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A screening for Schmallenberg Virus among sheep and goats in Tanzania
En studie om förekomsten av Schmallenberg virus hos får och getter i Tanzania

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SUMMARY

Schmallenberg virus (SBV) is a novel arthropod-borne orthobunyavirus emerging in Europe in 2011 to 2012. Acute SBV infection causes diarrhoea, fever and reduced milk production in dairy cattle, but it is mainly the reproductive disorders (abortions, malformed foetuses and stillborn animals) in ruminants that have caused substantial economical losses. The prevalence of the virus outside of Europe is poorly investigated. SBV or SBV-like antibodies were detected in Mozambique in 2013, which raised interest for a similar study in Tanzania.

In this study in Tanzania, blood samples were collected from 478 sheep and goats from 39 herds in 15 different villages in three districts, covering areas in the north, south and east of Tanzania. The epidemiology of the virus was investigated by tracing antibodies by ELISA and mapping of the virus by PCR was started. In total 309 serum samples were analysed by a competitive ELISA. The overall seroprevalence was 43% by this test. Seropositive herds were found in all areas. In Mikumi in the east, all herds were positive for SBV antibodies, in Ngorongoro in the north, 64% were positive and in Mahenge in the south were 85% positive. In total 127 of the tested samples were additionally analysed by an indirect ELISA. The results between the two ELISAs differed more than expected. The overall seroprevalence was 21% by the indirect ELISA. This divergent result is not supported by other studies comparing different SBV ELISAs, and the fact that the sensitivity and specificity are similar for both tests contradicts the result. In total 68 buffy coats from herds seropositive by both ELISAs were analysed by PCR. All analysed samples were negative in the Pan-Simbu RT-PCR.

The findings in this study indicate that SBV is endemic in Tanzania. However cross-reactivity with viruses related to SBV cannot be ruled out as cause of the positive ELISA results.

SAMMANFATTNING

Schmallenberg virus (SBV) är ett nytt vektorburet virus som upptäcktes och spred över Europa 2011-2012. Akut infektion med SBV orsakar diarré, feber och sänkt mjölkproduktion hos mjölkkor. Det är dock främst reproduktionsstörningarna (abort, missbildade och dödfödda avkommor) hos idisslare som har orsakat stora ekonomiska förluster. Förekomsten av viruset utanför Europa har studerats i mycket liten utsträckning. År 2013 upptäcktes antikroppar mot SBV eller ett SBV liknade virus i Mozambique, vilket väckte intresse för genoförandet av en liknande studie i Tanzania.

I denna studie i Tanzania samlades blodprov från 478 får och getter från 39 flockar, i 15 olika byar i tre olika distrikt, från norra, södra och östra Tanzania. Viruset undersöktes epidemiologiskt genom spårning av antikroppar med ELISA och en kartläggning av viruset med PCR påbörjades. Totalt analyserades 309 serumprover med en kompetitiv ELISA. Den totala seroprevalensen var 43 % med detta test. Seropositiva flockar fanns i alla provtagna områden. I Mikumi i öst, var samtliga flockar seropositiva, I Ngorongoro i norr, var 64 % av flockarna positiva och i Mahenge i söder, var 85 % positiva. Av de analyserade proverna analyserades 127 även med en indirekt ELISA. Resultatet mellan de två ELISorna skiljde sig mer än väntat. Den totala seroprevalensen var 21 % med den indirekta ELISAn. Denna skillnad mellan olika ELISor har inte setts i tidigare studier. Båda testerna har liknande sensitivitet och specificitet vilket gör resultatet än mer motsägelsefullt. Från flockar som var seropositiva med både den kompetitiva och den indirekta ELISAn analyserades 68 buffy coats med PCR. Alla prover var negativa med Pan-Simbu RT-PCR.

Fynden i denna studie indikerar att SBV finns endemiskt i Tanzania. Dock kan korsreaktivitet med virus som är närbesläktade med SBV inte uteslutas som orsak till de positiva ELISA resultaten.

CONTENT

INTRODUCTION

The aim of this study was to investigate the prevalence of Schmallenberg virus in small domestic ruminants in Tanzania. This was carried out by tracing antibodies by ELISA and mapping the virus through PCR.

Schmallenberg virus (SBV) is a novel vector-borne orthobunyavirus, first detected in northwestern Europe 2011 (Hoffmann *et al*., 2012). According to a publication based on a Swedish master thesis in veterinary medicine published in 2014, a high prevalence of SBV antibodies have been detected in domestic ruminants in the northern parts of Mozambique (Blomström *et al*., 2014). This finding raised interest for a similar study in Tanzania, since SBV is a relatively new and undefined virus in Africa, and in the rest of the world outside of Europe.

The original plan was to investigate the prevalence of SBV even in small wild ruminants. Unfortunately we had no possibility to get hold of samples from wild animals during this study period.

LITERATURE REVIEW

Schmallenberg Virus

History

In summer and autumn 2011, a significant increased prevalence of an unspecific disease with clinical signs including decreased milk production, fever and diarrhoea in adult cows were reported from several dairy farms in northwestern Germany and in the Netherlands (Hoffmann *et al*., 2012). In November and December the same year many ewes in the Netherlands gave birth to malformed lambs (van den Brom *et al*., 2012). No such outbreak of malformations in lambs has ever occurred earlier in Europe. The ewes did not show any clinical signs during gestation or at parturition (van den Brom *et al*., 2012). In spring 2012, reports of similar cases in goat flocks started to be reported (Helmer *et al*., 2013). No clinical signs were reported in sheep and goats during the period when acute infection in adult cattle first was observed (Beer *et al*., 2013). In 2012, there were two major peaks of confirmed cases of SBV infections: in February it resulted largely from sheep herds, and in May from cattle (Afonso *et al*., 2014).

In a few months time the virus had spread over western Europe with outbreaks in Belgium, France, Germany, Luxemburg, The Netherlands, parts of England and Switzerland. One year after the first cases of SBV in Europe, the virus had spread as far as to northern Sweden (Chenais *et al*., 2013). Until 30th May 2013, 8730 confirmed cases of SBV from 22 European countries had been reported (EFSA, 2013). However, the number of SBV infected herds may be up to 300 times greater than the number of confirmed cases reported (Afonso *et al*., 2014). SBV has been detected in domestic sheep, goats, cattle, in twelve wild species, dog and in 19 different species in zoos (EFSA, 2014).

By metagenomic analysis and full-length sequencing, this novel virus turned out to be most similar to three viruses detected in Japanese cattle; Shamonda virus, Aino virus and Akabane virus (Hoffmann *et al*., 2012). The new virus was named after the town Schmallenberg in Germany where it was first detected (Hoffmann *et al*., 2012). The outbreak of SBV was the first time a virus of the Simbu serogroup was detected in Europe (Hoffmann *et al*., 2012).

Genetic properties

SBV belongs to the Simbu serogroup, genus orthobunyavirus in family *Bunyaviridae* (Hoffmann *et al*., 2012). Viruses of this family are spherical and enveloped. The surface of the envelope is covered with glycoprotein peplomers and it encircles three circular, helical nucleocapsid segments. The

genome consists of three single-stranded RNA segments; small (S), medium (M) and large (L) (Quinn *et al*., 2002).

A phylogenetic analysis indicates that SBV is a reassortant, with the M RNA segment from Sathuperi virus and the S- and L RNA segments from Shamonda virus (Yanase *et al*., 2012). However, full-length genome sequencing highly indicates that SBV being a Sathuperi virus and is an ancestor of Shamonda virus, not a reassortant (Goller et al., 2012).

