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Epidemiology of Viruses in the Livestock in Tanzania – A Minor Field Study with Focus on Peste des Petits Ruminants Virus

Ida Herbe

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Virusepidemiologi hos tama idisslare i Tanzania – en Minor Field Study med fokus på Peste des Petits Ruminants Virus

Ida Herbe

***Supervisor**: Jonas Johansson Wensman, Department of Clinical Sciences*

***Assistant Supervisor**: Gerald Misinzo,* Sokoine Univeristy of Agriculture, Morogoro, Tanzania
***Examiner**: Stefan Alenius, Department of Clinical Sciences*

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Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences

SUMMARY

Peste des Petits Ruminants (PPR) is a disease of major socioeconomic impact. It is an acute and highly contagious viral disease of small ruminants caused by the agent Peste des Petits Ruminants Virus (PPRV), a *Morbillivirus* closely related to Rinderpest virus (RPV) which was declared eradicated from the world in 2011. PPR has a high morbidity and mortality rate and is characterised by high fever, nasal and ocular discharge, pneumonia, necrosis and ulceration of the mucous membranes and inflammation of the gastrointestinal tract causing severe diarrhoea. The role of wildlife in the epidemiology of the disease is still unclear. The disease is currently affecting sheep and goat in 70 countries worldwide and this year (2014) FAO has announced a program to eradicate the virus by 2030.

In Tanzania, the disease was first reported in 2008 and has since then spread to different parts of the country. Tanzania is currently the southern border of the disease in the world but the risk of spread even further south is considered to be high.

This Minor Field Study is a part of a bigger three-year project with focus on evaluating the role of wildlife in the PPR epidemiology. I have investigated the presence of PPRV-antibodies in sheep and goat in two different regions of Tanzania where the domestic animals intermingle with the wildlife. A total number of 476 animals were sampled and analysed and the overall seroprevalence of PPRV was 43.2% in sheep and 49.0% in goats. The results indicate the presence and activity of the virus in both vaccinated and non-vaccinated areas of the country.

SAMMANFATTNING

Peste des Petits Ruminants (PPR) är en sjukdom med stor socioekonomisk påverkan. Det är en akut och mycket smittsam virussjukdom som drabbar små idisslare. Sjukdomen orsakas av Peste des Petits Ruminantsvirus (PPRV), ett *Morbillivirus* nära besläktat med boskapspestviruset som förklarades utrotat från jordens yta år 2011. PPR har hög morbiditet och mortalitet och karaktäriseras av hög feber, nos- och tårflöde, lunginflammation, nekroser och ulcerationer av slemhinnor samt kraftig diarré orsakad av en inflammerad mag- och tarmslemhinna. I dagsläget härjar sjukdomen bland får och getter i 70 länder världen över. FAO presenterade i år ett program för hur viruset ska lyckas utrotas till år 2030.

I Tanzania påvisades sjukdomen första gången år 2008 men har sedan dess spritt sig till olika delar av landet. Tanzania är i dagsläget sjukdomens mest södra utbredningsområde i världen men det finns en överhängande risk att sjukdomen i framtiden kan komma att sprida sig till länder söder om Tanzania.

Denna studie är en del av ett treårigt projekt vars fokus ligger på att undersöka de vilda djurens roll i sjukdomens smittspridning. Jag har undersökt närvaron av PPRV-antikroppar hos får och getter i två olika regioner av Tanzania där de tama djuren har kontakt med de vilda. Totalt togs och analyserades 467 prover och den totala seroprevalensen hos får var 43,2 %, och 49,0 % hos getter. Resultaten talar för att viruset är närvarande och aktivt i både vaccinerade och ovaccinerade områden av Tanzania.

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INTRODUCTION

Aims

The purpose of this study was to investigate the prevalence of Peste des Petits Ruminants Virus (PPRV) in goats and sheep that have contact with wild small ruminants in chosen parts of Tanzania. To achieve the aim, I investigated the epidemiology of the disease by tracing antibodies by competitive ELISA (cELISA) and mapping the virus through real time polymerase chain reaction (RT PCR). This is a part of the mapping of PPRV in Africa that in the end hopefully will contribute to the global eradication of PPRV.

