



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Veterinary Medicine
and Animal Science**

Department of Biomedical Sciences and
Veterinary Public Health

Schmallenberg virus among ruminants in the Gaza province, Mozambique

Maria Rystedt



*Uppsala
2017*

Degree Project 30 credits within the Veterinary Medicine Programme

*ISSN 1652-8697
Examensarbete 2017:74*

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Schmallenbergvirus hos idisslare i Gazaprovinen, Moçambique

Maria Rystedt

Supervisor: *Anne-Lie Blomström, Department of Biomedical Sciences and Veterinary Public Health*

Assistant Supervisor: *Harindranath Cholleti, Department of Biomedical Sciences and Veterinary Public Health*

Examiner: *Mikael Berg, Department of Biomedical Sciences and Veterinary Public Health*

Degree Project in Veterinary Medicine

Credits: 30

Level: *Second cycle, A2E*

Course code: EX0751

Place of publication: Uppsala

Year of publication: 2017

Number of part of series: *Examensarbete 2017:74*

ISSN: *1652-8697*

Online publication: <http://stud.epsilon.slu.se>

Key words: *Schmallenberg virus, Simbu serogroup, ruminants, arbovirus, seroprevalence, cross reactivity, PCR, Mozambique*

Nyckelord: *Schmallenbergvirus, Simbu serogroup, idisslare, arbovirus, seroprevalens, korsreaktion, PCR, Moçambique*

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SUMMARY

Schmallenberg virus was discovered in North Rhine-Westphalia in Germany in 2011. The virus spread over most of Europe during the following years, including United Kingdom and the Nordic countries. Its origins are yet to be discovered. Schmallenberg virus belongs to the Simbu serogroup of the *Bunyaviridae* family, genus *Orthobunyavirus*. Where, exactly, on the phylogenetic tree of Simbu serogroup viruses it should be placed is not concluded, but its closest relatives seem to be Shamonda virus, Aino virus, Akabane virus and Sathuperi virus.

The susceptible species are primarily ruminants, but Schmallenberg virus and its antibodies have occasionally been found in dogs. No evidence of zoonotic potential has been found. The virus is mainly transmitted by different species of biting midges of the genus *Culicoides*. Transplacental transmission can occur during the early part of pregnancy in ruminants, after placentomes have been formed. Infectious Schmallenberg virus has also been found to be shed in semen of cattle and sheep, thus horizontal transmission is possible in theory but has not yet been proven experimentally.

Clinical symptoms in adult cattle, sheep and goats are usually mild or absent, lasts only a few days and includes fever, reduced milk yield and diarrhoea. Transplacental transmission can, but does not always, lead to congenital Schmallenberg. If it does, the most common symptoms are malformations such as arthrogryposis (joint deformity/contracture) and different malformations of the vertebral column and brain. Reduced body weight in affected calves at birth seems to be correlated to the severity of the malformations. Infected foetuses are sometimes aborted or stillborn.

Schmallenberg virus antibodies are detectable by indirect methods such as ELISA and virus neutralization test. Cross reactions with antibodies directed against other viruses of the Simbu serogroup are known to occur. In the present study, seroprevalence for Schmallenberg virus in the Gaza province in southern Mozambique was investigated in cattle, sheep and goats, using competitive Schmallenberg ELISA. The overall seroprevalence in cattle was approximately 90 %, in sheep close to 60 % and almost 75 % in goats. All investigated herds and flocks had seropositive animals.

Detection of Schmallenberg viral RNA is possible by real-time RT-PCR, either specific for just Schmallenberg virus, or a general pan-Simbu real-time RT-PCR. Some samples in the present study that were positive in competitive ELISA were analysed using pan-Simbu real-time RT-qPCR. None of the included samples gave a positive result in the PCR.

The findings in this study show that antibodies able to produce a positive result in Schmallenberg virus ELISA circulates the Gaza province in Mozambique. The virus responsible for the positive results might be Schmallenberg virus, but it can as well be one or many other Simbu serogroup viruses. Cross reactivity cannot be ruled out, but is on the contrary quite possible, since other members of the Simbu serogroup are known to circulate in Africa.

Further studies in Mozambique, or other parts of southern Africa, would be of interest to determine which virus or viruses that are causing the positive ELISA results. Virus neutralization tests can be used to investigate which Simbu serogroup virus or viruses that are circulating the area. Schmallenberg virus and Simbu serogroup virus detection is of even greater interest, but this might prove difficult due to the short viremic period of Schmallenberg virus and other Simbu serogroup viruses. Since Schmallenberg virus shedding in semen can continue for more than two months in some individuals, virus detection in semen might be a viable way. Virus detection in the vector can also be possible to achieve, especially if very many biting midges are included.

SAMMANFATTNING

Schmallenbergviruset upptäcktes i Nordrhein-Westfalen i Tyskland år 2011. Det spred sig över Europa, inklusive Storbritannien och Norden, under de följande åren. Var viruset ursprungligen kom ifrån är ännu okänt. Schmallenbergvirus tillhör Simbu-serogruppen i familjen *Bunyaviridae*, genus *Orthobunyavirus*, men exakt var på det fylogenetiska trädet det ska placeras in är ännu inte helt klarlagt. Dess närmsta släktingar tycks vara Shamondavirus, Ainovirus, Akabanevirus och Sathuperivirus.

Mottagliga djurslag är framför allt idisslare, men Schmallenbergvirus och antikroppar riktade mot viruset har påträffats även hos hundar. Det finns inga indikationer på att viruset har någon zoonotisk potential. Huvudsakligen överförs Schmallenbergvirus genom olika arter av svidknott, tillhörande genuset *Culicoides*. Transplacental överföring av viruset kan ske under tidig dräktighet hos idisslare, efter att placentomen har bildats. Infektiöst Schmallenbergvirus har också påträffats i sperma från tjur och bagge och viruset skulle därför i teorin kunna överföras horisontellt vid parning. Detta har dock inte påvisats experimentellt ännu.

Nöt, får och getter visar vanligtvis milda eller inga kliniska symptom vid smitta. Symptomen kan innefatta feber, minskad mjölkproduktion och diarré hos vuxna djur. Transplacental överföring kan, men behöver inte, resultera i kongenital Schmallenberg. De vanligaste symptomen hos intrauterint smittade kalvar, lamm och killingar är arthrogryposis (ledmissbildningar och ledkontrakturer) och missbildningar i ryggraden och hjärnan. Lägre födselvikt hos smittade kalvar tycks korrespondera med gravare missbildningar. Infekterade foster kan även aborteras eller vara dödfödda.

Indirekta metoder såsom ELISA och virusneutraliseringstest kan användas för att detektera antikroppar mot Schmallenbergvirus. Det är känt att korsreaktioner med antikroppar riktade mot andra virus i Simbu-serogruppen kan förekomma. I denna studie har seroprevalensen för antikroppar hos kor, får och getter i Gazaprovinen i Moçambique undersökts med Schmallenberg-ELISA. Seroprevalensen hos samtliga undersökta kor var ungefär 90 %, hos får nära 60 % och strax under 75 % hos getter. Alla undersökta besättningar hade seropositiva djur.