The S segment of the genome of SBV is much more conserved than the M segment which has a hypervariable region (mutation hot-spot) (Fischer *et al*., 2013a). The role of this hypervariable region is unclear and needs to be further investigated, but since similar regions in other Orthobunyaviruses play a key role in the evasion of the immune response, Fischer *et al*. (2013a) suggest that it might be the same case for the SBV genome. Analysis of samples from different regions of Germany indicates that the variation of the M segment is probably independent of geographical region and host species, and may develop during virus replication within the specific individual host (Fischer *et al*., 2013a). If the sequence divergence is related to the vertebrate host or the arthropod vector is unclear (Fischer *et al*., 2013a).

Clinical signs

In adult animals the virus causes a short viremia of 5-6 days (Hoffmann *et al*., 2012). In adult cattle, the clinical signs are unspecific including fever, diarrhoea and drop in milk production (Hoffmann *et al*., 2012). Adult sheep do not show any clinical signs, besides maybe dystocia when giving birth to malformed lambs (van den Brom *et al*., 2012). However, there have been retrospective reported cases of lethargy and diarrhoea in some goats and sheep during the period of time when SBV infection of the flock might have taken place (Helmer *et al*., 2013).

It is the reproductive disorders (abortions, malformed fetuses and stillborn animals) that is the major concern, since SBV infection is not associated with any mortality in adult cattle, sheep or goats (Afonso *et al*., 2014). Other orthobunyaviruses are also associated with congenital defects in ruminants (Quinn *et al*., 2002).

In utero infection with SBV of the fetus may result in malformations and major damage to muscles and CNS (Bayrou *et al*., 2014; Peperkamp *et al*., 2014). The clinical picture shown by the in utero infected calf is likely to depend largely of the age of the fetus at the time of infection (Bayrou *et al*., 2014). In utero SBV infected lambs and goat kids show similar signs as SBV infected calves (Helmer *et al*., 2013; van den Brom *et al*., 2012). The kind of malformations seen in the offspring is malformed spine, joint contractions (arthrogryposis), neurological signs and hypoplasia of skeletal muscles. Most commonly, affected offspring has multiple malformations of the vertebral column (Peperkamp *et al*., 2014). In lambs is scoliosis the most frequent malformation of the spine, and in calves torticollis is the most frequent malformation of the spine (Bayrou *et al*., 2014; Peperkamp *et al*., 2014). In a Dutch study, 97% of 102 affected lambs and 96% of 204 affected calves had arthrogryposis (Peperkamp *et al*., 2014), which is consistent with Dominguez *et al*.'s (2012) result that the most common deformity in lambs is arthrogryposis (Figure 1). When arthrogryposis is seen, it is common that multiple joints and limbs are affected. It is also common with distortion of the head (Bayrou *et al*., 2014).

Figure 1. *Lamb with torticollis and arthrogryposis. Photo: SVA*

Besides the malformations, newborn SBV infected calves weigh significantly less than normal calves (Bayrou *et al*., 2014). When twins (or more offspring) are in utero SBV infected it is not uncommon that only one of the offspring shows clinical signs of SBV (van den Brom *et al*., 2012; Wernike *et al*., 2014a).

There is a divided opinion about if the seroprevalence is depending one the animals' age. According to Méroc *et al.* (2013), the seroprevalence is significantly associated with age. In their study of the seroprevalence among Belgian cattle, adult animals had higher prevalence than younger. The authors suggest it to be an effect of that younger animals are being kept more indoors, and thus less exposed to vectors than older cattle. This idea is supported by the report by Helmer *et al*. (2013) of a significant correlation between housing conditions (indoors or outdoors) in goat flocks, with lower prevalence among animals kept indoors. However, in the Netherlands no difference in seroprevalence in different ages has been seen (Elbers *et al*., 2012).

Diagnosis

Both ELISAs and RT-PCR for detection of antibodies and the viral genome were available on the market only a few months after the discovery of the virus. Validated commercial RT-PCR was available after about three months (Bilk *et al*., 2012), and different ELISAs were being used in several countries in 2012 (Anon., 2012). Virus neutralisation test (VNT) is the gold standard for diagnosis of SBV, with sensitivity and specificity close to 100% (Loeffen *et al*., 2012).

Wernike *et al*., (2014a) claims that the virus' RNA is more often detected in SBV infected aborted, stillborn and/or malformed lambs and goat kids than in aborted, stillborn and/or malformed calves. The authors suggest the longer gestation period for cattle, than for sheep and goats, to probably be the explanation of this difference, in cattle the virus is no longer infecting the offspring at time of delivery. Similar findings supports this hypothesis (Peperkamp *et al*., 2014). However, virus nucleic acids remain detectable at term in calves born with clinical signs of SBV (Bayrou et al., 2014; Garigliany et al., 2012).

Postmortal findings

Common findings, besides the malformations, at post mortem examination of in utero SBV infected calves and lambs, are the tendons spanning affected joints are shorter than expected and corresponding muscles displays altered colour and decreased mass (Bayrou *et al*., 2014).

Neurons are the primary target cells in SBV infected newborn calves, which can be seen both macroscopically and microscopically in most affected lambs and calves (Peperkamp *et al*., 2014). Severe CNS lesions are characterised by cavitation in the white matter, with major neuron loss in the cortex of cerebrum and cerebellum, in the brainstem nuclei and in the gray columns of the spinal cord. The lesions are in general more severe in lambs than in calves (Peperkamp *et al*., 2014).

Microscopically you can see that the spinal cord has a significant decreased number of neurons and the muscles have an increased fibre size and abnormal infiltration of connective tissue and adipocytes (Bayrou *et al*., 2014). Encephalitis with lymfohistiocytic and perivascular cuffs can be diagnosed histologically (Hahn *et al*., 2013).

At post mortal examination the viral RNA is often present in the CNS and placental fluids, and might also be present in other tissues, such as lung, spleen, rib cartilage, colon and meconium (Bayrou *et al*., 2014; Bilk *et al*., 2012). Hence, CNS tissue, placental fluids and the umbilical cord are suitable to use for confirming SBV infection, which is convenient since these materials can easily be collected from the farms without need for necropsy (Bilk *et al*., 2012).

Transmission

Vectors

SBV is highly suggested to be an arthropod-borne virus similar to other members of the family *Bunyaviridae* (Quinn *et al*., 2002). SBV has been detected in several *Culicoides* spp in several countries. The viral RNA has been detected in *C. obsoletus, C. Dewulfi* and *C. Chiopterus* in Belgium (De Regge *et al*., 2012), Denmark (Rasmussen *et al*., 2012), France (Balenghien *et al*., 2014), Germany (Hoffmann *et al*., 2012), Italy (Goffredo *et al*., 2013), Netherlands (Elbers *et al*., 2013) and Poland (Larska *et al*., 2013b). SBV has been detected in the salivary glands of the midges, which is a proof that the virus are able to amplify in the midge and not simply be positive after a blood meal from a SBV viremic animal (De Regge *et al*., 2012; Rasmussen *et al*., 2012). Other viruses of the Simbu serogroup have been isolated from both *Culicoides* midges and mosquitoes (Quinn *et al., 2002)*. For SBV, there have been no findings in mosquitoes, which indicates that they most likely do not have a major, if any, role of SBV transmission (Wernike *et al*., 2014b).

Vertical transmission

Transplacental infection is possible when the first placentome is present until the fetus is immunocompetent, d 30-150 after conception in cattle (Bayrou *et al*., 2014) and d 28-56 in sheep and goats (Helmer *et al*., 2013). The age of the fetus are highly dependent for the outcome of the clinical signs of the infection (Bayrou *et al*., 2014). If the mother animal is infected earlier in gestation it can result in embryonic death, decreased fertility and stillbirth. If infected later in gestation, the fetus is able to fight the virus with its matured immune system, but abortion, stillbirth and mummification can also be observed (Helmer *et al*., 2013).

