambique has been unstable. A violent emancipation from the Portuguese in the 1970ies was directly followed by a civil war not ending until 1994. From the declaration of peace in 1994 to this day the political situation has been unsettled and a large proportion of the population is living in grave poverty. According to the FAO about 70 % of the population in Moçambique is living at the level of absolute poverty. Agriculture is the main activity in Moçambique. This engages about 80 % of the population, mostly dominated by breeding and herding livestock, keeping poultry and growing crop at a small scale (FAO 2012).

Moçambique is situated in an area of the world that is exposed to multiple climate threats due to the global warming (GRACE *et al.*, 2012). During the annual rain period parts of Moçambique get flooded and consequently uninhabitable, and some years a large amount of people are forced away from their homes. This is assumed to happen more frequently as the global temperature is increasing (GRACE *et al.*, 2012).

It is proven that many re-emergences or emergences of diseases have been catalyzed by wars, unstable political situations and natural disasters e.g. flooding (MORENS *et al.*, 2004) and that it is most probably that EIDs will originate from areas at lower latitudes with deficient reporting (JONES *et al.*, 2008). This makes Moçambique a possible hotspot for emerging diseases.

With a steadily growing population of more than 25 000 000 people (WHO 2013) the need for animal products, such as milk and meat, is increasing in Moçambique. With the increasing quantity of livestock, new areas are exploited and used for pasture and farms. This leads to new contacts and interaction between domestic animals, humans, wildlife and arthropod vectors. Interface like this is known to be one of the factors of greatest importance when diseases are emerging (Siembeda, *et al.*, 2011). New contacts are also established due to farming, hunting, camping, deforestation and other types of change or destruction of habitats. This creates many new opportunities for diseases to emerge (DESSELBERGER 2000).

Emerging virus surveillance and readiness

Today the majority of the resources for surveillance of EIDs are focused to countries where the diseases are least likely to emerge. To be able to spot new diseases before they emerge at a large scale the resources for monitoring should be re-allocated from the low risk areas, where they are focused today, to high risk areas such as poor tropical countries. This would be profitable for the high risk countries as well as for the low risk countries (JONES *et al.*, 2008). For an efficient surveillance system the key is to have early warning systems that work at both national levels as well on an international ones (SANJEEV *et al.*, 2012).

Active search for emerging viruses make it possible to prevent future outbreaks. This can for example be done thru big screenings of plants, animals and humans, focusing on risk groups or risk areas, using new techniques such as metagenomics (KING *et al.*, 2006, DAZAK *et al.*, 2000).

Metagenomics

Background

Viruses can be detected and identified using many different techniques. Before the arrival of molecular methods, virus detection such as serology, inoculation studies, vaccination, electron microscopy, filtration and cell culture were used (MOKILII *et al.*, 2012). Except for the traditional methods of virus detection there are two types of molecular methods to discover virus:

- Sequence-dependent methods e.g. PCR and microarrays - which requires a prior knowledge of the viral DNA and RNA to detect novel virus (MOKILI *et al.*, 2012).
- Sequence-independent viral metagenomic methods – which do not require any prior knowledge of the virus in the sample (MOKILI *et al.*, 2012).

Viral metagenomics is unlike other methods for viral detection since no prior knowledge of the viral genome is required. This makes it possible to characterize whole viroms in a cell culture-independent and unbiased way (BLOMSTRÖM 2011). Delwart described metegenomics in 2007 as “*a conceptually related set of methods relies on sequence-independent amplification, subcloning and sequencing of purified viral nucleic acids followed by in silico searches for sequence similarities to known viruses*”. When applied on samples collected from the environment or used to analyze unmanipulated biological material it is called

viral metagenomics. Viral metagenomics might also be used as an umbrella term to describe non-specific amplification and sequencing of viral DNA and RNA from samples where unknown viruses are suspected to be present (DELWART 2007).

The first use of viral metagenomics was in 2002 in San Diego where the virome of two marine locations in the littoral zone was characterized (BREITBART *et al.*, 2002). Since then, many studies have been done in this field, but only a small part of the virome is yet detected.