Det är möjligt att hitta Schmallenbergvirus-RNA med hjälp av realtids-RT-PCR, antingen specifik för Schmallenbergvirus eller en mer generaliserad realtids-RT-PCR som inkluderar flera virus ur Simbu-serogruppen. Vissa av de ELISA-positiva proverna i denna studie analyserades med hjälp av pan-Simbu realtids-RT-qPCR, men inget prov gav positivt utslag för något Simbuvirus.

Resultatet av denna studie bekräftar att antikroppar, som kan ge positivt utslag i Schmallenberg-ELISA, cirkulerar i Gazaprovinen i Moçambique. Viruset som föranlett detta antikroppssvar kan vara Schmallenbergvirus, men det kan lika gärna vara orsakat av ett eller flera andra Simbuvirus. Korsreaktivitet kan inte uteslutas, utan är tvärtom ganska sannolik, eftersom det är välkänt att andra virus ur Simbu-serogruppen cirkulerar i Afrika.

Vidare studier i Moçambique eller andra delar av södra Afrika är av intresse för att kunna undersöka vilket eller vilka virus som orsakat det positiva ELISA-resultatet. För att undersöka vilka Simbuvirus som finns i området kan virusneutraliseringstest användas. Att upptäcka RNA från Schmallenbergvirus eller andra Simbuvirus är av ännu större intresse, men detta kan visa sig vara svårt att uppnå med tanke på den korta viremiperioden som dessa ger upphov till. Eftersom Schmallenbergvirus utsöndras i sperma i mer än två månader hos vissa individer kan virusdetektion i sperma kanske vara en möjlig väg. Virusdetektion i vektorn är också en möjlighet, särskilt om det går att inkludera ett stort antal svidknott i en sådan studie.

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INTRODUCTION

In 2011, Schmallenberg virus was discovered in North Rhine-Westphalia in Germany, Europe, (Hoffman *et al.*, 2012) and it became endemic on the continent during the following years (EFSA, 2012b, 2013). The origin of the virus is shrouded in mystery. In this master thesis, there were three major aims:

The first one was to introduce Schmallenberg virus and summarize what research has concluded about the virus since its introduction in Europe.

The second one was to screen cattle, sheep and goats in the Gaza Province in Mozambique for Schmallenberg virus antibodies. The southern parts of Africa have been pointed out as a hotspot for emerging infectious diseases and a previous screening study in 2013 (Blomström *et al.*, 2014) has shown a great abundance of animals positive in Schmallenberg competitive ELISA in the Zambezia province in Mozambique.

The third one was to detect viral RNA from viruses in the Simbu serogroup from possibly infected animals in the Gaza province in Mozambique, thus providing evidence that one or many Simbu serogroup viruses are present in the area.

LITTERATURE REVIEW

Discovery of Schmallenberg virus

During the summer and autumn of 2011, reports of a mild clinical disease including fever, diarrhoea and decreased milk production started coming from North Rhine-Westphalia in Germany and the Netherlands (Hoffman *et al.*, 2012). All infectious agents known to cause this kind of symptoms were ruled out and blood samples from infected animals were analysed using metagenomics, only to discover a previously unknown virus belonging to the Simbu serogroup of the *Bunyaviridae* family, genus Orthobunyavirus. The virus was named Schmallenberg virus after the geographical location where it was originally discovered (Hoffman *et al.*, 2012). Orthobunyaviruses of the Simbu serogroup had until then not been known to be prevalent in Europe at all, though widely spread in the rest of the world (Saeed *et al.*, 2001).

Phylogeny

Shortly after discovery, Hoffman *et al.* (2012) sequenced the complete genome of the new virus and found it belonged to the genus Orthobunyavirus. The small (830 nucleotides), medium (4415 nucleotides) and large (6865 nucleotides) segments were compared to other Orthobunyaviruses and it was discovered that the S segment most resembled Shamonda virus, detected in Japanese cattle, with a similarity of 97 %. The M segment resembled Aino virus, found in Japanese cattle, to an extent of 71 % and the L segment was similar by 69 % to Akabane virus, also found in Japanese cattle. Based on these findings, Schmallenberg virus was classified as a Shamonda-like virus in the Simbu serogroup of genus Orthobunyavirus, family *Bunyaviridae* (Hoffman *et al.*, 2012). However, Yanase *et al.* (2012) suggested that Schmallenberg virus is a reassortant virus with the S and L segments originating from Shamonda virus and the M segment originating from Sathuperi virus.

Goller *et al.* (2012) concluded that Schmallenberg virus belongs to the species Sathuperi virus and most likely is not a reassortant virus and in fact may be an ancestor to Shamonda virus (and not the other way around as previously suspected). The similarities between Shamonda and Schmallenberg virus is explained by Shamonda virus being a reassortant between Schmallenberg virus (S and L segments) and another unknown virus (M segment).

Detection tools and cross reactivity

Commercial kits are available for detection of Schmallenberg virus antibodies in multi-species serum/plasma and milk by indirect ELISA. Detection kits for Schmallenberg virus antibodies in multi-species serum/plasma by competitive ELISA are also available (ID vet, 2017). Very high specificity has been shown for indirect ELISA when compared to virus neutralization tests in a European study (Bréard *et al.*, 2013). Using virus neutralization tests to determine presence or absence of antibodies is generally considered as “gold standard” and can of course also be used.

In a study by Mathew *et al.* (2015) in Tanzania, cattle serum samples that were positive in indirect Schmallenberg virus ELISA were tested using Simbu serogroup virus neutralization tests for ten different Simbu serogroup viruses. All tested samples were positive for one or more of the viruses included, and only a few had the highest titer for Schmallenberg virus. In some of the samples, high titers could be seen for one or more viruses and low titers for one or more other viruses. This suggests that members of the Simbu serogroup is prone to cross react and may confuse the interpretation of indirect detection methods like ELISA and virus neutralization tests.

Another study in Jordan used indirect Schmallenberg virus ELISA to investigate the prevalence in serum from cattle, sheep and goats and bulk milk from cattle. Positive results were obtained from some of the samples and a virus neutralization test was done. The results suggested that it was not Schmallenberg virus antibodies responsible for the positive result, but instead Aino virus antibodies (Abutarbush *et al.*, 2015).

Direct detection of Schmallenberg virus is possible using real-time RT-PCR. Protocols exclusively designed to detect Schmallenberg virus are available, as well as a protocol for detection of multiple viruses from the Simbu serogroup (Fischer *et al.*, 2013).

Geographical distribution

Europe

It is quite certain that the virus was not prevalent, or at least only prevalent to a very low extent, in the core area of the outbreak before summer 2011, since samples from Belgium and Germany collected in 2010 and spring and summer 2011 were negative in serological tests (Garigliany *et al.*, 2012; Wernike *et al.*, 2013c).

Schmallenberg virus spread in the southern parts of Belgium during the second half of 2011 (Garigliany *et al.*, 2012), and in spring 2012 it was considered endemic in the country with a seroprevalence of approximately 90 % in cattle (Méroc *et al.*, 2013; Wernike *et al.*, 2014a), almost 85 % in sheep and slightly above 40 % in goats (Méroc *et al.*, 2014). In winter 2012, the seroprevalence in the Netherlands was above 70 % in cattle, with the highest within-herd seroprevalence seen in herds in the central and eastern parts of the country (Wernike *et al.*, 2014a). Germany showed a spatial pattern of spread during winter 2011 and spring 2012 of higher seroprevalence in the regions closest to the core area of the Schmallenberg virus outbreak in the north-western part of the country, and lower in the counties in eastern and south-eastern parts. The seroprevalence in Germany, all counties included, among cattle, sheep and goats was 61 %, 24.7 % and 26.4 % respectively during winter 2011 and spring 2012. The seroprevalence in North Rhine-Westphalia, where the community of Schmallenberg is located, was almost 98 % in cattle (Wernike *et al.*, 2014a).