This master thesis is a smaller part of a three-year project, which is a collaboration between Sweden, Pakistan, Tanzania and the UK, funded by the Swedish Research Council (VR U-forsk). The aim of the large project is to study the prevalence of PPRV in wild and domestic small ruminants in the wildlife-livestock interface to evaluate the role of wildlife in the epidemiology of the disease and virus transmission. The larger project will also investigate the complete genome sequence of PPRV and study the host's response to infection. This master thesis contributed to the larger project by collecting data by fieldwork, performing lab work and data analysis.

Study region

Tanzania is a country in East Africa with 26 different regions and a population of almost 49 million people on 88.58 million of ha (*FAOSTAT website*, 2014, *Landguidens website*, 2014). It borders to Kenya and Uganda in the north, Rwanda, Burundi and Kongo-Kinshasa in the west, Zambia, Malawi and Mozambique in the south and the Indian Ocean in the east. Tanzania is famous for its rich nature and wildlife. The country has two rain seasons, a lighter one in November/December and a heavier one in April/May. The average temperature varies over the year between 19-24 °C, and the climate is hot and dry on the inland areas and tropical monsoon on the islands. Morogoro, situated on the mainland approximately 175 km west of the coastal city Dar es Salaam, is the centre for agricultural sciences in the country (*Landguidens website*, 2014). Most of the research around PPRV in domestic and wild animals in Tanzania is performed at the Sokoine University of Agriculture (SUA) in Morogoro.

In 2013 the rural population in Tanzania was 72.3% (*FAOSTAT website*, 2014). Agriculture is the most important sector in the country and is estimated to be 26% of the gross domestic products (*Landguidens website*, 2014). The amount of people working in the agricultural sector was 74.4% in 2013, and 55% were woman (*FAOSTAT website*, 2014). Livestock keeping is important in Tanzania and sheep and goats are among the main farm animals owned by the poor, as in most developing countries. Sheep and goats are sometimes called "the cattle of the poor". PPR is one of the so-called transboundary animal diseases and it threatens to spread south into the Southern African Development Community (SADC). At present Tanzania is its southern border (Muse et al., 2012b). In 2014 Chazya *et al*., performed a risk assessment on the overall risk of introducing PPRV into northern Zambia from Tanzania via live goat trade. They concluded that risk at the time of the assessment was "high."

LITERATURE REVIEW

Peste Des Petits Ruminants

PPR is considered as a disease of major economic impact and has to be notified to the World Organisation for Animal Health (OIE), because of its dramatic clinical incidence and that it is associated with animal and product movements (Balamurugan *et al.,* 2010). Since small domestic ruminants cover the family running income for most pastoral households, PPR largely affects their present and future income and generating ability. The disease therefore also has negative impact on the livelihoods and food security of the entire pastoral community (Muse *et al.,* 2012a).

Genetic proprieties

PPR virus is classified in the family *Paramyxoviridae* under genus *Morbillivirus* along with other members including rinderpest virus (RPV) of cattle and buffalo, measles virus (MV) of humans, canine distemper virus (CDV) of dogs and wild carnivores (Gibbs *et al.,* 1979), and morbilliviruses of marine mammals (Rajak *et al.,* 2005). Gibbs showed in 1979 that PPRV is antigenically related to RPV but that the relation is incomplete.

Morbilliviruses are enveloped, non-segmented, single stranded, negative sense RNA viruses with genomes approximately 16 000 nucleotides long (Diallo, 1990). PPRV has the second longest genome among morbilliviruses. It consists of six structural proteins (Figure 1) and two non-structural proteins (Bailey *et al.,* 2005). The nucleoprotein (N) surrounds the genomic RNA, and it is coupled to the large protein (L) and the phosphoprotein (P). Three other proteins form the viral envelope; the matrix (M) protein, the haemagglutininneuramidase (HN) protein and the fusion (F) protein. The F and HN proteins are responsible for the attachment and the entry of the virus into the host cell (Munir *et al.,* 2013), while the M protein plays an important role in ensuring efficient incorporation of nucleocapsids into virions during the virus budding process (Chauhan *et al.,* 2009).