Viral metagenomic analysis contains these main steps:

- Sample preparation – preparing of the sample by removal of contaminating nucleic acids, e.g. nucleic acids from the DNA or from bacteria, and concentration of the viral nucleic acids (DELWART 2007).
- Sequence-independent amplification – multiplication of the nucleic acids in the sample. This shows the actual genetic viral constitution of the sample (DELWART 2007).
- High-throughput sequencing – determination of the order of nucleotides within a nucleic acid molecule using data bases containing a huge amount of genetic information, so-called platforms. These large scale sequencing platforms include a variety of amplification and sequencing techniques and make it possible to do a large number of sequence reads. A great advantage with this technique is that the sequencing can be done without an initial cloning of the genetic material. (BLOMSTRÖM 2011).
- Bioinformatics – through *in silico* analyses the huge amount of data is assessed, comparing previously known viral sequences and “markers” to the sequences from the sample, aiming to find sequences from known and unknown viruses. This might be the hardest and most time consuming part of a metagenomic study (BLOMSTRÖM 2011).
- Follow-up – often further characterization and isolation of the identified virus has to be done as well as studies on prevalence, experimental infections and development of diagnostic tests. The follow-up is of great importance to understand the importance and significance of the finding (BLOMSTRÖM 2011).

Area of use

Throughout history viruses have caused large outbreaks and pandemics with high economical costs as well as many deaths. To avoid this, or at least to decrease the impact of a highly pathogenic virus in case of an emerging new disease, it is of great importance to discover, characterize and identify it fast (GRENINGER *et al.*, 2010).

Ever since the first virus was discovered, tools for identification and detection have been evolving. With the sequence-independent techniques of today it is possible to characterize whole viral communities e.g. from seawater samples (BREITBART *et al.*, 2002), samples from the gastrointestinal tract (ALLANDER

et al., 2007) as well as to characterize pandemics rapidly (GRENINGER *et al.*, 2010).

Viruses are known to be important sources of EIDs both in animals and in humans. Preventively screenings and early detection using are the keys in an effective prevention from emerging infectious diseases (CLEAVELAND *et al.*, 2001). This is a good application for the use of viral metagenomics.

The main advantage of using viral metagenomic techniques is its unbiased way to rapidly identify novel viruses (DELWART 2007). Hopefully the future will provide better methods to separate viral and host DNA/RNA as well as developed bioinformatics.

According this, metagenomic techniques, are an optimal way to survey the virom in mosquitoes in the Zambezia province.

MATERIAL AND METHODS

Collection of samples

The sample collection was done in the Zambezia province in Moçambique during two weeks in September 2013. Blood were taken from the jugular vein from 86 cows, 402 sheep and 336 goats. The blood was collected using a vacutainer system and serum tubes. When separated from the whole-blood, the serum was transferred into micro tubes containing about 2 ml each. The blood and serum were kept in a cooling box with ice during transportation and in a refrigerator at + 4°C in the lab.

For the SBV screening adult females were chosen for the serology since they were in majority as well as the oldest animals. Therefore they were the ones were the most likely to be exposed to the virus. Table 1, table 2 and table 3 are presenting the sampled numbers of animals and coordinates for the farms for cattle, sheep and goats respectively.

Tabell 1

Species	Farm	Coordinates	Samples
Bos indicus	Cuacua - Rogerio	S: 17.80069 E:035.41220	36
Bos indicus	Nicoadala - Amed	S: 17.60421 E:036.82723	49

Table 1 is presenting the farm's coordinates and the number of sampled cattle on each farm.

Tabell 2

Species	Farm	Coordinat es	Sampl es
Sheep	Cuácuá -	S:	98

	Rogério	17.79748 E:035.4144 2	
Sheep	Mopeia south	S: 17.84602 E: 035.74150	55
Sheep	Nicuada ala - Mucelo	S: 17.70626 E: 036.89647	196
Sheep	Quelim ane - Padeiro	S: 17.82279 E: 036.92272	44
Sheep	Nicuada ala - Amed	S: 17.60421 E: 036.82723	8

Table 2 is presenting the farm's coordinates and the number of sampled sheep on each farm.

Tabell 3

Species	Farm	Coordinates	Samples
Goat	Cuacua - Rogério	S: 17.79748 E: 035.41442	9
Goat	Deda	S: 17.98278 E: 035.76248	5
Goat	Quelimane - Padeiro	S: 17.82279 E: 036.92272	36
Goat	Nicuada - Amed	S: 17.60097 E:036.8293 2	104
Goat	Nicuada - Mingano	S: 17.80573 E: 037.13612	38
Goat	Quelimane - Dona ana	S: 17.83348 E: 036.93911	63

Table 3 is presenting the farm's coordinates and the number of sampled goats on each farm.