The outbreak in northern France started in cattle in September-October 2011 and spread to sheep and goats in the northern parts of France during autumn 2011 and the southern parts of the country during spring 2012. During January 2012, the first cases of congenital Schmallenberg were reported in lambs, goat kids and calves in the North-Eastern part of the country (Dominguez *et al.*, 2014). About two years after its discovery in 2011, Schmallenberg virus had already spread over most of Europe including United Kingdom, except Scotland, and the Nordic countries, except Iceland (EFSA 2012b, 2013). Serological tests in cattle and sheep as far east as Romania were positive during autumn 2013 (Danes *et al.*, 2014).

Reports of possible recirculation of Schmallenberg Virus after the initial epidemic in 2011-2013 have come from many countries, including Germany (Wernike *et al.*, 2015a), Belgium (Delooz *et al.*, 2016), England, Wales (APHA, 2017b) and Ireland (Collins *et al.*, 2016). In April 2017, Schmallenberg virus was confirmed for the first time in sheep flocks in Scotland in April 2017 (APHA, 2017a), possibly a result of recirculation in southern United Kingdom and Ireland. Many animals in Europe today have not been exposed to infection or vaccine, i.e. seroprevalence is decreasing (Wernike *et al.*, 2015b). Recirculation, though not as economically costly as the introduction of Schmallenberg virus in 2011, may occur.

Nordic countries

Schmallenberg viral RNA was isolated, using RT-PCR, from midges from one collection site close to the German border in Denmark in 2011 (Rasmussen *et al.*, 2012). In 2012, viral RNA was isolated from midges collected in four sampling spread over the country. Hence, the spread of Schmallenberg virus in Denmark mainly took place during the vector season of 2012 (Rasmussen *et al.*, 2013).

Serological surveys and bulk milk surveys in Sweden shows that Schmallenberg virus spread in cattle and sheep herds mainly during the vector season of 2012, a year later than in central Europe. After the vector season of 2012, lasting less than four months, almost 75 % of the investigated bulk milk samples were ELISA positive. The first positive serological sample from sheep and bulk milk from cattle was both found in the county of Blekinge in south eastern Sweden and was collected before the vector season of 2012. The first case of congenital Schmallenberg in an aborted lamb was confirmed in the county of Halland in November 2012. RT-PCR confirmed the presence of viral RNA from malformed and/or aborted offspring of cattle and sheep during winter 2012-2013 (Chenais *et al.*, 2013). Schmallenberg virus antibodies has been found as far north as Piteå, at latitude 65.2°, only 320 km south of the northern polar circle in Sweden (Chenais *et al.*, 2013).

The spread of Schmallenberg virus in Norway seems to have been limited compared to Sweden and Denmark. It was shown to circulate in the south-eastern parts of the country during the vector season of 2012. Serological surveillance showed negative results in the northern and western parts of the country (Åkerstedt *et al.*, 2015). The first case of congenital Schmallenberg was detected in April 2013 in a calf (Wisløff *et al.*, 2014). No evidence of Schmallenberg virus circulation could be found in midges during 2013 (Åkerstedt *et al.*, 2015).

In Finland, Schmallenberg virus antibodies were found in the southern parts of the country in August 2012 (Chenais *et al.*, 2014). Schmallenberg virus antibodies could be found in 39 % of the tested dairy herds in spring 2013 (Chenais *et al.*, 2014; Evira, 2016).

Southern Africa

If Schmallenberg virus is present in countries outside Europe is presently unknown, however, serological studies using ELISA and virus neutralization tests, as presented below, have shown

antibody positive results. Since members of the Simbu serogroup are known to circulate in many parts of Africa (Theodoridis *et al.*, 1979; Zeller & Bouloy, 2000; Mathew *et al.*, 2015) and because of the issue with cross reactivity, as discussed above, Blomström *et al.* (2013) and Mathew *et al.* (2015) concluded that the seropositive ELISA results could be caused by other members of the Simbu serogroup rather than by Schmallerberg virus.

South Africa

Leask *et al.* (2013) hypothesized that Schmallerberg virus might be a possible cause of a few stillborn and deformed lambs in South Africa in 2006 and 2008. In 2006, two lambs from the same ewe were born with deformities consistent with congenital Schmallerberg, but no further investigation was made. In 2008, six lambs from the same flock with 50 pregnant ewes were either stillborn or had Schmallerberg-like deformities. All teratogenic infectious agents known to circulate the area were ruled out through testing, except Rift valley fever. Rift valley fever was not tested due to lack of signs of liver pathology in the lambs. Since Schmallerberg virus was not yet known, hence it was not tested for. Leask *et al.* (2013) suggested based on these findings that Schmallerberg virus may have circulated in South Africa already in 2006 or 2008. Unfortunately, this thesis could not be tested, since there no longer were any samples available from the affected animals.

Mozambique

In 2013, a screening study for Schmallerberg virus antibodies using competitive ELISA in cattle, sheep and goats took place in the Zambezia province in Mozambique. Seropositive animals were found in all herds on all farms. 100 % of the cattle tested were positive and with serum 8x diluted 87 % remained positive. For sheep, the within-herd seroprevalence varied between 43-97 % with 71 % remaining positive when diluted 8x. The within-herd seroprevalence for goats varied between 72-100 % (Blomström *et al.*, 2014).

None of the farmers had observed any clinical signs of Schmallerberg virus in any of the animals or their offspring. Blomström *et al.* (2014) drew the conclusion that vector activity is evenly distributed because of the humid and warm climate, hence making the exposure of a vector borne virus/viruses, possibly causing the positive result, continuous.

Tanzania

A screening study of cattle serum from samples collected 2008-2009 and 2012-2013 in Tanzania has been made, using indirect ELISA for Schmallerberg virus antibodies. In the samples from 2008-2009, 55 % gave a positive result. Of the samples from 2012-2013, 61 % were positive, and 87 % of the herds investigated in 2012-2013 had at least one ELISA positive animal (Mathew *et al.*, 2015).

Some of the ELISA positive samples were tested by virus neutralization test. Of the samples from 2012-2013, 51 % were positive and of the samples from 2008-2009, 21 % were positive (Mathew *et al.*, 2015).

Epidemiology

Susceptible species

Schmallerberg virus antibodies have been found in serum in several ruminant species, including alpaca, anatolian water buffalo, elk, bison, red deer, fallow deer, roe deer, muntjac, chamois, domestic cattle, sheep and goats. Schmallerberg viral RNA has been confirmed in cattle, goats and sheep infected both naturally and experimentally (EFSA, 2012a).

Dogs living in areas where Schmallenberg virus is known to circulate have been found positive for Schmallenberg virus antibodies in serological tests (Johansson Wensman *et al.*, 2013; Salieau *et al.*, 2013) and in one case Schmallenberg viral RNA has been isolated from the cerebellum of a dog expressing neurological symptoms (Salieau *et al.*, 2013).