Only a few percent of infected fetuses develop abnormalities or antibody response (Wernike *et al*., 2014a). In a French study, overall 85% of the ewes in SBV positive flocks gave normal birth, differing 50-95% between herds (Dominguez *et al*., 2012). The authors suggest this difference being due to that ewes in the different herds were exposed to the virus during different stages of gestation. In only about 25% of seropositive goat flocks the animals showed clinical signs of SBV infection in Germany in 2012 (Helmer *et al*., 2013).

Since there are no reports today of viremia in infected offspring, there is no evidence that the virus can be transmitted from the infected offspring to vectors (EFSA, 2014).

Semen

Infectious SBV can be excreted in semen (Schulz *et al*., 2014). The excretion of SBV in semen is intermittent with high individual variation (Ponsart *et al*., 2014). Although the highest levels is excreted the first week after infection (Van Der Poel *et al*., 2014), SBV can still be detected in bull semen several months after natural infection (Ponsart *et al*., 2014). The SBV RNA has been detected both in the seminal cell fraction and the seminal plasma from SBV seropositive bulls, but only in the seminal plasma from seronegative bulls (Hoffmann *et al*., 2013).

It is unlikely that the embryo gets infected when the mother is inseminated with semen containing SBV, but it might enable vector transmission if the mother gets viremic (Schulz *et al*., 2014).

Prophylaxis and treatment

Today there are no therapies available for SBV, hence relying on supportive care.

There are a number of inactivated vaccines developed for SBV, and two vaccines are commercially available. Bovilis® SBV (MSD Animal Health) was the first available vaccine targeting SBV in cattle

and sheep (Merck Animal Health, 2013). The vaccination regime according to the manufacturer is for cattle two injections with four weeks interval, for sheep one injection is sufficient for protection. The other commercially available vaccine is SBVvax (Merial, France) which has a similar vaccination regime (Merial, 2013). Hechinger *et al*. (2014) support that one single injection gives complete protection of sheep from SBV-infection.

Besides vaccination there are a few measures that can be taken to protect naive animals or herds that only have a low within-herd seroprevalence: The mating period can be rescheduled to late autumn (mainly relevant for sheep and goats), the animals can be held more indoors year around, or at least from sunset to sunrise, and proper treatment with repellents over the summer months can be performed. To prevent infection during gestation, susceptible animals can also be moved into endemic areas in time to develop immunity before they are first mated/inseminated (Helmer *et al*., 2013). SBV specific antibodies persist for at least two years in adult cattle (Elbers *et al*., 2014). Calves loses their maternal antibodies after five to six months (Elbers *et al*., 2014).

Introduction and spread of SBV in Europe

Since autumn 2011, when SBV was first detected in northwestern Europe (Hoffmann *et al*., 2012), the virus has spread over large parts of Europe until 2013 (Afonso *et al*., 2014). In the core region (Germany, Belgium and the Netherlands) the seroprevalence turned rapidly high after the first reports. Sporadic cases from other parts of Europe such as Italy, Spain and Denmark began to be reported. In winter 2011-2012, the seroprevalence was 72.5% among Dutch dairy cattle (Elbers *et al*., 2012). In January to March 2011, the between herd seroprevalence was 99.8% and estimated to 86.3% within herds for cattle in Belgium (Méroc *et al*., 2013). The median intra-herd seroprevalence in goats in Germany in 2011/2012 was 36.7%. The virus has been found in high altitude areas (>2000 m) (Fernandez-Aguilar *et al*., 2014) and far north, over latitude 65 ° N in Sweden (Chenais *et al*., 2013).

It is most likely that SBV was introduced to Europe in the spring or early summer of 2011 (Beer *et al*., 2013). How it got to Europe is unclear but it has been suggested that since SBV was first detected in the same area as Bluetongue virus (Conraths *et al*., 2012), there may be an unidentified way of transmission for exotic viruses into Europe (Beer *et al*., 2013). According to Beer *et al*. (2013) the affected region has some unique features such as several international airports and harbours, high human populations density with daily importation of large amounts of fresh goods, vegetables, fruits and flowers from all over the globe. In the region there is also a high density of cattle and sheep, and *Culicoides* are present in the region. The relative risk for introduction of an emerging infectious disease to Europe has proven to be greatest in the northwestern parts (Jones et al., 2008).

In a partly retrospective study, SBV antibodies have been detected in Turkey in blood collected 2006-2010 from slaughtered cattle, sheep, goats and buffalo, indicating that SBV were present in Turkey before the outbreak in Europe (Azkur et al., 2013).

Schmallenberg virus in wild animals

Only a few studies are published on SBV in wild animals (Barlow *et al*., 2013; Chiari *et al*., 2014; Fernandez-Aguilar *et al*., 2014; Laloy *et al*., 2014; Larska *et al*., 2013a; Linden *et al*., 2012). The first study investigated the seroprevalence among roe deer and red deer shot in Belgium in 2010-2011 (Linden *et al*., 2012). No SBV antibodies were detected in the samples from 2010, but during the autumn of 2011 an increased seroprevalence (20 to 88.9%) of SBV was detected. This finding shows a rapid spread of the virus during 2011 among wild animals. However, no clinical signs were seen among the adult animals or fetuses at necropsy. This finding is supported by other reports of seropositive deer in 2011-2012, with no reproductive disorders or other clinical findings in different

parts of Europe: England (Barlow *et al*., 2013), France (Laloy *et al*., 2014) and in alpine Italy (Chiari *et al*., 2014). SBV RNA has also been detected in a Polish elk calf with pneumonia (Larska *et al*., 2013a). The calf had not seroconverted. In the same study SBV seropositive bison and deer were found in autumn to winter 2012-2013.

Schmallenberg in Africa

The prevalence of SBV in Africa is poorly investigated. In autumn 2013, Blomström *et al*., did a serological screening study in Mozambique using ID Screen® Schmallenberg virus competition multi-species ELISA. The result indicates that there is a continuous exposure of SBV in northern Mozambique since all tested farms were seropositive, but none of the animals showed any clinical signs. However, in the ELISA cross-reactions with other viruses cannot be excluded, why the result cannot be certain to be due to SBV. The results also show a higher seroprevalence in goats than in sheep, in contrast to studies in European farms (Blomström *et al*., 2014).

There are no publications concerning SBV in Tanzania. In a Tanzanian-Norwegian unpublished screening survey, Tanzanian cattle and goats have been positive in an SBV-ELISA, indicating the circulation of SBV or an SBV-like virus (Coletha Matthew and Maria Stokstad, personal communication).

Leask *et al*. (2013) suggests in a retrospective study that SBV may be present in South Africa. The report presents two cases that occurred in 2006 and 2008. In the first case, one ewe gave birth to three lambs, of which one was malformed. No other reproductive disorders from that farm were reported. In the second case, several ewes gave birth to malformed lambs. Wesselborn, bluetongue, rift valley fever and Akabane virus were ruled out. After the reports of SBV in Europe, the authors suggest that the malformations might have been caused by SBV, and that other cases might wrongly have been attributed as Akabane virus (Leask *et al*., 2013).

Socioeconomic impact

Due to trade restrictions, SBV has caused major economic losses in Europe. Between 2011 and 2012 there was a 20% decline in export value for purebred breeding animals (EFSA, 2014). The trade with bovine semen were also affected. In 2012, the trade with bovine semen dropped by 8.9 million doses, corresponding 11-26% (EFSA, 2014).

Even if the indirect impacts, like trade restrictions, are the major causes of the economical losses in Europe due to SBV, it also caused severe effects on individual level for some farmers. The most reported cases of SBV infections are those from malformed fetuses (Afonso *et al*., 2014). Farms that experienced a high number of malformed lambs are also the ones that suffered the highest economical losses (Beer *et al*., 2013). Only a small number of herds reported acute cases in adults (6% for cattle, 1% for goats and 3% for sheep) (Afonso *et al*., 2014). In cattle farms the reduction in milk yield caused bigger economical losses than the malformed offspring did (Beer *et al*., 2013). Even if only a limited number of goat farms have been affected by SBV, some of the affected farms have been suffering great economic losses (up to 50%) due to loss of kids and drop in milk production (Helmer *et al*., 2013).