Mosquitoes were collected in the area of Cuacua-Rogério (S: 17.80069 E: 035.41220) using WHO's light trap, originally designed to trap mosquitoes from the genus *Anopheles*. The traps were placed shielded from wind directly adjacent to the animal's night time shelter. A picture of the trap is to be seen below in figure 2. The traps were used for 5 nights. In table 4 the number of collected mosquitoes from each species on the particular dates is on display.

Tabell 4

Date	Site	Coordinates	Light trap	Anopheles	Culex	Monsonia	Outros	Total
2013-09-25	Cuacua Lodge	S: 17 79 748; E 03541442	Trap 1	46	105	62	33	246
2013-09-26	Cuacua Lodge	S: 17 79 748; E 03541443	Trap 1	25	19	66	0	110
	Cuacua Lodge	S: 17 79 748; E 03541444	Trap 2	2	98	77	0	177
	Cuacua Lodge	S: 17 79 748; E 03541445	Trap 3	6	59	31	0	96
	Cuacua Lodge	S: 17 79 748; E 03541446	Trap 4	65	700	107	0	872
2013-09-27	Cuacua Lodge	S: 17 79 748; E 03541447	Trap 1	8	70	39	0	117
	Cuacua Lodge	S: 17 79 748; E 03541448	Trap 2	166	1365	778	0	2309
2013-09-28	Cuacua Lodge	S: 17 79 748; E 03541449	Trap 1	12	500	100	0	612
	Cuacua Lodge	S: 17 79 748; E 03541450	Trap 2	15	50	400	0	465
	Cuacua Lodge	S: 17 79 748; E 03541451	Trap 3	2	40	25	0	67
Total				347	3006	1685	33	5071

Table 4 shows the exact number and site of the collected mosquitoes.



Figure 2 shows the light trap used for collecting mosquitoes.

After the trapping the mosquitoes were euthanized with ether, separated according to genus (*Culex*, *Monsonia* and *Anopheles*) and put in ethanol 99, 8 %. During the transportation the mosquitoes were kept in a cooling box with ice. At the lab they were stored in a refrigerator at + 4°C.

Questionnaire:

The questions were asked by Dr José Fafetine in Portuguese to the farmers/ the animal keepers. Their answers were translated to English by him as well.

The asked questions were:

- How many animals do you have?
- Have you seen any health issues in your animals during the last year, or before?
- Any abortions?
- Any misshaped fetuses?
- Cases of death in newborns?
- Do you vaccinate any animals?

Lab techniques

The lab work was done at the veterinary faculty in Maputo, University of Eduardo Mondlane, Moçambique.

ELISA

An ELISA-kit from ID. vet was used screen for SBV amongst sheep, goat and cattle in Zambezia province. The kit is called “ID screen Schmallenberg virus Competition Multi-species” and is developed to detect SBV antibodies in cattle, sheep and goat. Information about the ELISA can be found at ID vets’ website: www.id-vet.com. The protocol included in the kit was followed and initially 50 µl undiluted serum was used. The microplate was read at 450 nm. A part of the tested samples were re-tested in various dilutions. As dissolvent PBS, diluted 1x, was used. The different dilutions were: 1:2, 1:4, 1:8, 1:100, 1:200 and 1: 400.

Metagenomics

The samples used for metagenomic analyzes were the collected mosquitoes. The steps of the metagenomic analyses done in Moçambique were:

- Sample pre-preparation
- DNA extraction
- DNA labelling
- Random amplification of labelled DNA

Due to outer circumstances such as dysfunctional pipettes and power failure the samples will be sent to Sweden and the remaining steps will be done there.

Sample pre-preparation

Since the mosquitoes were stored in ethanol they were washed in distilled water and dried on filter paper before further use. The mosquitoes were divided in to 5 aliquots per genera with 20 mosquitoes in each micro tube. Then 1, 5 ml DNase buffer (100 U) was added to the aliquots and using an electric mortar (VWR International) the samples were homogenised. The homogenate was centrifuged 2 x 5 minutes at 4000 rpm and then the supernatant was transferred to clean tubes. The supernatant were divided in to 4 aliquots, 2 of the volume of 200 µl for DNA extraction and 2 of the volume of 250 µl for RNA extraction, to which 750 µl Trizol was added. The RNA tubes were stored in a -80 degrees freezer until transported to Sweden for RNA extraction and metagenomic analyzes. To the DNA tubes 2µg RNase A and 100 U DNase I was added and then incubated in a 37°C water bath for 2 hours.