Zoonotic potential

Hoffman *et al.* (2012) suggested that the risk of zoonotic transmission of Schmallenberg was “very low to negligible” due to the presumed close relationship to Shamonda virus and the absence of reports of clinical signs in humans.

Further investigation of humans exposed to the virus through infected animals, aborted material and vectors was done through surveys during 2012. Molecular and serological tests were also conducted. None of the studies found any seropositive human blood samples or any other evidence of transmission to humans. The public health risk was hence deemed extremely low or non-existent (Ducombe *et al.*, 2012; Reusken *et al.*, 2012; ECDC, 2017).

Transmission

Vector

Biting midges have been found to act as vectors for Schmallenberg virus. Especially members of the *Culicoides obsoletus* complex, but also other *Culicoides spp.* seems to be able to transmit Schmallenberg virus (De Regge *et al.*, 2012; Rasmussen *et al.*, 2012; Elbers *et al.*, 2013; Rasmussen *et al.*, 2013; Balenghien *et al.*, 2014).

Culicoides midges seems to prefer cattle stables over horse stables. A greater diversity of *Culicoides* species and a greater number of specimens have been found in cattle stables than in horse stables, but if this is due to the cattle themselves or preferences for temperature, breeding sites or some other unknown factor is not yet concluded (Kameke *et al.*, 2017).

During winter, vector activity is probably absent in central and northern Europe. Domiguez *et al.* (2014) saw a clear connection between the number of reported cases of congenital Schmallenberg in France during 2011-2013 and the seasonality in midges. Kameke *et al.* (2017) showed that the first *Culicoides* midges for the season can be found inside cattle stables and sheep barns in mid-March in Germany.

A German study found no evidence that mosquitoes can act as a vector for Schmallenberg virus. Approximately 50 000 specimens, mainly of the species *Culex pipens/torrentium* and *Aedes vexans* were collected in Schmallenberg virus abundant areas of Germany in 2011 and tested negative in a Schmallenberg virus specific real-time PCR (Wernike *et al.*, 2014b).

Vertical

Schmallenberg virus can cross the placenta in cattle and infect the foetus as soon as the first placentome is formed, at day 30 post conception. The age of the foetus at the time of infection impacts the clinical outcome. Infection between day 120-150 and later of pregnancy in cows will likely not produce an affected offspring, since the foetus already is immunocompetent and can prevent the virus from spreading. This leaves a window between day 30 and 120-150 in cattle during which infection can be obtained and may result in a calf born with congenital Schmallenberg (Bayrou *et al.*, 2014). Experiments with inoculation of Schmallenberg virus in pregnant ewes at day 45 and day 60 post conception resulted in colonization of the placentomes and transmission of Schmallenberg virus to the foetus, although the study found no clinical disease in the lambs born (Martinelle *et al.*, 2015).

Garigliany *et al.* (2012) concluded that based on data from the outbreak in southern Belgium 2011-2012, the risk of transplacental infection in an immunologically naïve herd of cattle was 28 %.

Horizontal

Experimental subcutaneous injection of cattle (Hoffman *et al.*, 2012; Wernike *et al.*, 2013a), sheep (Wernike *et al.*, 2013b; Martinelle *et al.*, 2017) and goats (Laloy *et al.*, 2015), as well as intradermal injection of sheep (Martinelle *et al.*, 2017) and intravenous (Hoffman *et al.*, 2012) injection of cattle with Schmallerberg virus results in viremia in the inoculated animals.

Intramuscular injection (Wernike *et al.*, 2013b) and intradermal inoculation (Martinelle *et al.*, 2017) of sheep has been tried experimentally without causing viremia, but the former has nevertheless managed to seroconvert some of the individuals (Wernike *et al.*, 2013b).

Oral transmission has been tried experimentally in cattle, but no Schmallerberg viral RNA could be detected in the blood for several weeks after inoculation and the animals did not seroconvert (Wernike *et al.*, 2013a).

Semen

Schmallerberg viral RNA has been found in bovine semen (Hoffman *et al.*, 2013; Kesik-Maliszewska & Larska, 2016; Ponsart *et al.*, 2014; Van der Poel *et al.*, 2013). In some individuals, excretion is intermittent (Hoffman *et al.*, 2013). Laloy *et al.* (2015) investigated the semen of two experimentally infected bucks, but did not find any Schmallerberg viral RNA.

Schmallerberg viral RNA has been detected both in seminal plasma and in the seminal cell fraction of bulls (Hoffman *et al.*, 2013; Van der Poel *et al.*, 2013). The highest concentrations of viral RNA in bull semen can be found during the first week of infection (Van der Poel *et al.*, 2013). Shedding of Schmallerberg viral RNA in bull semen has been shown to continue for 2-3 months after the viremia has ended, but the period of excretion varies a lot between individuals (Hoffman *et al.*, 2013; Ponsart *et al.*, 2014).

Schmallerberg viral RNA-positive semen has been proven to be infectious by subcutaneous injection in cattle. Venereal transmission of the virus has not yet been evaluated, but it has been theorized that dams might be exposed for infection through lesions caused by artificial insemination (Schulz *et al.*, 2014). Ponsart *et al.* (2014), succeeded to inoculate and seroconvert mice with infected semen from bulls that had already seroconverted, thus showing that semen can keep its infectivity even if an immune response has taken place in the host.

Pathogenesis

The complete pathogenesis of Schmallerberg virus is not yet known. It has been shown to target and replicate in neurons in the developing brain and central nervous system of *in utero* infected calves and lambs, leading to cavitory lesions. In experimentally infected mice, the white matter in the brain of baby mice undergoes vacuolar changes when infected. In the spine, it is instead the grey matter that is targeted (Varela *et al.*, 2013).

Viremia

Hoffman *et al.* (2012) inoculated calves with a virus isolate and discovered their blood was PCR-positive between day 2 and 5 post inoculation. This result has been repeated multiple times following inoculation in Schmallerberg antibody naïve cattle, showing a short viremic period

of 2-4 days, starting at day 2-6 post infection (Van der Poel *et al.*, 2013; Wernike *et al.*, 2013a; Schulz *et al.*, 2014).

A viremic period of 3-5 days, starting at day 2-7 post infection, has been reported in experimentally infected sheep (Wernike *et al.*, 2013b; Poskin *et al.*, 2014; Martinelle *et al.*, 2015; Poskin *et al.*, 2015). It has also been concluded that a higher infection dose is more likely to infect an animal than a lower dose, but does not extend the time of viremia (Poskin *et al.*, 2014).

The viremic period in goats is also short, 3-4 days, and starts between day 1 and 3 post infection (Laloy *et al.*, 2015).

Immune response and immunity

An innate immune response has been shown to occur immediately post infection in cattle (Wernike *et al.*, 2013a). Seroconversion, i.e. occurrence of Schmallenberg virus specific antibodies in blood, in infected cattle takes place at day 8-14 post infection (Wernike *et al.*, 2013a; Schulz *et al.*, 2014). In sheep, seroconversion happens at day 6-22 post infection (Wernike *et al.*, 2013b; Poskin *et al.*, 2014; Poskin *et al.*, 2015) and in goats between day 7-14 post infection (Laloy *et al.*, 2015).