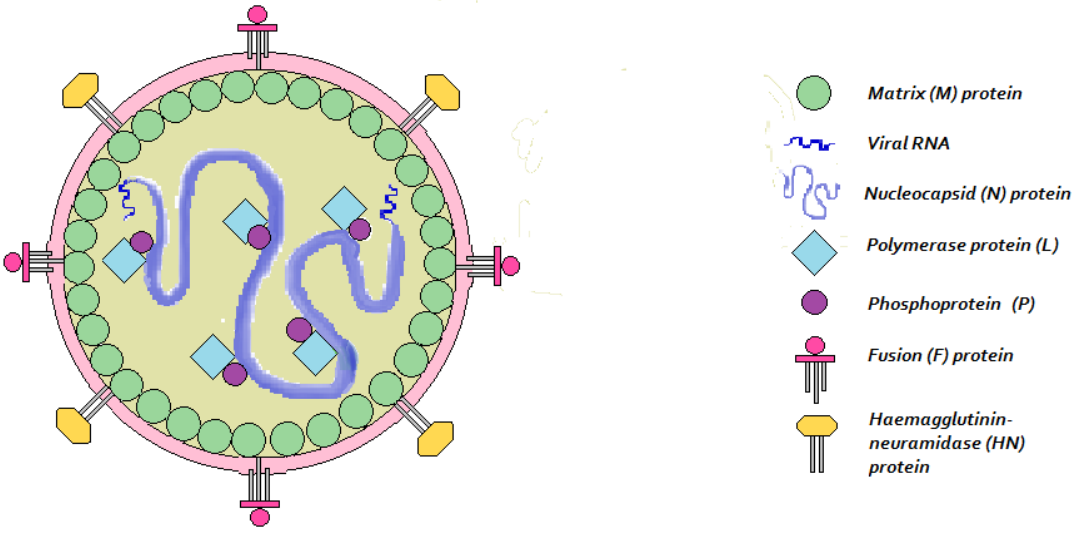

Figure 1. *Schematic structure of the PPRV virion.*

Life cycle and survival

At first, the virus attaches to the host cell membrane. The binding is mediated by the haemagglutininneuramidase protein and the sialic acid on the cell membrane of the host (Munir *et al.,* 2013). The interaction continues with the fusion protein that allows fusion of the virion envelope with cellular membranes (Chauhan *et al.,* 2009). The virus is uncoated and the nucleocapsid is released into the cytosol of the infected cell via endocytosis. Replication takes place in the cytosol and results in full-length, positive stranded antigenomes that are transcribed into negative-stranded virus genome copies. After translation of viral proteins in the cytoplasm and the endoplasmatic reticulum, all structural and non-structural proteins self-assemble with the genomes near the cell surface and the budding process begins (Munir *et al.,* 2013).

It is known that PPRV is fairly fragile and cannot exist for long periods of time in the environment (OIE, 2013, Gitao *et al.,* 2012), but details in the matter are lacking (Munir *et al.,* 2013). The virus is sensitive to drought and is inactivated in pH below 4 and above 11. It survives for a long time in chilled and frozen tissues (OIE, 2013). PPRV has a calculated half-life of 2.2 min at 56°C (Munir *et al.,* 2013), 2 hours in 37°C and it is destroyed at 50°C for 60 minutes (*Peste des Petits Ruminants*, 2013). It is susceptible to most disinfectants such as alcohol, ether, phenol and sodium hydroxide.

Lineages

PPRV has only one known serotype (Luka *et al.,* 2011), but it can be divided into four distinct lineages (I, II, III and IV) based on partial sequence analysis of the genes (Dhar *et al.,* 2002). A classification system based on the fusion (F) protein gene was originally used. The F gene classification system has helped a lot in the understanding of the spread of PPRV over time and geographically. In the 1990s, a new sequence analysis was developed based on the nucleocapsid (N) protein (Kerur *et al.,* 2008). In a comparison of the both methods made by Kerur *et al*., (2008) it was revealed that the N gene analysis grouped the virus into the four lineages in a better way and therefore giving a better epidemiologic picture about PPRV. In 2010 Balamurugan *et al*., concluded that the HN gene is the best one to analyse outbreaks in endemic areas. Due to the ability of PPRV to mutate it is desirable to use more than one viral gene for phylogenetic interpretation (Munir *et al.,* 2013).