No data are available for the impact on international level for beef or goat farms (EFSA, 2014).

Concerning The public health, the risk is considered as negligible because neither SBV nor closely related viruses have shown any zoonotic potential (Hoffmann *et al*., 2012; Reusken *et al*., 2012).

Tanzania – the study area

Tanzania is an East African country located just south of the equator. By the coast the climate is tropical and in the inland the climate is warm and dry. Even if Tanzania has a stable economic growth, it is still one of the poorest countries in the world. About 33% of the population lives below the income threshold for poverty (landguiden, 2014), and the prevalence of undernutrition was 39% in 2010-2012 (FAOSTAT, 2014). In total 72% of the population lives in rural areas, which is a declining share. Agriculture accounts for about 25% of the GDP and 74% of the total labour force, of these women account for 55% (FAOSTAT, 2014). There are about 19 200 000 cattle, 13 700 000 goats and 3 600 000 sheep in Tanzania (The united republic of Tanzania Livestock and Fisheries development, 2010).

This study is based on three field trips. Two field trips were to two different districts in the Morogoro region in the east and south of Tanzania, and one field trip was to Ngorongoro Conservation Area in the Arusha region in the north (see Figure 2). In the Morogoro region we sampled herds close to the Mikumi National Park, where there is lot wildlife. The park is not gated, meaning the wildlife can be found outside the park and intermingle with domestic animals. The second sampled area in Morogoro region was around the town Mahenge in the south. Agro-pastoral farming is practiced in both of these areas. In Ngorongoro Conservation Area, pastoral farming is practiced, and the Maasai people and their animals live in harmony with the wildlife. In Morogoro region, there are about 650 000 cattle, 300 000 goats and 60 000 sheep, and in Arusha region 1 700 000 cattle, 800 000 goats and 800 000 sheep (The united republic of Tanzania Livestock and Fisheries development, 2010).

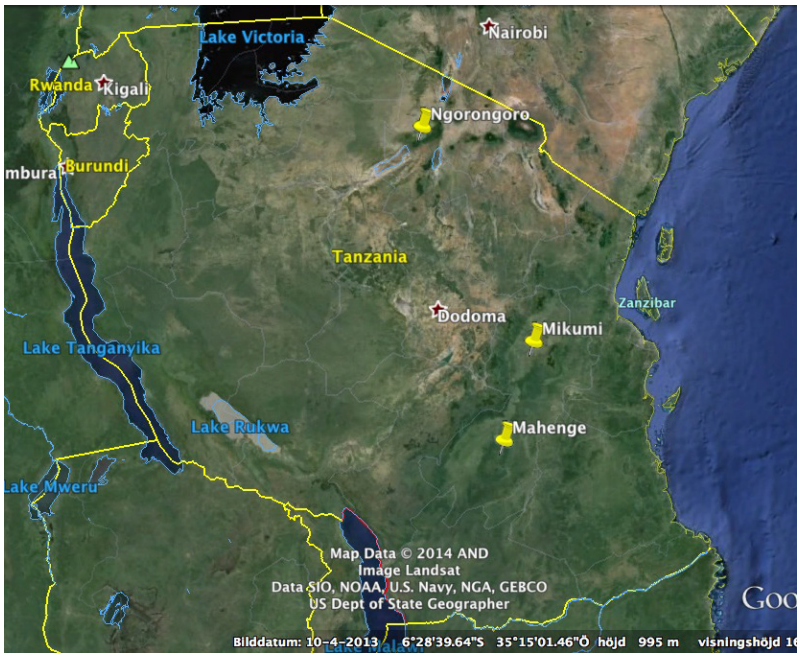

Figure 2. *A map of Tanzania with the areas sampled, Mahenge, Mikumi and Ngorongoro, marked with yellow pins and text.*

MATERIAL AND METHODS

The original plan was to investigate the prevalence in both domestic and wild animals. Unfortunately we did not get hold of any samples from wild animals during this study period, so only samples from small domestic ruminants have been analysed.

Sampling design

The sampling was performed during three field trips to three different areas, one trip to each area, in late September until mid-October 2014. The areas visited were Mikumi in the east, Ngorongoro in the north and Mahenge in the south of Tanzania. In total 478 animals from 39 herds in 15 villages were sampled. Animals sampled were sheep and goats in ages of two weeks to nine years. As far as possible, 10 to 20 animals in each herd were sampled. If available, both sheep and goats were sampled, aiming to include both young and adult animals in each herd. The sampled animals were manually selected at the sampling occasion. The age of the animals was either reported by the farmer, or estimated by looking at the size of the animal or its teeth. The age estimation was performed either by the farmer, a veterinary assistant or by one of the students. Breed, sex and age of each sampled animal were registered. All sampled animals are presented in Table 1.

Blood samples, serum and EDTA-blood, were collected from all sampled animals from the jugular vein, using a vacutainer system (Figure 3). The samples were stored in a cooling box during the field trips. After separation of serum, plasma and the buffy coat, the samples were stored in cryo tubes in a freezer at -45° C until analysis. Most samples were separated the same day as sampled. Due to field conditions, some samples were not separated until up to three days after being collected.

The fieldwork was collaboration between three different projects, and the sampling sites were selected to cover a wide geographical area of Tanzania, with sampling sites the northern, eastern and southern parts of the country. Our local supervisor, local veterinarians and the chiefs of the villages made the selection of herds sampled in each area by convenience. In the areas sampled in the north and east, the domestic small ruminants intermingle with wild small ruminants, which was of interest for all of the three projects. Assuming that all animals in one herd would have encountered the same antigens, we prioritized to sample a few animals in many herds, rather than many animals in few herds.

Figure 3. *Sampling during field conditions.* *Photo: Ida Herbe and Lovisa Levin*

Table 1. *Presentation of all the sampled animals from each herd*

Area/ Region/District	Village	Herd	Sheep	Goats	Total
Mikumi/Morogoro/Mvomero	Mtipule	Herd 1	6	4	10
		Herd 2	7	12	19
		Herd 3	7	8	15
		Herd 4	13	8	21
	Msongozi	Herd 5	5	3	8
		Herd 6	16	6	22

		Herd 7	8	11	19
		Herd 8	4	7	11
	Mkata	Herd 9	5	10	15
		Herd 10	7	9	16
		Herd 11	3	8	11
		Herd 12	2	8	10
		Total	**83**	**94**	**177**
Ngorongoro/Arusha/Ngorongoro	Kijakaybosi	Herd 13	4	2	6
	Endulen	Herd 14	2	8	10
	Esere	Herd 15	3	8	11
		Herd 16	5	7	12
		Herd 17	5	7	12
	Meshili	Herd 18	10	0	10
	Kesile	Herd 19	14	6	20
		Herd 20	0	10	10
		Herd 21	0	10	10
		Herd 22	0	15	15
		Herd 23	0	11	11
	Irkeepusi	Herd 24	13	0	13
		Herd 25	7	3	10
		Herd 26	3	0	3
		Total	**66**	**87**	**153**
Mahenge/Morogoro/Tanga	Tanga	Herd 27	6	6	12
		Herd 28	10	2	12
		Herd 29	6	6	12
	Lupunga	Herd 30	11	10	21
	Ipera	Herd 31	6	6	12
		Herd 32	9	3	12
Area/ Region/District	**Village**	**Herd**	**Sheep**	**Goats**	**Total**
		Herd 33	6	6	12
	Lupiro	Herd 34	8	4	12
		Herd 35	0	3	3
	Mwaya	Herd 36	5	0	5
		Herd 37	10	10	20
		Herd 38	9	0	9
	Nkongo	Herd 39	1	5	6

	Total	87	61	148
Total all herds		236	242	478

Analysis

The lab work was performed at the Genome Science Centre at the Faculty of Veterinary Medicine, Sokoine University of Agriculture (SUA), Morogoro, Tanzania.