DNA extraction

DNA extraction was done using “QIAamp DNA mini kit” where the included protocol was followed exactly except from the last step, the elution. The DNA was eluted in 50 µl elution buffer (included in the kit).

DNA labelling

The DNA labelling was done using a “mini cycler”.

Used reagents were:

- 2 µl FRoV26-N (10 µM) (GCCGGAGCTCTGCAGATATCNNNNNN)
(ALLANDER *et al.*, 2005)

- 1, 5 µl dNTP (10mM each)
- 1, 5 µl NEB2 buffer (10X)
- 10 µl DNA
- 0, 5 µl Klenow Fragment (3'-5' exo-)

A master mix was made and the mix distributed to micro tubes together with the DNA. The tubes were put in the micro cycler at 94°C for 2 minutes and after that put directly on ice for 2 minutes. Next 0, 5 µl Klenow Fragment (3'-5' exo-) was added and the micro tubes were put in the cycler again: 37°C for 60 minutes 94°C for 2 minutes and after that directly put on ice for 2 minutes. Thereafter 0, 5 µl Klenow Fragment (3'-5' exo-) was added and the tubes once again put in the cycler: 37°C for 60 minutes and 75°C for 10 minutes. The labelled DNA was stored in the micro cycler at + 4 °C over the night until the next morning when the random DNA-amplification was done.

Random amplification of labelled DNA

PCR master mix:

- 5 µl buffer (10X)
- 5 µl MgCl₂ (25 mM)
- 1 µl dNTP (10 mM each)
- 4 µl FR20 (10 mM) (ALLANDER *et al.*, 2005)
- 0,5 µl AmpliTaq Gold polymerase (5U/µl)
- 32 µl H₂O

+ 2.5 µl template

For each labelled DNA aliquote 5 separate reactions were run from the *Culex* samples and the *Monsonia* samples. From the *Anopheles* samples only 4 reactions were run due to pipette problems.

The used PCR-program was:

95°C for 12 minutes; 40 cycles of - 95°C for 30 seconds, 58°C for 30seconds, 72°C for 90 seconds; and a final 10 minutes elongation step at 72°C
5 µl PCR product was run on 1, 5% gel with etidium bromide (130V, 90 mA for 20 minutes)

The remaining PCR product was stored at -20°C until sent to Sweden.

Sources of errors

- The pipettes in the lab were not calibrated and therefore the volumes were not right at all times.
- Unknown numbers of power breakdowns during the incubation times which disturbed the temperatures.

RESULTS

ELISA

The tested animals were adults and the majority of them were females. The sero-prevalence varied between the different farms, but animals from all species on every farm were tested positive for SBV-antibodies in the “ID screen Schmallenberg virus competition Multi-species” ELISA-kit. 10 animals from each species that first tested positive when the serum was undiluted all tested negative when the serum was diluted 1:100, 1:200 and 1:400.

Cattle

Cattles from two different farms were sampled. All of the tested animals, 36 from Cuácua – Rogerio and 43 from Nicoadala – Amed, were sero-positive for SBV-antibodies. In the tables below, table 5 and table 6, the prevalence's from the two farms are presented. The average prevalence was 100% among the cattle. The results in table 1 are from ELISA-reactions with undiluted serum.

Tabell 5

Species	Farm	Positive
Bos indicus	Cuácua – Rogerio	100% (36/36)
Bos indicus	Nicoadala – Amed	100% (43/43)

Table 5 shows the proportion of animals positive for SBV-antibodies among the tested cattle.

When the serum was diluted 1:2 times 100% of the tested animals were positive. When the serum was diluted 1:4 93% of the animals tested positive and 3% tested negative. In dilution 1:8 87% of the tested animals were positive and 13% were negative for SBV-antibodies. In table 5 the sero-prevalence in diluted serum is presented.