History and expansion

PPR was first described on the Ivory Coast as a disease of its own by Gargadennec and Lalanne in 1942 and because of its similarities in the clinical picture it was thought to be a variant of RPV that had adapted to sheep and goats and lost its virulence for cattle (Gibbs et al., 1979, Banyard et al., 2010). It is probable that early cases of rinderpest (RP) described in small ruminants actually was PPRV infection (Baron *et al.,* 2011). The disease has had many names referring to its clinical manifestations; “goat plague”, “stomatitis/pneumo-enteritis complex”, “pseudo rhinderpest” and “kata” (Munir *et al*., 2013). In 1976, Hamdy was the first to differentiate between PPRV and RPV (Taylor, 1984). In 1979 Gibbs *et al.,* proved it to be the fourth member of the genus *Morbillivirus*. Until 1979 the disease was described mostly in West African countries but then it started to appear in countries in Eastern and Northern

Africa as well. Between 1983 and 1987 the disease had found its way to the Middle East, and India was the first Asian country to confirm PPR in 1987 (Munir *et al.,* 2013). The development of trade relations, tourism, transport and migration of wild animals is described to have contributed to the spread of the disease (Kaukarbayevich, 2009). A lavishly spread took place between 1993-95 in the Middle East, South Asia and the Arabian Peninsula (Munir *et al.,* 2013). Currently, the disease is present in Central, Eastern and Western Africa, Asia, and the Middle East (Figure 2) (Banyard *et al.,* 2010, Geerts, 2009), affecting sheep and goats in over 70 countries (FAO, 2014). Out of the four known lineages, lineage IV is restricted to Asia and the other three are found in Africa and the Middle East (Dhar *et al.,* 2002).

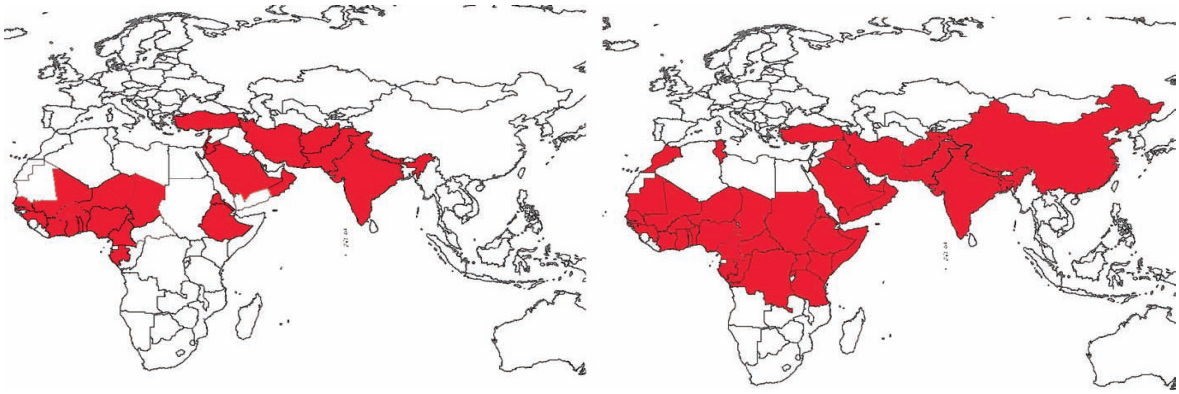

Figure 2. *World distribution and spread of PPR from (left) 1998 to 2000 and (right) 2008 to 2010. The colour indicates countries where PPR cases have been reported. Source: (Baron et al., 2011) Permission granted from publisher .*

Peste Des Petits Ruminants in Tanzania

The virus was detected in Tanzania for the first time in 2008 in the northern part of the country. It was introduced from the neighbouring countries in the north (Karimuribo *et al.,* 2011). However there are suspicions that disease outbreaks occurred in the country long before 2008 (Lembo *et al.,* 2013). A comprehensive serological study showed the absence of PPRV antibodies in Tanzanian sheep and goats in 1998 (Wambura, 2000), and in 2004 the presence of antibodies in Northern Tanzanian sheep and goat was confirmed (Karimuribo *et al.,* 2011).