ELISA

A subset of selected samples were analysed for screening of SBV. Samples for the ELISA were manually selected to represent equal proportion of the sampled animals from each farm, both young (less than one year) and adult animals, both sexes and both species from all herds sampled, as far as possible. Due to limited resources all collected samples could not be analysed.

First, the samples were analysed by the ID Screen® Schmallenberg virus Competition Multi-species ELISA (ID-vet, France). According to the manufacturer, this ELISA is developed for ruminants and other species. After retrieving the results, some of the samples were additionally analysed by the SVANOVIR® SBV-ab ELISA (Svanova, Sweden), which is an indirect ELISA, developed for cattle, sheep and goats. The selected samples used in the indirect ELISA represented positive, negative and doubtful as classified by the competitive ELISA. Due to limited number of indirect ELISAs available, only a selection of samples could be analysed by both ELISAs. Samples analysed with the two different ELISAs are presented in Table 2.

Table 2. *Presentation of all animals analysed by competitive and indirect ELISA. The presented ages are for the samples analysed by the Competitive ELISA*

Area/ Region/District	Village	Herd	Age		No. analysed by Competitive ELISA		No. analysed by Indirect ELISA	
			< 1 yr.	>1 yr.	Sheep	Goats	Sheep	Goats
Mikumi/Morogoro/Mvomero	Mtipule	Herd 1	0	6	2	4	1	4
		Herd 2	2	8	4	6	3	5
		Herd 3	0	9	6	3	5	3
		Herd 4	2	8	6	4	4	2
	Msongozi	Herd 5	0	5	3	2	1	1
		Herd 6	3	10	8	5	7	3
		Herd 7	1	10	7	4	5	4
		Herd 8	1	6	3	4	1	2
	Mkata	Herd 9	0	9	3	6	3	2
		Herd 10	0	10	4	6	3	2
		Herd 11	0	8	2	6	0	2
		Herd 12	0	7	1	6	0	1
		Total	**9**	**96**	**49**	**56**	**33**	**31**
Ngorongoro/Arusha/Ngorongoro	Kijakaybosi	Herd 13	2	2	2	2	2	1
	Endulen	Herd 14	7	0	1	6	1	4
	Esere	Herd 15	3	5	2	6	1	5
		Herd 16	1	7	3	5	2	3
		Herd 17	0	7	4	3	2	1
	Meshili	Herd 18	0	7	7	0	2	0
	Kesile	Herd 19	2	10	7	5	3	1
		Herd 20	0	7	0	7	0	1
		Herd 21	0	7	0	7	0	2
		Herd 22	0	7	0	7	0	4
		Herd 23	7	0	0	7	0	2
	Irkeepusi	Herd 24	0	8	8	0	1	0
		Herd 25	2	7	6	3	1	0
		Herd 26	0	3	3	0	1	0
		Total	**24**	**77**	**43**	**58**	**16**	**24**
Mahenge/Morogoro/Tanga	Tanga	Herd 27	1	7	3	5	1	3
		Herd 28	8	0	6	2	0	1

Area/ Region/District	Village	Herd	Age		No. analysed by Competitive ELISA		No. analysed by Indirect ELISA	
			< 1 yr.	>1 yr.	Sheep	Goats	Sheep	Goats
		Herd 29	5	4	5	4	0	2
		Herd 30	1	11	6	6	1	2
	Ipera	Herd 31	0	8	3	5	2	0
		Herd 32	1	8	7	2	1	0
		Herd 33	0	9	4	5	0	1
	Lupiro	Herd 34	1	7	5	3	0	0
		Herd 35	1	2	0	3	0	1
	Mwaya	Herd 36	1	3	4	0	1	0
		Herd 37	2	10	5	7	3	1
		Herd 38	3	4	7	0	1	0
	Nkongo	Herd 39	0	6	1	5	0	2
		Total	**24**	**79**	**56**	**47**	**10**	**13**
Total all areas			**57**	**252**	**148**	**161**	**59**	**68**

The manufacturers' protocols were followed for both kits. In brief, the ID-vet ELISA testing procedure was as follow. Serum, positive (PC) and negative (NC) controls were added to microwells coated with recombinant SBV nucleoprotein antigen. If anti-nucleoprotein antibodies were present, an antibody-antigen complex, which masks the nucleoprotein epitopes, was formed. After incubation, conjugate that fixes to the free nucleoprotein epitopes was added. After washing steps, substrate solution and finally stop solution was added (Figure 4). The microplate was read at 450 nm. The optical density (OD) was recorded and the competition percentage (S/N%), where S is the OD of the sample and N is the OD of the negative control, was calculated.

$$S/N\% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

The result was interpreted as follows: S/N% less than or equal to 40% were considered positive, greater than 50% negative, and between 40-50% as doubtful.

Since the Svanova ELISA is an indirect ELISA the added conjugate forms complex with the antibodies, instead of the antigen as in the competitive ELISA. The testing procedure was otherwise quite similar to the competitive ELISA. The microplate was measured at 450 nm. In this test, each sample was added to two wells, one coated with the SBV antigen and one with a control antigen. The optical density values were corrected by subtracting the values of the two wells for each sample. The percent positivity (PP) values were related to the value of the positive control.

$$PP = \frac{OD_{Corr\ (sample\ or\ NC)}}{OD_{Corr\ (PC)}} \times 100$$

PP < 10 were considered as negative and PP > 10 as positive.

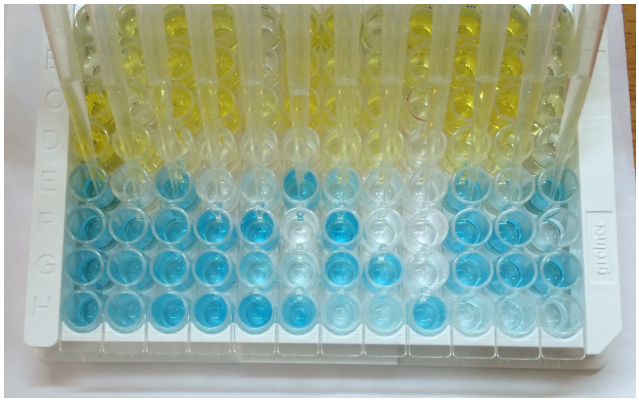

Figure 4. *Stop solution is added to an ELISA plate. Photo: Lovisa Levin*

When the results were received from both ELISAs, the two tests were compared with each other. This was done by calculate the sensitivity (Se) and specificity (Sp) for the two ELISAs, using each other as standard test. However, this calculation does not indicate the true Se and Sp of the two tests, but it gives a good indication of how well the two tests are consistent with each other. The formula below was used for the calculation.

	Standard test +	Standard test -
New test +	A	B
New test -	C	D

$$Se = \frac{A}{A + C} \qquad Sp = \frac{D}{B + D}$$

PCR

PCR analysis was not possible to do in Morogoro due to some unfortunate happenings. Buffy coats were inactivated on FTA cards and brought to Sweden where PCR analysis was performed. Sixty-eight samples from seven herds seropositive by both ELISAs were selected for pan-Simbu real-time RT-PCR (Fischer *et al.,* 2013b). RNA was eluted from the FTA cards by the following procedure. A disc, approximately 2 mm in diameter, was cut out from the FTA card. The discs were put in RNA processing buffer consisting of: 10 mM Tris-HCl, (pH8.0), 0.1 mM EDTA, 800U/ml RNase Out (Invitrogen) and, 2mMDTT. It was incubated at room temperature for 15 min.