Tabell 6

Cattle	Dilution 1:2	Dilution 1:4	Dilution 1:8
Positive	100% (30/30)	93% (28/30)	87% (26/30)
Intermediate		3% (1/30)	
Negative	0% (0/30)	3% (1/30)	13% (4/30)

Table 6 shows the sero-prevalence for SBV-antibodies in diluted serum from cattle

Sheep

Sheep from 5 different farms were tested for SBV-antibodies. The tested animals were all adults, mostly females. Sheep from all farms were tested sero-positive for SBV-antibodies. The prevalence ranged from 43% to 97% in the different farms,

with an average prevalence of 63%. In the tables below, table 7 and table 8, the prevalence's from the farms are presented. The results in table 7 are from ELISA-reactions with undiluted serum.

Tabell 7

Species	Farm	Positive	Negative
Sheep	Cuácuá – Rogerio	69% (24/35)	31% (11/35)
Sheep	Mopeia south	97% (33/34)	3% (1/34)
Sheep	Nicuadala - Amed	43% (3/7)	57% (4/7)
Sheep	Nicuadala - Mucelo	56% (19/34)	44% (15/34)
Sheep	Quelimane - Padeiro	49% (17/35)	51% (18/35)

Table 7 shows the proportion of animals positive and negative for SBV-antibodies among the tested sheep

When the serum was diluted 1:2 86% of the animals were positive and 14% were negative. In dilution 1:4 82% of the animals were tested positive and 18% tested positive. When the serum was diluted 1:8 71% of the animals were positive for SBV-antibodies and 18% were negative. The sero-prevalence

Tabell 8

Sheep	Dilution 1:2	Dilution 1:4	Dilution 1:8
Positive	86% (24/28)	82% (23/28)	71% (20/28)
Intermediate			11% (3/28)
Negative	14% (4/28)	18% (5/28)	18% (5/28)

Table 8 shows the sero-prevalence for SBV-antibodies in diluted serum from sheep

Goat

Goats from 6 farms were tested for SBV-antibodies. All of the tested animals were adults and most of them were females. There were animals which tested positive on all of the farms. The prevalence ranged from 72% to 100% with an average prevalence of 84%. In the tables below, the prevalence's from the farms are presented. The results in table 9 are from ELISA-reactions with undiluted serum.

Tabell 9

Species	Farm	Positive	Negative
Goat	Cuácuá - Rogerio	89% (25/28)	11% (3/28)

Goat	Deda	100% (3/3)	0% (0/3)
Goat	Nicudadala - Amed	74% (23/31)	26% (8/31)
Goat	Nicudadala - Mingano	90% (19/21)	19% (2/21)
Goat	Quelimane - Dona Ana	72% (23/32)	28% (9/32)
Goat	Quelimane - Padeiro	81% (21/26)	19% (5/26)

Table 9 shows the proportion of animals positive and negative for SBV-antibodies among the tested goats

Serum from 30 goats was diluted 1:2, 1:4 and 1:8. When the serum was diluted 1:2 97% of the tested goats were positive for SBV-antibodies and 3% were negative. In dilution 1:4 93% were positive and 7% were negative. When the serum was diluted 1:8 87% of the goats were positive and 13% were negative for SBV-antibodies. The results in table 10 present the sero-prevalence in diluted serum.

Tabell 10

Goat	Dilution 1:2	Dilution 1:4	Dilution 1:8
Positive	97% (29/30)	93% (28/30)	87% (26/30)
Intermediate			
Negative	3% (1/30)	7% (2/30)	13% (4/30)

Table 10 shows the sero-prevalence for SBV-antibodies in diluted serum from goat

Gel electrophoresis

The figure below, figure 2, shows the result from the gel electrophoresis done on the PCR-products from the 3 mosquito pooles. From the left: ladder 100 base pairs, *Culex* 1, *Culex* 2, *Culex* 3, *Culex* 4, *Culex* 5, *Monsonia* 1, *Monsonia* 2, *Monsonia* 3, *Monsonia* 4, *Monsonia* 5, and *Anopheles* 1, *Anopheles* 2, *Anopheles* 3, *Anopheles* 4. The molecular size of the smears from all samples is around 100 bp.



Figure 2: Gel electrophoresis on PCR-products from mosquito samples. From the left: ladder 100 bp, C1, C2, C3, C4, C5, M1, M2, M3, M4, M5, A1, A2, A3 and A4.