In 2011, PPRV was detected in sheep and goats during an outbreak in the Tandahimba district that borders to Mozambique in the southern part of Tanzania (Muse *et al.,* 2012a). This indicates that there is an on-going spread of the disease. It was also shown that the source of this outbreak was through the introduction of new animals purchased from the Pugu livestock market located about 679 km south of Dar es Salaam city (Muse *et al.,* 2012b). Later PPRV was detected in sick animals during a suspected outbreak in the Morogoro region in 2013 (Kgotlele *et al.,* 2014a), and virus was found present in samples from sheep and goat in 2013 in Ngorongoro district, Northern Tanzania, and Mvomero district in Eastern Tanzania (Kgotlele *et al.,* 2014b). So far, PPRV isolated in different parts of Tanzania all originate from lineage III (Kivaria *et al.,* 2013), indicating that the virus found in the southern parts came from the northern parts of the country.

Tanzania is today the southern border of the disease and there is a risk that PPR might continue to spread southwards in the near future (Chazya *et al.,* 2014) (Muse *et al.,* 2012b). Due to this risk of PPRV spreading south into the SADC, an emergency vaccination programme were launched and implemented in the northern half of Tanzania in 2010 (Munir *et al.,* 2013). In 2011, September, a vaccination campaigns with a focus on sheep and goats along livestock marketing routes were implemented (OIE, 2012). Also in the area around Mikumi National park (central and eastern part of Tanzania), most herds were vaccinated in the late spring of 2013. This was due to mandatory vaccination campaigns in the area (Gerald Misinzo, Sokoine University of Agriculture, Tanzania, personal communication). One risk factor for further spread of the virus over the border from Tanzania to Mozambique is export of live animals in combination with insufficient surveillance systems. Illegal movement of animals is another risk. It is suggested that the import of goats and sheep from Tanzania to Mozambique should be prohibited until efficient and adequate measures to reduce the risk of virus spread have been put in place (Chazya *et al.,* 2014). Today it is not known whether wild ruminants in Tanzania are infected with PPRV (Lembo *et al.,* 2013) however, wildlife such as Goitered Gazelle (*Gazella subgutturosa subgutturosa*) are known to be susceptible to PPR (Gur and Albayrak, 2010). Movement of wild animals over borders is hard to control.

In the United Republic of Tanzania, the cumulative annual loss due to PPR is estimated to be around US$67.9 million (FAO, 2014).

Hosts

PPR is a disease of mainly sheep and goat, but it is known that wild ruminants can get infected (Albina *et al.,* 2013). Sheep and goats of both sexes and all ages are susceptible (Muse *et al.,* 2012a), but the disease is most prevalent in young animals less than one year old (Radostits O, 2009). It has also been observed that kids and lambs after the age of three months are highly susceptible to infection, probably due to a decline in passive immunity (Munir *et al.,* 2013) and that their clinical symptoms are severe. It is suggested that goats are more susceptible to PPRV infection than sheep (Taylor, 1979). Upon experimental infection with different virulent strains of PPRV, both sheep and goats developed clinical signs and lesions typical of PPR, although sheep displayed milder clinical disease compared to goats (Truong *et al.,* 2014). The study showed that goats got more enlarged lymph nodes and a more robust inflammatory response (Interferon gamma levels) than sheep. Moreover, virus RNA was detected in the blood (viremia) of all infected goats but in none of the infected sheep. The antibody response did not differ between the two species (Truong *et al.,* 2014).

Cattle, African buffaloes (*Syncerus caffer*) and Defassa waterbuck (*Kobus ellipsiprymnus defassa*) may seroconvert in enzootic areas when exposed to the virus, but PPRV is not considered pathogenic to any of these species (Couacy-Hymann *et al.,* 2005). Ruminants from the subfamilies of *Gazellinae, Caprinae* and *Tragleaphinae* may also express serious illness and mortality (Kinne *et al.,* 2010). A clinical outbreak in a zoological collection on the Arabian Peninsula showed that species that intermingle could get affected if exposed to PPRV (Furley *et al.,* 1987). Animals affected were gazelles (*Gazellinae*), ibex and sheep (*Caprinae*), and gemsbok (*Hippotraginae*). The virus was likely introduced by imported goats. In 2001, antibodies were detected in cattle and camels in Ethiopia, and camels had a high incidence of

respiratory disease reported (Abraham *et al.,* 2005). An outbreak of PPR in the east part of Sudan among camels had mortality rates up to 50%. In general, the clinical feature of PPR in camels during this outbreak was no different from those reported in sheep and goats. It seemed that the severity of PPR in camels was much higher in adult camels in comparison to calves and young camels. (Khalafalla *et al.,* 2010)