The PCR kit SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR (Invitrogen Life technologies) was used for detection and quantification of viral RNA in a Corbett Rotor-Gene cycler (Corbett Technologies). The reaction conditions were as follow: a mastermix of 0.4 µl SuperScript® III RT / Platinum®*Taq Mix*, 10 µl 2X SYBR® Green Reaction Mix, 0.4 µl of panOBV-L-2959 F 10 µM (Fischer *et al.,* 2013b), 0.4 µl panOBV-L-3274R 10 µM (Fischer *et al.,* 2013b), 4 µl Nucleic acids and DEPC-treated water to a final volume of 20 µl was added. According to Fischer *et al.* (2013b), the following thermoprofile was applied: 50 ºC for 30 min (cDNA-synthesis) and 95 ºC for 5 min (heat activation/inactivation), followed by 40 cycles of 95 ºC for 15 s (denaturation) and 55 ºC for 30 s (annealing), 72 ºC for 30 s (extensions) and 78 ºC for 15 s (fluorescence data collection). After the cycles followed 40 ºC for 1 min. The melt curve analysis was as follow: 95 ºC for 15 s, 60 ºC for 30 s, followed by 80 cycles of 10 s from 60 ºC and increased temperature by 0.5 ºC for each cycle.

Questionnaire

During the fieldwork the local farmers were asked for information about their livestock, animal health and clinical signs, treatments etc.

The questions were asked to the farmers by different people (mostly local veterinarian and veterinary assistants) acting as interpreters during the three field trips. The same person translated the answers into English.

The asked questions were:

- How many animals do you have?
- Have you had an outbreak of Peste des petits ruminants (PPR)?
- Have you seen any health issues in your animals the last year?
- Have your animals had any abortions and/or malformed offspring?
- Have your animals got any vaccinations?

RESULTS

ELISA

Competitive SBV-ELISA

In total, 309 samples (148 sheep and 161 goats) were tested with the competitive ELISA. Eighty-two percent of the tested herds were seropositive for SBV. In Mikumi area in the east, all herds were seropositive. The within-herd prevalence ranged from 33-100% for sheep and 0-100% for goats. In Ngorongoro area in the north, 64% (12/17) of the herds were seropositive, with within-herd prevalence ranging from 0-29% for sheep and 0-40% for goats. In Mahenge in the south, 85% (11/13) of the herds were seropositive. The within-herd prevalence ranged from 0-80% for sheep and 0-100% for goats. The overall within-herd prevalence for seropositive herds ranged from 13% to 100%, and the overall seroprevalence for all tested animals was 43% . More detailed results are seen in Table 3 and Figure 5.

Indirect SBV ELISA

In total, 127 samples (59 sheep and 68 goats) were also analysed with the indirect ELISA. Of these, 32 samples were tested two times, and six samples were tested three times. Eight of the samples tested multiple times resulted as both positive and negative. For one of the samples with diverging result, both tests were performed on the same ELISA plate. The other samples with diverging results were tested on different ELISA plates. Samples analysed several times that got different result are registered as doubtful. Seventy-five percent (9/12) of the herds in Mikumi were seropositive, with within-herd prevalence ranging from 0-100% for both sheep and goats. In Ngorongoro, 21% (3/14) of the herds were seropositive. The within-herd prevalence ranged from 0-100% for sheep and 0-50% for goats. In Mahenge, 58% (7/12) of the herds were seropositive, and the within-herd prevalence ranged from 0-100% for both sheep and goats. The overall seroprevalence was 21%. Results are presented in Table 3 and Figure 5.

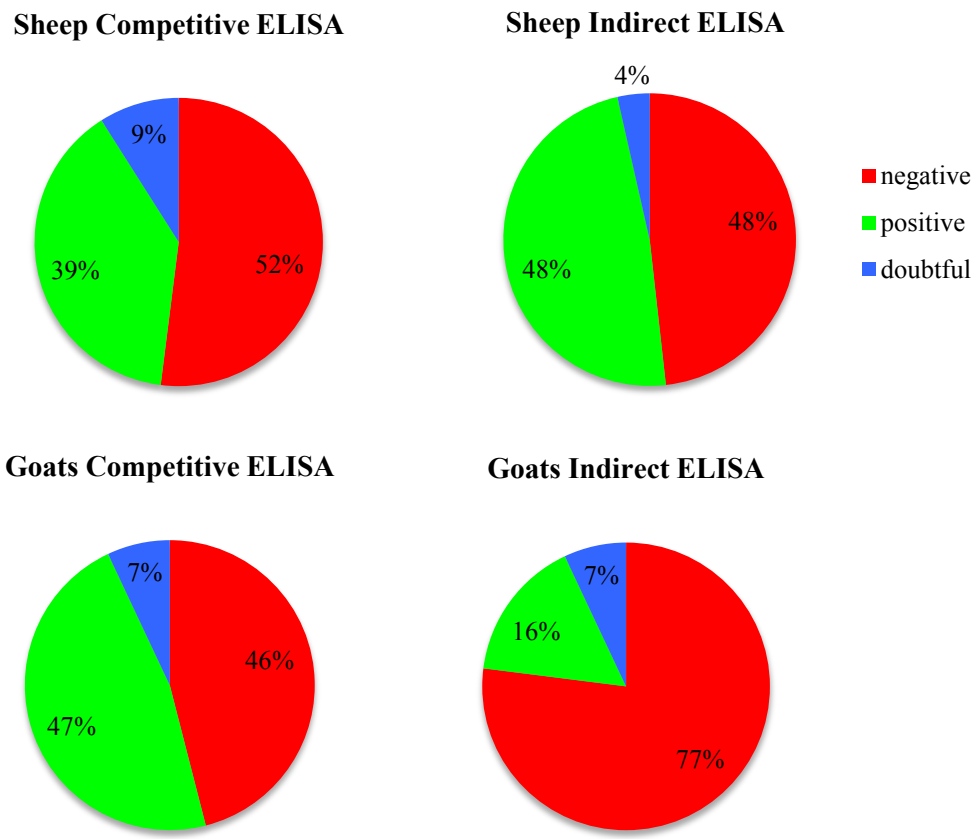

Figure 5. *Seroprevalence among sheep vs. goats on individual level with competitive and indirect ELISA.*

Table 3. *Presentation of the seroprevalence by competitive vs. indirect ELISA for all herds. For the samples tested both negative and positive with the indirect ELISA both alternatives are calculated for the seroprevalence*

			Competitive ELISA		Indirect ELISA	
Area/ Region/District	**Village**	**Herd**	**No. analysed**	**Seropreva lence (%)**	**No. analysed**	**Seropreva lence (%)**
Mikumi/Morogoro/Mvomero	Mtipule	Herd 1	6	50	5	0
		Herd 2	10	60	8	13
		Herd 3	9	44	8	0-25
		Herd 4	10	60	6	33
	Msongozi	Herd 5	5	20	2	50
		Herd 6	13	38	10	20
		Herd 7	11	55	9	22-33
		Herd 8	7	57	3	0-33
	Mkata	Herd 9	9	100	5	60-80
		Herd 10	10	100	5	40-60
		Herd 11	8	88	2	50
		Herd 12	7	100	1	0
Ngorongoro/Arusha/Ngorongoro	Kijakaybosi	Herd 13	4	0	3	0
	Endulen	Herd 14	7	0	5	0
	Esere	Herd 15	8	13	6	0
		Herd 16	8	25	5	20-40
		Herd 17	7	29	3	0
	Meshili	Herd 18	7	29	2	0
	Kesile	Herd 19	12	0	4	0
		Herd 20	7	29	1	0
		Herd 21	7	0	2	0
		Herd 22	7	29	4	0-25
		Herd 23	7	0	2	50
	Irkeepusi	Herd 24	8	13	1	0
		Herd 25	9	0	1	100
		Herd 26	3	0	1	0
Mahenge/Morogoro/Tanga	Tanga	Herd 27	8	88	4	25
		Herd 28	8	0	1	0
		Herd 29	9	22	2	50

			Competitive ELISA		Indirect ELISA	
Area/ Region/District	Village	Herd	No. analysed	Seropreva lence (%)	No. analysed	Seropreva lence (%)
	Lupunga	Herd 30	12	67	3	100
	Ipera	Herd 31	8	63	2	0
		Herd 32	9	44	1	100
		Herd 33	9	33	1	100
	Lupiro	Herd 34	8	63	0	-
		Herd 35	3	0	1	0
	Mwaya	Herd 36	4	75	1	0
		Herd 37	12	67	4	50
		Herd 38	7	43	1	0
	Nkongo	Herd 39	6	83	2	50
Total			**309**	**43**	**127**	**21**

Competitive vs. indirect ELISA

In total 127, samples were tested with both the competitive and the indirect ELISA. The results are presented in Table 5. The samples tested multiple times with the indirect ELISA, that got both positive and negative results are reported as indirect ELISA doubtful.