Answers to questionnaire

Chimura

Date: 2013-09-25

Cuacua – Rogerio S: 17.79748 E: 035.41442

- 130 sheep and 137 goats
- History of disease: During 2013 2 adult goats and 7 adult sheep have died, all of them females. 5 juvenile males and 9 juvenile females died during the wet season. The farmer believed that these deaths among the juveniles were due to too little milk and too damp environment. 5-10 sheep about 10 months old died from assumed snakebites.
- The animals are not vaccinated.

Chimura

Date: 2013-09-26

Cuacua- Rogerio S: 17.90069 E: 03541220

- The number of cattle and water buffalos is unknown.
- History of disease: 1 adult female cattle and 2 adult buffalos have died during 2013 with unknown symptoms.
- The cattle were vaccinated against rift valley fever in July 2012 and Lumpy skin disease 2013.

Deda

Date: 2013-09-27

S: 17.98278 E: 03576248

- 120 goats and 28 cattle

- History of disease: during 2013 20 juvenile goats died with neurological symptoms and 5 goats have aborted. Among cattle there have been 2 abortions.
- No malformations in neonatals or foetuses have been seen.

Mopeia South

Date: 2013-09-27

S: 17.89602 E: 035.74150

- 64 sheep
- History of disease: 1 adult sheep died during partus.
- No abortions.
- A few juvenile labs have died due to trampling from cattle.

Quelimane – Padeiro

Date: 2013-09-30

S: 17.82279 E: 03692272

- 58 cattle, 64 sheep and 36 goats.
- History of disease: A few goats and sheep have had a fever (no thermometer was used) during the rainy season and were treated for trypanosomiasis.
- 5 juvenile goats and sheep have had diarrhoea.
- No abortions and no malformations among newborns.

Nicoadala – Mucelo

Date: 2013-09-30

S: 17.70626 E:036.89647

- The farm has 223 sheep over three months and an unknown number of lambs less than 3 months. The farm also has cattle.
- History of disease: 5 sheep and 4 cattle have had late abortions but no malformed newborns. The farmer believes that they aborted due to a poor food supply. The farm has had some problems with tympanism in cattle and sheep. 3 cattle, 7 months old died with some kind of symptoms from their mouth. Some adult sheep died during rainy season due to diarrhoea. No deaths in neonatals.
- The cattle were vaccinated against lumpy skin disease this year and rift valley fever another year.

Nicoadala- Amed

Date: 2013-10-01

S: 17.60097 E: 036.82723

- 108 goats and 10 sheep.

- History of disease: Problems with laminitis. No abortions. Some juveniles died due to trampling. Some deaths which farmer believes are due to tick born diseases since they have not used tick repellents this year. Some deaths due to attack by stray dogs.

Nicoadala- Amed

Date: 2013-10-01

S: 17.60421 E: 03682723

- 536 cattle
- History of disease: Problems with laminitis. During rain period some animals gets pneumonia. Sometimes there are problems with diarrhoea and sometimes with poor food supply. In both cattle and sheep more young animals die during the rainy season and more adults during dry season.
- The cattle were vaccinated this year against lumpy skin disease and last year against rift valley fever.

Nicoadala – Mingano

Date: 2013-10-02

S: 17.80573 E: 037.13612

- 42 goats and 82 cattle
- History of disease: Some deaths among the cattle due to supposed tick borne infections. During the last month there have been 4 early abortions among goats and 2 among cattle. No malformations among neonatals. Some neonatals have died presumably due to lack of milk from the dam.
- Vaccination of cattle against rift valley fever last year and this year for lumpy skin disease and trypanosomiasis.

Quelimane - Dona ana

Date: 2013-10-02

S: 17.83348 E: 036.93911

- 70 goats and 29 cattle
- History of disease: possible tympanism in 2 adults and 17 juveniles between 1 and 2 months. Symptoms: swollen abdomen and salivation. One cow aborted last week, no abortions in goats. The cattle are vaccinated against something, the animal keeper is uncertain against what.

DISCUSSION

Schmallenberg virus

A vast majority of the tested animals from each farm tested positive for SBV-antibodies. The highest prevalence for SBV-antibodies was among the cattle, 100% of the tested animals were positive. The average prevalence among

sheep and goats were 63% (43-97%) respectively 84% (72-100%). The epidemiology, with a high sero-prevalence on the positive farms, seen among the ruminants in Zambezia is corresponding with the patterns of the serology surveys conducted in Europe (ELBERS *et al.*, 2012, MEROCC *et al.*, 2013).