The role of wildlife in the epidemiology of PPR is still rather uncertain and it is crucial to have it further evaluated. This because domestic and wild ruminants mingle together at several points, allowing inter-species transmission of PPRV that might interfere with the current disease surveillance program but might also be a serious threat to the survival of endangered species of wild ruminants (Munir, 2014). One hypothesis is that the infection in wild animals is not self-sustained and epidemics, that it cannot survive within the wild population itself. Instead it is proven that infection among wildlife is the result of infection spread by domestic small ruminants neighbouring the natural parks (Couacy-Hymann *et al.,* 2005, Bao *et al.,* 2011).

Phylogenetic analysis of nucleoprotein and fusion genes indicates that all PPRVs isolated from wild ungulate outbreaks belong to lineage IV. The reason for this is discussed but yet unclear (Munir, 2014).

Transmission and epidemiology

PPR is a highly contagious disease and the virus spreads fast among animals that have close contact (Ezeibe *et al.,* 2008). Where communal grazing system is practiced, animals from different herds intermingle and graze together. This type of close contact and movement of animals from affected to unaffected areas plays an important role in transmitting the disease. One of the most common ways for an animal to get the infection is by inhaling small droplets in the air containing the virus (Roeder, 1999). The virus can also spread through conjunctival penetration, why animals sharing the same bedding, food and water troughs can get infected from licking and eating. Affected animals shed virus in exhaled air, secretions and excretions (ocular and nasal discharge, milk, semen, urine and faeces). Suckling lambs and kids acquire passive immunity via the colostrum and this immunity lasts until 3-4 months of age (Munir *et al.,* 2013) Using cELISA the levels of antibodies is detectable until the 3rd month (Libeau *et al.,* 1992). Infected animals can spread the virus before the onset of clinical signs, during the incubation period. Incubatory carriers are therefore suggested to play a role in the transmission of the virus (Couacy-Hymann *et al.,* 2007b). Ezeibe (2008) showed that infected goats shed virus detectable with haemagluttination test up to 11 weeks after complete recovery from PPR. Studies in Pakistan show that PPR is a seasonal disease with the highest prevalence during the dry cool season (Abubakar *et al.,* 2009).

Pathogenesis

PPRV has a strong affinity to epithelial cells and lymphoid tissues, and the predominant sites of replication are the lymph nodes and the digestive tract organs (Truong *et al.,* 2014). The virus damages the epithelial cells of the intestinal and respiratory tract, causing lesions and therefore respiratory and intestinal disorders. It also causes a significant immunosuppression by damaging the lymphocytes (Rajak et al., 2005,Sahinduran et al., 2012). In early stages of

infection, leucopoenia, lymphopenia and a suppressed antibody response can be seen. The immunosuppression caused by the virus makes the animal more susceptible to secondary infections, such as pneumonia. It also increases the risk for activation of latent infections such as intestinal and blood parasites, increasing the mortality rate (Pastoret P-P., 2006). In susceptible flocks the morbidity may be 100% and mortality up to 90% (Gibbs et al., 1979). PPR severity can vary a lot depending on the virulence factor of the virus but also on many predisposing factors such as breed, sex, age and health status of the animal before infection (Couacy-Hymann et al., 2007a). Both morbidity and mortality rates have shown to be higher in young animals than in adults (Abubakar et al., 2008). Despite the immunosuppressive effect of the virus, recovering animals always develop a strong life-long immunity for the disease (Albina et al., 2013).