Table 5. *Comparison between the result from the two ELISA kits run for 127 samples*

	Competitive ELISA positive	Competitive ELISA negative	Competitive ELISA doubtful	Total
Indirect ELISA positive	21	4	2	27
Indirect ELISA negative	28	49	15	92
Indirect ELISA doubtful	7	0	1	8
Total	56	53	18	127

The numbers in Table 5 were used to calculate the sensitivity (Se) and specificity (Sp) for the two ELISAs, using each other as standard test.

For the competitive ELISA the result was as follow. If doubtful results were calculated as negative, the Se would be 78% and the Sp 65%. If doubtful results were calculated as positive, the Se would be 89% and the Sp 53%.

For the indirect ELISA, if doubtful results were calculated as negative the Se would be 78% and the Sp 92%. If doubtful results were calculated as positive the Se would be 42% and the Sp 7%.

Pan-Simbu RT-PCR

In total, 68 buffy coats (eluted from FTA cards) from animals in seven of the seropositive herds were analysed. All analysed samples were negative in the Pan-Simbu RT-PCR.

Answers to the questionnaire

The results from the questionnaires and impressions of the herds' health status are reported below for each sampling site.

Most farmers had cattle and other animals like dogs and chicken besides sheep and/or goats. All farmers kept the animals for household and in all areas some farmers also kept their animals for milk- and meat production and for selling live animals at markets.

Mikumi

Region: Morogoro, district: Mvomero

Twelve herds in three villages were sampled in this area. The herd size ranged from 50 to 400 sheep and goats. In two villages, all farmers considered the animals' health status to be good. All herds besides one were vaccinated against PPR in spring 2013. In one village there had been problems with abortions, diarrhoea and coughing among the animals in 2013. Three of four farmers of those herds reported an outbreak of PPR in that year. In the fourth herd, the animals had similar signs during the same period of time, but the farmer did not claim it to be due to PPR. In this village, the animals in three herds were having nasal discharge and/or were coughing at the sampling occasion. In the other two villages the animals were healthy.

Ngorongoro

Region: Arusha, district: Ngorongoro

In this area, 14 herds in six villages were sampled. The herd size ranged from 50 to 650 sheep and goats. No herd was vaccinated against PPR. Two herds were vaccinated against anthrax. Seven farmers claimed there was an outbreak of PPR in their herds in 2011. Three farmers reported outbreaks in their herds in 2012, 2013 and 2014. In one of these villages, the farmers mentioned that there had been an outbreak recently as well. During the outbreak, the animals showed clinical signs such as fever, coughing, diarrhoea, weakness and abortion, nasal discharge, skin and oral lesions, alopecia and swollen heads. Malformed offspring during the time of the PPR outbreak were reported from two villages. In the most affected herd, seven offspring were malformed. In two herds, there had been problems with diarrhoea. In one village, animals in all of the herds were coughing and having nasal discharge at the sampling occasion. In one herd, some animals also had diarrhoea. All farmers said that if many of their animals would get sick or die, it would negatively affect the families' health and economy.

Mahenge

Region: Morogoro, district: Ulanga

In this area, 13 herds in six villages were sampled. The herd size ranged from three to 200 sheep and goats. Nine herds were vaccinated against PPR, and the other herds were unvaccinated. In this area, most farmers reported problems with diarrhoea with fatal outcome during the rain seasons, probably due to parasites. In one village, many goat kids were born week and died after a few days during the last rain season. In 2014, there was an outbreak of PPR in four of the herds in two villages. Many animals died and many aborted during this outbreak. Other clinical signs were diarrhoea, fever, coughing and nasal discharge. In 2013 one herd had an increased prevalence of malformed offspring, with swollen and stiff joints. Another farmer in one of these villages reported that the animals had diarrhoea, fever, coughing and nasal discharge, but the farmer did not claim it to be to PPR. One farmer considered his animals' health status to be good. In one herd, the health status was very poor at the sampling occasion. The animals, especially the sheep, were having thick nasal discharge, stood

with low head and were coughing. In two other herds, the animals were weak and had nasal discharge and in another herd the animals had diarrhoea.

DISCUSSION

The result of this study indicates that SBV or an SBV-like virus is circulating and most likely being endemic in Tanzania, assuming the result in this study represents the true prevalence in Tanzania. In total 82% seropositive herds (competitive ELISA result) for SBV is slightly less than in northern Mozambique (Blomström *et al*., 2014) that borders south to Tanzania.

The results of the two compared ELISAs differ a lot. According to the manufacturers (Comtet & Pourquier, 2013; Svanova, 2014), the sensitivity is 97.6% for the competitive ELISA, and 98% for the indirect ELISA. The specificity is 100% for both tests. Thus, the accuracy is similar for the two ELISAs. The results in this study contradict that the sensitivity and specificity of the ELISAs are that similar. Both tests cannot have 100% specificity since not all samples negative by one test were negative by the other test.

When I compared the two ELISAs by calculating the sensitivity and specificity for the test, using each other as standard test, it resulted with quite low results. The worst result was a specificity of seven percent, calculated for the indirect ELISA, when doubtful results were regarded as positive. Only 127 samples were tested by both ELISAs, which cannot give completely reliable result, but it indicates that either one or both of these two tests are not as accurate as specified by the manufacturers. The calculation of sensitivity and specificity do not indicate the true sensitivity and specificity of the tests, but gives an indication on how much the two tests differ from each other. However, if results were confirmed by dilution of the samples, the results might have been different, and hence more accurate. For this study, decision was taken not to dilute the samples for the benefit of running more samples.

Van der Poel *et al*., (2014) compared the accuracy of eight different ELISAs with virus neutralisation test (VNT) performed in different countries without specifying which ELISAs were tested. They found some discrepancies between the ELISA tests, and suggest that results would be more accurate if all doubtful ELISA results were considered as positive. In this study, the discrepancy would have been even higher if results classified doubtful in the competitive ELISA were considered positive, since 15/17 of these were classified as negative in the indirect ELISA. However, this is if the two tests are compared with each other, and not with VNT that is gold standard. If one speculates about this, if the doubtful samples were registered as positive the results might have been truer compared to VNT. It would of course be highly interesting to analyse the samples also by VNT. It would be interesting both of aspect to evaluate the ELISAs, and to investigate if it is truly SBV antibodies that we have found in this study.

The fact that the results between the two kits in this study differ so much is confusing and unexpected. It might be because of suboptimal handling of the samples. Some samples were not separated until a few days after collected, which may have affected the quality of the serum and buffy coat. During the time in Morogoro there were frequent power cuts, which affected both the storing of the samples and the analyses. However, there should not be false results due to power cuts during analysis (causing inability to incubate in right temperature) since the positive and negative controls were always correct, making a valid test. Poor quality of the samples should not be the explanation, since both ELISAs analysed the same samples. If the quality of a sample was poor, the result should have been negative by both ELISAs, and not diverged between the two tests.