According to Wernike *et al.* (2013a), immunity lasted for at least eight weeks in experimentally infected cattle. A long-term study was done in Germany between 2011 and 2014, showing that 90 % of the tested cattle still were seropositive for Schmallenberg virus antibodies after three years, thus concluding that a long lasting, but not lifelong, immunity can be expected (Wernike *et al.*, 2015b). During experimental conditions, immunity in sheep has been shown to last for at least 15 months (Poskin *et al.*, 2015).

Clinical symptoms

Adult cattle present no or mild clinical symptoms when infected with Schmallenberg virus (Hoffman *et al.*, 2012; Van der Poel *et al.*, 2013; Schulz *et al.*, 2014). Symptoms are displayed for a brief period (days) and include fever, decreased milk yield and diarrhoea (Hoffman *et al.*, 2012; Van der Poel *et al.*, 2013). There seems to be no difference in the course of infection between the genders (Wernike *et al.*, 2013a).

Experimentally infected sheep and goats usually do not display any clinical symptoms at all during viremia or later course of infection (Wernike *et al.*, 2013b; Poskin *et al.*, 2014; Poskin *et al.*, 2015; Martinelle *et al.*, 2015; Laloy *et al.*, 2015). Some sheep show very mild clinical signs in the form of diarrhoea and snotty nose (Wernike *et al.*, 2013b) and fever (Poskin *et al.*, 2014).

Farmers in France retrospectively stated that they had seen a higher frequency of repeated oestrus and early embryonic death in their cattle, sheep and goats during the vector season of 2012 when Schmallenberg virus spread in the country (Dominguez *et al.*, 2014).

Congenital Schmallenberg

Transplacental infection with Schmallenberg virus can, but does not always, lead to abortion, stillbirth or malformations (Garigliany *et al.*, 2012; Martinelle *et al.*, 2015). If symptoms are expressed in the offspring, the most common ones are malformations such as arthrogryposis (joint deformity/contracture) and different malformations of the vertebral column and brain (Bayrou *et al.*, 2014; Dominguez *et al.*, 2014). Bayrou *et al.* (2014) found that Schmallenberg

virus infected calves had a significantly (on average 35 %) lower birth weight than unaffected calves, and stillbirths often occurred. A lower body weight at birth seems to be connected to more severe bodily malformations and a lower amount of skeletal muscles.

When Schmallenberg virus spread in naïve populations in France during 2011-2013, there was a great variability in morbidity rate between herds. The average risk of deformities due to congenital Schmallenberg in offspring was 3 % in calves, 8 % in lambs and 2 % in goat kids (Dominguez *et al.*, 2014). Martinelle *et al.* (2015) did not observe any signs of congenital Schmallenberg in any of the offspring to experimentally infected ewes, suggesting that the risk of congenital Schmallenberg is rather low, even if transplacental transmission has taken place.

Prophylaxis

To minimize the risk of congenital Schmallenberg, Statens Veterinärmedicinska Anstalt (2017) suggests exposure of young ruminants to Schmallenberg virus before they become sexually mature and breed. This is acquired by keeping them on pasture during vector season and letting them be naturally exposed to the vector and the virus, go through viremia and then seroconvert, i.e. acquire immunity.

Another way of diminishing the risk of congenital Schmallenberg in temperate climates is to allow young sheep and goats to breed in the beginning of the vector free period, thus eliminating the risk of infection and transplacental transmission during the susceptible part of pregnancy (SVA, 2017).

Vaccine

The first vaccine against Schmallenberg virus in cattle and sheep came in 2013. It is called Bovilis® SVB and is an inactivated vaccine with adjuvants to boost the immune response (MSD Animal Health, 2013). Only non-pregnant animals should be injected with the vaccine. In cattle, two doses are administered four weeks apart and in sheep only one dose is necessary. Immunity is acquired after three weeks. In 2014 and 2015 two other inactivated vaccines with adjuvants also got approved on the European market (EMA, 2017; Merial, 2017).

In Sweden, vaccine has been available since 2013 (SJV, 2017) but only by licence (SVA, 2017). It is not a general recommendation to vaccinate all animals in Sweden since the disease is considered endemic in the country, and hence does not provide a threat to production economics (SVA, 2017).

MATERIAL AND METHODS

Literature review

The database PubMed was used to search for relevant articles. Words used for search included, but were not limited to: Schmallerberg, midges, cattle, sheep, goats, transmission, Orthobunyavirus, Simbu. Many of the found articles also gave suggestions in their reference section about other relevant articles.

Sample collection

The blood samples were collected in the Gaza province, in the districts of Macia, Xai-Xai, Chibuto and Chokwe between 2014-10-22 and 2014-10-30 (Figure 1). The samples from Macia were collected in 2014-10-03 by a veterinary colleague at the Veterinary Faculty in Eduardo Mondlane University in Maputo.

Blood samples

In total, 730 blood samples were collected from seven farms in four different districts of the Gaza province. From cattle, 494 samples were collected, 77 from sheep and 159 from goats (Tables 1, 2 and 3). The vacutainer system was used for collecting the samples in EDTA collection tubes. The cattle samples were collected from the tail vein and samples from sheep and goats from the jugular vein on either side of the neck. The cattle were picked randomly and the quota of samples collected was approximately 25% of the cattle population on each farm. All genders and age (>4 months) were represented. Regarding the sheep and goats, almost all animals of both genders on each farm were included, except for very young animals (<6 weeks old) and some adult animals on Gogoti that managed to escape from their paddock before sampling could take place. The samples were stored in an ice box at approximately 4° C during transportation to the laboratory.

FTA cards

Some of the blood samples were put on FTA cards immediately after sampling. Due to a limited amount of FTA cards only samples from some of the farms were included. All cattle samples from Nguluzane and Bassopa were included and a few of the samples from Ndonga. All sheep and goat samples from Nguluzane and Bassopa were included and some of the samples from Psungo. After separating the serum fraction from the blood cells in the lab, the corresponding serum of each sample was also put on the FTA cards, which were shipped to Sweden for further analysis in other projects.

Informal interviews

During the sample collection, a veterinary colleague named Belisario Moiane asked the farmers and their staff questions about the history of disease on the farms. The questions were aimed to determine whether the farmer or staff had noticed any clinical symptoms, abortions, birth malformations or disturbances in the overall reproduction.