Clinical picture

The disease often has an acute course (Taylor, 1984), but can also be described as peracute, subacute and subclinical (Munir *et al.,* 2013). The incubation period is often between four and five days, and the onset of the acute form of disease is well marked by sudden dullness and pyrexia (Obi *et al.,* 1983, Bundza *et al.,* 1988). The fever peaks at levels of 40 to 41 ºC on the second or third day of illness. The fever can last up to eight days and then gradually decrease (Taylor, 1984). Anorexia, sneezing and increased serous nasal discharge is also considered early signs of infection (Figure 3). Other clinical signs include dyspnoea, tachypnoea, diarrhoea, enteritis, serous-mucopurulent nasal and ocular discharges, soft, moist and productive cough, conjunctivitis, and necrosis and erosions of the oral cavity (Obi *et al.,* 1983, Bundza *et al.,* 1988). Pregnant animals may abort in all stages of pregnancy, but the foetuses show no sign of malformation (Abubakar *et al.,* 2008). It is common with secondary bacterial infection and most affected animals develop pneumonia during the course of the disease. Death usually takes place towards the end of the period of acute disease (Taylor, 1984), due to dehydration and secondary infections. Recent studies show that DIC (disseminated intravascular inflammation) due to coagulopathy is a probable cause of death as well (Sahinduran *et al.,* 2012). Case fatality rates in naïve herds can be up to 90 %, while in endemic areas as low as 20 % (Chauhan *et al.,* 2009, Abraham *et al.,* 2005).

Figure 3. *Animals showing signs of nasal discharge in the Mahenge region*. Photographer: Lovisa Levin.

Pathology

The carcass of an animal that suffered from PPR is usually emaciated, with the hindquarters soiled with soft, watery faeces and the eyeballs sunken in the cranium due to dehydration (Roeder P. L., 1999). Purulent nasal and ocular discharges with a foul smell is also seen (Muse *et al.,* 2012a). Post mortem examination often shows ulcerative to necrotic lesions in the mucosa of the oral and nasal cavity, but also in the pharynx, upper esophagus, abomasum and small intestine. The sites most affected in the oral cavity is the dental pad, the hard palate, the buccal papillae and the dorsal surface of the tongue (Munir *et al.,* 2013). The lungs often show evidence of pneumonia including lung congestion and consolidation, increased thickness of inter-alveolar walls and moderate infiltration of inflammatory cells in bronchiolar perivascular and sub-epithelial layers (Muse *et al.,* 2012a). Pneumonia is most prominent in the cranioventral lobes. Most of the digestive tract shows signs of hemorrhagic enteritis with hemorrhagic, edematous, ulcerative mucosa, congested intestinal blood vessels and enlarged intestinal lymph nodes (Kihu, 2014).One finding considered pathognomonic is the so called "zebra striping", which is congestion of the longitudinal folds of the caecum, proximal colon and rectum (Roeder P. L., 1999, Munir *et al.,* 2013).

Diagnosis

One of the problems limiting efforts to control the spread of the disease is how to quickly and correctly identifying outbreaks (Baron et al., 2014). A provisional diagnosis of PPR can be made from epidemiological and clinical features. PPRV infection should always be suspected if signs such as discharges, and deaths with breathing problems occur in sheep and goats, but