In total, 39% of the sheep and 47% of the goats tested were SBV positive, according to the results from the competitive ELISA. The proportion of more seropositive goats than sheep agrees with the result in Mozambique (Blomström *et al*., 2014), but differs from the results seen in European livestock (Helmer *et al*., 2013). The difference between the continents may be due to genetic differences in African and European sheep and goat breeds. I consider the difference more likely to be a result of different housing systems for the animals on the two continents. In Africa, the animals are often kept mixed species and are held outdoors. In Europe, it is most common to keep either sheep or goats, and not both species together, and some herds are kept indoors. However, according to the results from the indirect ELISA, the seroprevalence is higher for sheep than goats, which is more similar to the results seen in Europe. If the results by the indirect ELISA are more accurate, the result in this study supports the theory that sheep are more susceptible to SBV than goats. However, since the results are divergent between the two tests, no such conclusions can be made.

The largest differences between the two ELISAs were mostly seen for goats. A possible explanation for this is that the tests are not developed on samples from African animals. The competitive ELISA can detect antibodies from all species, and therefore should not cause any issues. However, it is not sure that the indirect ELISA can detect antibodies from other species than specified by the manufacturer. Even if both sheep and goats are specified by the manufacturer, it might be so that the antibodies of the African breeds (especially the goats) are so different from the ones the test are developed on, so that the test do not detect all of these antibodies. Further studies needs to be performed to evaluate this hypothesis.

The overall seroprevalence was higher in the southern and eastern parts, than in the northern parts of Tanzania. The lower seroprevalence in the north might be an indication for a northern border for the presence of the virus in Africa. Since the seroprevalence seems to be higher in northern Mozambique (Blomström *et al.,* 2014), which borders south to Tanzania, than in Tanzania, it might also be an indication of a northern border of the SBV distribution in Africa. The lower seroprevalence in the north might be due to less movement of animals between herds and animals grazing over wider areas. However, further studies needs to be performed to draw any conclusions about this. It would be of great interest to continue the investigation of the prevalence of SBV in both Tanzania and in neighbouring countries, to see if the trend of lower seroprevalence in north stands strong.

It is not possible to draw any conclusions from the seroprevalence on within-herd level, because the sample selection from each herd was small, especially for the indirect ELISA. From some herds only one or a few samples were analysed.

Unfortunately it is not sure that SBV is present in Tanzania, since there is a risk for cross reactivity with other Simbu viruses. By the results in this study, it can only be suggested that SBV or an SBV-like virus is present in Tanzania. To be certain that SBV is present in the country the antigen needs to be detected. This could be done by PCR. In this study, no SBV RNA could be found by pan-Simbu RT-PCR. Since only 68 samples were analysed and the fact that SBV causes a viremia of only 5-6 days (Hoffmann *et al*., 2012), it is not surprising that the viral RNA was not found. Animals that have seroconverted may due to the short viremia no longer be viremic, and the virus cannot be found in their blood. Despite this, animals from seropositive herds were selected for the PCR, since it was considered to be more likely to find the virus in these herds than in seronegative herds. In all tested seropositive herds there were seronegative animals, which could theoretically have been viremic but not yet seroconverted, and hence be positive by PCR. However, all tested samples were PCR-negative. Since no SBV RNA was found, it is not possible to exclude that the ELISAs were positive due to cross-reactivity. A possible source of error for the PCR analysis is the elution from the FTA-cards

might not have worked. If possible, in the further studies, it would be highly advantage to run PCR on the original buffy coats in Morogoro.

If SBV truly is present in Tanzania it most likely do not cause any clinical illness. In the interviews, some farmers answered that there had been abortions and malformed offspring in their herds. All farmers, besides one, claimed it to be due to PPR. PPR had only been diagnosed clinically (if all by a veterinarian) and not confirmed by a laboratory. Theoretically the abortions and malformations could be due to SBV. However, it is most likely that the signs were due to PPR rather than SBV of at least two reasons. Firstly, the animals also suffered of other signs like diarrhoea, coughing and nasal discharge, which suggests PPR rather than SBV. Secondly, the relative high seroprevalence among Tanzanian sheep and goats indicates that SBV or an SBV-like virus is endemic in Tanzania. In endemic areas, most animals will be infected before they breed, and then the disease can pass quite unnoticed. If the animals sampled previously were naive to SBV, an outbreak of abortions and malformed offspring would be expected. Since no major outbreaks of abortions and malformations were reported, SBV is more likely endemic, rather than recently introduced, in Tanzania. The main reproductive disorder reported by the farmers were abortions, not malformations that are the major SBV concern, which also indicates that PPR is more likely to be the cause rather than SBV.

The reliability of the answers from the questionnaires is questionable. Unfortunately, we did not have much time in each herd since the farmers waited for us to let their animal go grazing. Some of the interviews were therefore performed very quickly. The fact that different persons were interpreters might also affect the results. Some days we received almost identical answers from the different herds, which is a bit suspicious. It is a sensitive question to ask how many animals someone has, since it is correlated to economical status. Therefore, these numbers are not completely reliable.

The age of the animals is just estimations, either made by us students, local veterinarians/veterinary assistant or the farmers, hence it may not be completely accurate. This may cause statistical errors if animals are older or younger than registered. However, for this study the animals age were not that important since we just investigated the prevalence in Tanzania.

The prevalence of SBV on other continents than Europe is unknown. However, this study and two other studies (Blomström *et al*., 2014; Leask *et al*., 2013) suggest it to be endemic in Tanzania, Mozambique and maybe even in South Africa. In endemic areas, SBV is not an issue since most animals being immune. The event of introducing SBV to a naive area would probably give the same outcome as in Europe. The fact that SBV, or an SBV-like antibodies were present in Turkey, without causing any clinical signs a couple of years before the outbreak in Europe (Azkur *et al*., 2013), and the presence in Africa, indicates that the virus might be widely distributed in many countries without being noticed.

SBV has been detected in several wild species without any signs of causing clinical illness. Wild ruminants are conceivable to be reservoirs and might also be the origin of SBV. The virus has been suggested to origin from Africa (Beer *et al*., 2013). Africa has many favourable conditions for the development of new diseases. There is a warm climate, the animal population is big and it is moving over large areas, there is close contact between wild and domestic animals, enabling circulation of viruses, and there is plenty of different possible arthropod vectors etc. It would be of great interest to find out where the virus has it origin, to understand more about its properties.

What can we expect in the future? Is SBV an infectious agent to fear? The introduction of SBV to Europe can be seen as a worst-case scenario, which is unlikely to be repeated (EFSA, 2014). SBV spread over Europe very rapidly (Afonso *et al*., 2014), with nearly 9000 confirmed cases until May

2013 (EFSA, 2013). The incidence of SBV infection peaked in spring 2012 and declined afterwards (Afonso *et al*., 2014). SBV could cause great consequences in Europe in 2011-2013, since the livestock was naive. Now that SBV is endemic in Europe, it is not of any great importance, since animals that are seropositive before time of breeding would not get malformed offspring or abortions. If the seroprevalence of SBV for some reason would decrease again, new outbreaks could occur. It is not likely that SBV would cause major outbreaks again. The rapid spread of both SBV and bluetongue virus in Europe indicates that we should not be surprised if a new infectious emerging virus would introduce and quickly spread over Europe in a not too faraway future.

CONCLUSIONS

SBV or SBV like antibodies have been found in this study. This finding indicates that SBV, or an SBV like virus, is widely present in Tanzania without causing any clinical illness. I suggest that further studies of this topic should be made to determine what Simbu virus it is circulating in Africa. In this study, we could unfortunately not analyse any samples from wild animals. This was of great interest since SBV antibodies have been found in wild animals in Europe. To confirm the positive ELISA results, it would be desirable to perform VNT. For further studies it is also of great interest to determine the origin of the virus.

I suggest the manufacturers to evaluate their ELISAs again since the result in this study was not consistent with the specified accuracy.

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