Table 1. Cattle blood samples with collection date, district of the farm, farm name, GPS coordinates and exact number of samples collected

Date	District	Farm	Coordinates	No. of samples
2014-10-03	Macia	Macia	Unknown	98
2014-10-22	Xai-Xai	Nguluzane	25° 5' 40,4" S 33° 39' 5,3" E	50
2014-10-25	Xai-Xai	Bassopa	25° 00' 59,1" S 33° 33' 48,0" E	61
2014-10-27	Guijá	Ndonga	24° 21' 12,5" S 32° 52' 31,1" E	95
2014-10-29	Chókwè	Chalucwane	24° 44' 15,5" S 33° 24' 56,2" E	150
2014-10-30	Chibuto	Gogoti	24° 34' 31,6" S 33° 24' 12,7" E	40

Table 2. Sheep blood samples with collection date, district of the farm, farm name, GPS coordinates and exact number of samples collected

Date	District	Farm	Coordinates	No. of samples
2014-10-24	Xai-Xai	Bassopa	25° 00' 59,1" S 33° 33' 48,0" E	51
2014-10-27	Guijá	Psungo	24° 15' 46,3" S 32° 53' 0,0" E	14
2014-10-30	Chibuto	Gogoti	24° 34' 31,6" S 33° 24' 12,7" E	12

Table 3. Goat blood samples with collection date, district of the farm, farm name, GPS coordinates and exact number of samples collected

Date	District	Farm	Coordinates	No. Of samples
2014-10-22	Xai-Xai	Nguluzane	24° 5' 40,4" S 33° 39' 5,3" E	66
2014-10-24	Xai-Xai	Bassopa	25° 00' 59,1" S 33° 33' 48,0" E	33
2014-10-27	Guijá	Ndonga	24° 21' 12,5" S 32° 52' 31,1" E	6
2014-10-27	Guijá	Psungo	24° 15' 46,3" S 32° 53' 0,0" E	10
2014-10-30	Chibuto	Gogoti	24° 34' 31,6" S 33° 24' 12,7" E	44

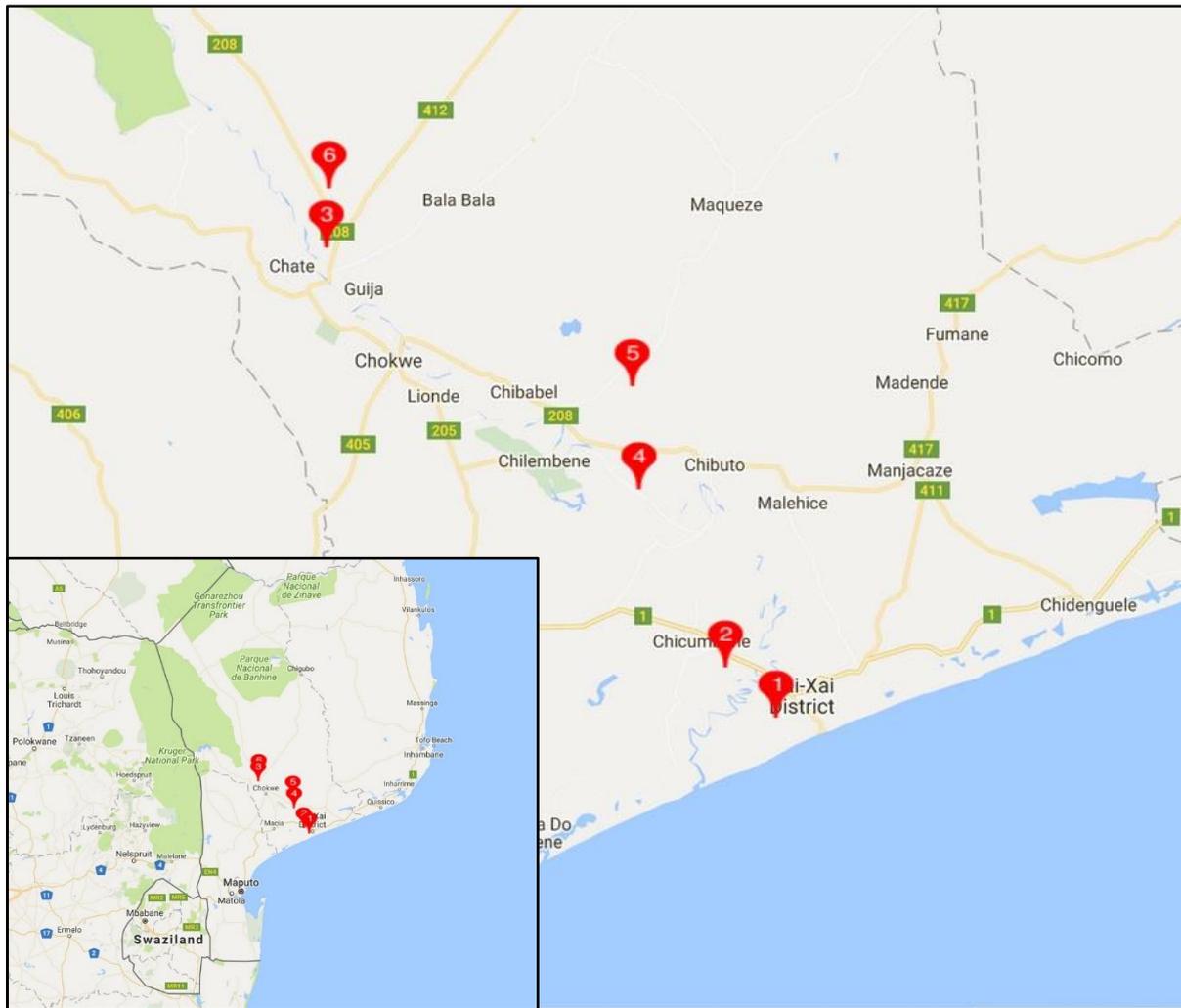


Figure 1. Geographical locations of the farms in the Gaza province in Mozambique where the samples were collected. 1) Nguluzane, 2) Bassopa, 3) Ndonga, 4) Chalucwane, 5) Gogoti, 6) Psungo. Macia is not plotted due to unknown coordinates. The small square in the left bottom shows a zoomed-out view displaying the southernmost part of Mozambique with neighbouring countries.

Lab analyses

Sample preparation

The EDTA tubes were centrifuged for a couple of minutes to separate the serum fraction. The serum was then poured into 1,5 or 2 ml tubes, labelled with sample number and stored at -20°C .

ELISA

A selection of samples was screened with a competitive ELISA designed to target antibodies directed against Schmallenberg virus nucleoprotein in cattle, sheep and goats. The kits came from ID Vet and is called “ID screen® Schmallenberg Virus Competition Multi-Species”. The instruction from the kits was followed and 50 μl undiluted serum was used in each well. Two positive and two negative controls (provided by the manufacturer) and two distilled water controls were included in every plate. The cut off values recommended by the manufacturer were used to determine the outcome. Unfortunately, there was no materials left to screen diluted serum from any of the positive samples as initially planned, hence, this was not done.

RNA extraction

The materials and protocols used came from Thermo Scientific in a kit called “Thermo Scientific GeneJET RNA Purification Kit”. Protocol C, from the information included the kit, “C. Human Blood Cells Total RNA Purification Protocol” was used for the serum. The protocol was followed, except for the first step that was skipped. Instead 200 µl of serum was directly added to 600 µl of lysis buffer. The last step was also modified, and instead of using 50 µl nuclease free water and centrifuging at 1200 x g for one minute, 20 µl nuclease free water was used twice, with centrifuging in between and after, both at 1200 x g for one minute. The extracted RNA was then labelled properly and stored at -80° C.

Spectrophotometry

The total concentration (ng/µl) of extracted RNA in the samples was measured to determine to what extent the samples needed to be diluted to be suitable as template for real-time RT-qPCR (reverse transcription quantitative PCR). The system used was called NanoDrop1000 and came from Thermo Scientific. All concentrations <50 ng/µl was considered suitable for real-time RT-qPCR without prior dilution.