Even when the serum was diluted 1:2, 1:4 and 1:8 a majority of the tested animals were positive. Although equality in epidemiologic pattern and the positive results with diluted serum make a true positive result for SBV-antibodies more probable, they do not exclude the possibility of false positive results due to cross-reacting virus in the same area.

Since SBV was recently discovered and since there are not any known viruses from the Simbu sero-group in Europe no studies on cross-reactivity with SBV have been conducted. Consequently, it is not known nor proven if there are any viruses that could cross-react with the SBV. In 2007 NAKOUNE´ YANDOKO *et al.*, stated that although there are serological immunoassays available for detection of some Orthobunyaviruses, cross-reactions in the tests are common. This does impair the interpretation of the positive SBV-ELISA results in the study. It is of common opinion that Shamonda virus and Sathuperi virus, members of the Simbu sero-group, are the closest relatives of the Schmallerberg virus (YANASE *et al.*, 2012). Both Sathuperi virus and Shamonda virus have been isolated in Africa both in midges and in a broad spectrum of ruminants (CAUSEY *et al.*, 1972, LEE *et al.*, 1979). Hence a cross-reaction with the Schmallerberg virus and Sathuperi virus or Shamonda virus is not unlikely.

To ascertain that SBV truly exist in Moçambique, further studies ought to be done, aiming to detect the virus and to determine the sequence of the genome. This could be done e.g. using PCR, cellculture isolation of virus or metagenomic methods. It is also of great interest to collect *Culicoides* midges in areas with SBV-positive animals to see if they carry the virus.

Since SBV-antibodies have been found in the wild cervid population in Belgium and it is expected that other wild ruminants are susceptible to the virus (LINDEN *et al.*, 2012), this must be considered a conceivable reservoir and maybe the origin of the SBV.

The possibility that SBV evolved in Africa should also be considered. Satuperi virus and Shamonda virus are distributed in the same geographic areas, in Africa and Asia, and have the same hosts (ruminants) and the same vectors (*Culicoides* midges) as SBV. Thus, SBV might be the result of a co-infection with Satuperi virus and Shamonda virus (YANASE *et al.*, 2012). If Africa is the origin of SBV it is also interesting to study its transmission route to Europe, especially since SBV emerged in the very same area where Blue tongue virus emerged 2009. This makes it likely that there is an unknown pathway for virus to Europe.

The fact that none of the interviewed farmers had experienced any problems neither with malformed or with misshaped newborns nor with abortions among the pregnant dams is conspicuous. When according to the serology; SBV is highly existent among their livestock. If assuming that the results are

true and that SBV exist in the area, its wide distribution might be the explanation to why it is not noticed – if the virus is endemic in an area, the majority of the animals will be infected before they breed, making the disease pass unnoticed. The weak symptoms or total absence of symptoms among adult animals also makes the disease hard to detect and might lead to an underreporting of the disease. Clearance of virus before birth in confirmed infected lambs have been recorded, which also make the disease pass unnoticed (MERO C *et al.*, 2013). According to this, SBV is of little importance in areas where it is endemic.

Metagenomics

According to the fact that a good smear for metagenomic use of a PCR product is expected to be ranging between 200bp to 20kb, the received smear is too short to be optimal. The smear ranging around 100 bp, shows that the material is partly degraded. This might be the result of remaining rests of inhibitory substances from the mosquitoes affecting the PCR. Due to the fact that the metagenomic analyzes are not yet done, it is not possible to evaluate the final result.

CONCLUSION

Neutralizing antibodies for SBV are frequently occurring among cattle, sheep and goats in the Zambezia province, Moçambique. If it truly are SBV antibodies the virus is endemic in the area and consequently not a problem. Further studies on this topic should be done aiming to isolate the virus and confirm the result. It is of great interest to determine the origin of the virus and possibly find its transmission rout to Europe.

Since Moçambique has been appointed to be a hot spot for emerging infectious diseases the viral metagenomic results are of great interest.

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TECHNICAL REPORT

"Schmallenberg" virus: analysis of the epidemiological data May 2013

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European Food Safety Authority (EFSA), Parma, Italy

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