Pan Simbu real-time RT-qPCR

Some of the eluted RNA from the serum samples was analysed with Pan Simbu real-time RT-qPCR (Fischer *et al.*, 2013). The materials used came from Thermo Fisher.

PCR mastermix (15 µl):

Express SuperScript qPCR SuperMix Universal	10 µl
Forward primer panOBV-L-2959F (10 µM)	0,5 µl
Reverse primer panOBV-L-3274R (10µM)	0,5 µl
Express SuperScript Mix for one-step qPCR	2 µl
DEPC-treated water	2 µl

15 µl mastermix and 5 µl template RNA was used for each reaction. At least one negative control from the manufacturer was included in each run. The protocol used was a bit modified compared to the one stated by Fischer *et al.* (2013) and included the following steps:

- 50° for 15 minutes
- 95° for 2 minutes
- 95° for 15 seconds
- 55° for 1 minute
- Repeat step three and four 40 times
- Melting curve: from 55° C to 95° Celsius with an increase of 0.5° C every minute
- 4° C forever

The PCR-products were stored at -20° C and shipped to Sweden.

Gel electrophoresis

Gel electrophoresis was not a part of the original plan, but was used on one occasion to evaluate the results from one of the real-time RT-qPCR runs. The gel used was 2 % agarose gel and the dye added to the gel was called “GR green DNA stain”. 5 µl of the respective PCR product were mixed with 1 µl “DNA loading dye” and the mix was applied to the wells in the gel. The electrophoresis ran for 20 minutes at 80 V and the result was inspected visually under ultra violet light to determine the outcome.

cDNA synthesis

Some of the samples with extracted RNA underwent first strand cDNA synthesis to make the product more stable and allow for shipping to Sweden and further analysis in other projects. The materials used came from Thermo Scientific in a kit called “Thermo Scientific Maxima H Minus Reverse Transcriptase”. The protocol from the kit was followed, including the optional step 2. The products were stored at -20° C until shipping.

Possible sources of error

- Pipette errors either because of uncalibrated pipettes or due to faulty pipetting
- Unexpected power breakdowns during lab procedures and storage of the samples
- Suboptimal temperatures for some of the reagents during shipping from Sweden to Mozambique that might have changed their properties

RESULTS

Informal interviews

None of the farmers or staff asked on the farms had noticed any clinical symptoms, elevated levels of abortions, birth malformations or disturbances in the overall reproduction during the weeks prior to the interviews.

ELISA

Four ELISA-plates were performed. In total, 328 serum samples were analysed, including 92 cattle samples, 77 sheep samples and 159 goat samples.

Cattle

Since the total number of cattle samples was high (494 samples) a selection of 92 samples were tested with ELISA. 15-16 samples from each farm were randomly selected regardless of the age and gender of the animals. All farms showed a high percentage of cattle positive for Schmallenberg virus antibodies and in two cases, Macia and Gogoti, 100 % of the animals were seropositive (Table 4). The seroprevalence among all the tested animals was 90.2 % and the mean seroprevalence among the farms was 90.3 %.

Sheep

All 77 sheep samples were tested. All three farms had animals that tested positive with the highest seroprevalence on Gogoti, 91.7 % (Table 5). The seroprevalence among all tested sheep was 59.7 %, which is lower compared to the mean seroprevalence among the farms that was 74.2 %.

Goats

All 159 goat samples were included in the ELISA. All farms had animals that tested positive (Table 6), but in one case, Ndonga, only one out of six (16.7 %) of the animals tested positive, while four (66.7 %) tested negative. The other farms all had at least ten animals tested and the second lowest proportion of positives was found on Bassopa, where 69.7 % of the animals were antibody positive. The highest percentage of positives was found at Psungo, where 90 % of the animals tested positive. The prevalence for the tested animals was 74.8 %, slightly higher than the mean prevalence among the farms that was 66.5 %.

Spectrophotometry

Most of the extracted RNA were analysed using spectrophotometry to determine if it needed dilution before being used as template for real-time RT-qPCR. The highest value measured was 2,3 ng/µl from one of the cattle samples. All the other samples measured were in the range 0-2,3 ng/µl, far below the 50 ng/µl limit. Samples from all three species were measured. Some samples were not tested due to lack of time and the expectancy that they also would be well below the limit for the need of dilution.

Pan Simbu real-time RT-qPCR

In total, 99 samples were analysed with Pan Simbu real-time RT-qPCR. All farms were represented, but not necessarily by all three species of animals. The chosen samples only came from animals showing a clearly positive result in the ELISA. All antibody positives were unfortunately not included due to a limited amount of reagents available for the PCR Mastermix.

Table 4. Proportion of cattle tested positive, doubtful or negative in Schmallerberg virus antibody specific competitive ELISA. Total number of tested samples presented to the right

Farm	Positive (%)	Doubtful (%)	Negative (%)	No. of samples tested
Macia	100	0	0	15
Nguluzane	73.3	26.6	0	15
Bassopa	93.3	6.7	0	15
Ndonga	87.5	6.3	6.3	16
Chalucwane	87.5	6.3	6.3	16
Gogoti	100	0	0	15
Total	90.2 %	7.6 %	2.2 %	92

Table 5. Proportion of sheep tested positive, doubtful or negative in Schmallerberg virus antibody specific competitive ELISA. Total number of tested samples presented to the right

Farm	Positive (%)	Doubtful (%)	Negative (%)	No. of samples tested
Bassopa	45.1	35.3	19.6	51
Psungo	85.7	7.1	7.1	14
Gogoti	91.7	0	8.3	12
Total	59.7 %	24.7 %	15.6 %	77

Table 6. Proportion of goats tested positive, doubtful or negative in Schmallerberg virus antibody specific competitive ELISA. Total number of tested samples presented to the right

Farm	Positive (%)	Doubtful (%)	Negative (%)	No. of samples tested
Nguluzane	78.8	9.1	12.1	66
Bassopa	69.7	12.1	18.2	33
Ndonga	16.7	16.7	66.7	6
Psungo	90	0	10	10
Gogoti	77.3	0	22.7	44
Total	74.8 %	6.9 %	18.2 %	159

Cattle

46 cattle samples from the farms Macia, Ndonga, Chalucwane and Gogoti were analysed. All the tested samples were negative.

Sheep

37 sheep samples from the farms Bassopa, Psungo and Gogoti were analysed. All the tested samples were negative.

Goats

16 goat samples from Nguluzane, Bassopa, Psungo, Ndonga and Gogoti were analysed. All the tested samples were negative.

Gel electrophoresis

Since one run of real-time RT-qPCR yielded somewhat curious results, a gel electrophoresis was performed, including PCR products from 12 of the cattle samples. The samples were picked based on that they showed signs of some amplification during the real-time RT-qPCR. The gel showed only diffuse bands of short nucleic acids (<100 bp), see Figure 2 below.



Figure 2. Gel showing only faint bands of short nucleic acids (<100 bp). From the left: Ladder (100 bp), negative control, C435, C439, C449, C454, C574, C591, C692, C700, C708, C727, C732, C735.

DISCUSSION

The results from the screening of Schmallerberg virus antibodies with competitive ELISA in the Gaza province of Mozambique are consistent with the results obtained by Blomström *et al.* (2014) from the Zambezia province. A clear majority of cattle, 90.2 % in the present study, compared to 100 % in Blomström *et al.* (2014), were ELISA positive in undiluted serum. The within-herd seroprevalence varied between 73.3 % and 100 % with a mean of 90.3 % in the present study and did not differ almost at all compared to the seroprevalence among all tested cattle (90.2 %). The study by Mathew *et al.* (2015) in Tanzania indicated a slightly lower seroprevalence in cattle screened by indirect ELISA, 61 % of the animals investigated were seropositive.

Among sheep, the within-herd seroprevalence varied between 45.1 % and 91.7 %, with a mean of 74.2 %, in the present study. The seroprevalence of all tested sheep was 59.7 %. Blomström *et al.* (2014) reported a within-herd prevalence between 43 % and 97 %.

In goats, the present study found a within-herd seroprevalence between 16.7 % and 90 %, with a mean of 66.5 %. Of all the goat samples, 74.8 % were positive. Blomström *et al.* (2014) reported a within-herd seroprevalence between 72 % and 100 % in goats of the Zambezia province in Mozambique.

One of the farms, Ndonga, had only one out of six positive goat samples. This farm had a seroprevalence of 87.5 % in cattle. Unfortunately, no data concerning the age of the tested animals from any of the farms was available. Either most of the goats from Ndonga were too young or had for some other unknown reason not been exposed to the virus giving the positive ELISA result in cattle and in the single goat.

None of the farmers in the informal interviews had noticed any clinical symptoms in their animals or any malformed offspring. This is also consistent with the findings of Blomström *et al.* (2014). If it truly is Schmallerberg virus causing the positive ELISA, vector activity is evenly distributed over the seasons and many of the animals will be infected before they breed, missing the period when transplacental transmission can occur (Garigliany *et al.*, 2012; Bayrou *et al.*, 2014; Martinelle *et al.*, 2015). Transplacental transmission does not always cause congenital Schmallerberg (Garigliany *et al.*, 2012; Martinelle *et al.*, 2015). Also, very few animals per unit of time can be expected to show clinical disease if Schmallerberg virus is endemic in the area, and most animals that have gone through a previous infection have an immunity lasting for several years (Poskin *et al.*, 2015; Wernike *et al.*, 2015b).

It is possible that the farmers either does not know what symptoms to look for in adult animals or that the animals are not as supervised as in European production facilities and the mild or even absent clinical symptoms in adult animals (Hoffman *et al.*, 2012; Van der Poel *et al.*, 2013; Wernike *et al.*, 2013b; Poskin *et al.*, 2014; Schulz *et al.*, 2014; Poskin *et al.*, 2015; Laloy *et al.*, 2015; Martinelle *et al.*, 2015) can pass unnoticed.

The question of which virus or viruses causing the positive ELISA results remains unanswered. Cross reactivity using indirect methods to detect viruses of the Simbu serogroup might occur (Abutarbush *et al.*, 2015; Mathew *et al.*, 2015), making it difficult to draw accurate conclusions. Many Simbu serogroup viruses circulate in Africa (Theodoridis *et al.*, 1979; Zeller & Bouloy, 2000; Mathew *et al.*, 2015). It might be one or several of them that are responsible for the high percentage of positive ELISA results, since cross reactions cannot be excluded. On the contrary, they are rather probable.

Further studies to investigate the prevalence of Schmallerberg virus specific antibodies in different regions of Mozambique and southern Africa is of course of interest. Commercial ELISA kits might not be the best way to do this due to their unknown validity in areas where other viruses of the Simbu serogroup might circulate or even be endemic and due to the risk of cross reactivity with such viruses. Virus neutralization tests might be another option, since it is considered the most accurate and detailed of the indirect detection methods, but the problem with cross reactivity remains. It would however be possible to draw some conclusions if several Simbu serogroup viruses were included in such a study, since the titers for the different viruses would be obtained and could be compared. It is also of great interest to further investigate any chosen antibody detection method for cross reactions among the Simbu serogroup to achieve a greater understanding concerning this matter.

To investigate the possibility of another virus causing the positive ELISA results, a Pan-Simbu real-time RT-qPCR was chosen for detection of viral RNA. All the Pan-Simbu real-time RT-qPCR runs yielded negative results. This was perhaps due to the study design where only seropositive animals were used for PCR. The viremic period of Schmallerberg virus and of many other Simbu serogroup viruses is very short, only a few days (Van der Poel *et al.*, 2013; Wernike *et al.*, 2013a; Wernike *et al.*, 2013b; Poskin *et al.*, 2014; Schulz *et al.*, 2014; Laloy *et al.*, 2015; Martinelle *et al.*, 2015; Poskin *et al.*, 2015). When an animal has seroconverted, which happens at earliest at day 6-8 post infection with Schmallerberg virus (Wernike *et al.*, 2013a; Wernike *et al.*, 2013b; Poskin *et al.*, 2014; Schulz *et al.*, 2014; Laloy *et al.*, 2015), the viremic period has already ended in most animals.

To try to find Schmallerberg viral RNA or RNA from another Simbu serogroup virus or viruses would be an interesting quest in future studies. This, however, can be very tricky to achieve. The chance of finding a viremic animal is very low due to the short viremia, even if great care is taken when choosing suitable animals. One option would be to screen very young animals with ELISA and only go through with PCR in seronegative or doubtful animals from farms with seropositive animals and hope to catch an animal with viremia.

A viable way might be to try to isolate Schmallerberg virus from sperm of young, seropositive bulls, since Schmallerberg virus shedding can continue for 2-3 months after viremia has ended in some individuals. Great individual variation is seen though, virus shedding might be intermittent and the highest concentrations of viral RNA is seen during the first week of infection (Hoffman *et al.*, 2013; Van der Poel *et al.*, 2013; Ponsart *et al.*, 2014).

Another option would be to finance a surveillance program where farmers could be on the lookout for offspring with signs of congenital Schmallerberg and send them in for analysis when they occur. Midges could also be taken into consideration for trying to detect Schmallerberg or Simbu serogroup viral RNA, but this approach would need very many specimens to be included to have a chance to succeed.

CONCLUSIONS

Antibodies targeted against Schmallerberg virus or a similar virus circulate the Gaza province in Mozambique and can be found in cattle, sheep and goats. Viral RNA from Schmallerberg virus or any other Simbu serogroup virus has not yet been isolated from seropositive animals in the area.

Further, carefully designed, studies need to be made to determine whether it truly is Schmallerberg virus or if it is another virus causing the positive ELISA results.

ACKNOWLEDGEMENTS

This work was financed by SIDA and the Swedish Agricultural University (SLU).

My supervisor Anne-Lie Blomström has been an excellent help during the entire process, especially by giving response and encouragement during the writing of this report. Thank you! Without your advice, this work would never have been finished.

Great thanks also to Harindranath Cholleti, my assistant supervisor that taught complicated lab procedures with an unlimited amount of patience.

Many thanks to Doctor José Fafetine and the staff at the Department of Veterinary Medicine at University Eduardo Mondlane, Maputo, Mozambique. They have been extremely supportive and helpful.

A special thanks to veterinarian Belissario Moiane, his family and an unnamed veterinary technician. They helped us arrange field trips, collect blood samples and showed us amazing rural areas of Mozambique.

Also, a great thanks to my study comrade Ulf Alling for coping with me for several months during very exotic circumstances.

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