not in cattle, with mainly young ones being affected and dying. The observation of characteristic post mortem changes further strengthen the provisional diagnosis (Roeder P. L., 1999). The disease has a clinical picture that can easily be confused with other diseases, hence, the list of differential diagnosis is long. Some of the diseases with similar clinical appearance are rinderpest, foot-and-mouth disease, bluetounge, contagious caprine pleuropneumonia, contagious ecthyma, Nairobi sheep disease, diarrhoea complex, pneumonic pasteurellosis, heartwater and mineral poisoning (Munir et al., 2013, Baron et al., 2011). Since the diagnosis is hard to differ from other diseases, laboratory confirmation is needed. Conventional diagnostic techniques include agar gel immune-diffusion test (AGIDT), counter-immunoelectrophoresis, indirect ELISA and cross virus neutralization test (VNT) (Singh *et al.,* 2004). All these tests except VNT cannot differ PPRV from RPV, and was therefore not preferred before the eradication of RP in 2011. Furthermore, the VNT is laborious and difficult when sample size is large (Singh *et al.,* 2004). In the 1990s other more simple and rapid tests for antibody detection were developed (Libeau *et al.,* 1995). The tests used today can be divided into those that look for the virus (PCR or immunocapture ELISA, icELISA), and those that look for antibodies against the virus (competitive ELISA, cELISA) (Baron *et al.,* 2011). Virus isolation from whole blood, tissues or discharges (for example ocular/nasal/faecal) using PCR is the most reliable way to detect acute infection (Roeder P. L., 1999). The finding of antibodies against the virus in serum is a convenient way for surveillance and to estimate how widespread infection has been in a flock or area (Baron *et al.,* 2011). Competitive ELISA is a fast way of getting accurate results from working in the field, but these do not tell if an animal once was infected with PPRV or if it was vaccinated. Also, it takes about 7 days for an infected animal to get measurable titres of antibodies in the blood (Truong *et al.*, 2014). Therefore, during the acute course of disease, antibodies against PPRV cannot be serologically detected. Most serologically based assays detect the N or the HN proteins of PPRV (Munir *et al.,* 2013). Recently, a field diagnostic assay for PPRV has been developed (Baron *et al.,* 2014). The assay is a pen side test based on immunochromatographic lateral flow technology and is carried out on a superficial swab sample. It is based on the specificity and affinity of monoclonal antibody C77, which recognizes the H protein in PPRV. The test has a sensitivity of 84% and a specificity of more than 95%, and feedback from field trials has been positive. The test is suggested to be one of the diagnostic tools for controlling the spread of the disease (Baron *et al.,* 2014).

Prevention and vaccine

The old fashion way of achieving immunity among flocks during an outbreak is immunization with hyper-immune serum, or a combination of hyper-immune serum and blood inoculated with PPRV (Liu *et al.,* 2014). This way, inoculated animals could stand clear of PPRV infection for 9 months post-inoculation. Attenuated RP vaccine has been used as a heterologous vaccine for a long time to protect small ruminants from PPRV, taking the close relationship of the two viruses in advantage (Diallo, 2003). Goats vaccinated with this vaccine were protected from PPR for at least one year, and they did not transmit the virus during this time (Taylor, 1979). Since the eradication of RP in 2011, the use of RP vaccine to protect small ruminants against PPR is now contraindicated to the Global Rinderpest Eradication Programme (Liu *et al.,* 2014). There have also been attempts to develop an efficient bivalent

vaccine that protects against PPRV and sheep and goat poxvirus. The development of such multivalent vaccines would help to enhance poverty alleviation (Munir *et al.,* 2013).

Live attenuated homologous PPR vaccines have now been available on the market for some years. They contain different PPRV isolates, for example Nigeria 75/1 and Shungri/96 that have been attenuated by serial passages in Vero cells (Saravanan *et al.,* 2010). The vaccines have been proven to be 100% potent and it is indicated that one single vaccination provides lifelong immunity in both sheep and goats. These highly successful vaccines are used in most PPRV endemic countries (Munir *et al.,* 2013). One of the biggest challenges with vaccination for control of PPRV in tropical and subtropical regions is the loss of potency due to absence of continuous cold-chain. There has been improvement in this area over time, freeze drying is one of them, but the vaccines still need a maintained cold chain for shipment and storage (Liu *et al.,* 2014).

There is also a need for a new generation of vaccines that can distinguish between antibody response in animals due to vaccination and natural infection. This can be done with DIVA (Differentiating Infected from Vaccinated Animals) vaccines (Munir *et al.,* 2013). Expectations on these new generations of vaccines are high. They should not have any side effects in vaccinated animals, they should be easy to produce at a low cost, have long lasting immunity, be thermo-resistant, and they need to have serological test especially accompanied to them. Although several studies have been carried out in recent years, there is currently no DIVA vaccine commercially available (Liu *et al.,* 2014).

MATERIAL AND METHODS

Study area and data collection

The study areas were targeted to fill in the blanks from previous studies on PPRV in Tanzania, but also to sample animals in areas where the domestic animals have contact with the wild ruminant population, covering the northern, eastern and southern parts of Tanzania (Figure 4). The areas were chosen together with our local supervisor Dr. Gerald Misinzo and the data collection took place during three time-limited field trips during September – October 2014. In all three areas, samples were obtained from goats and sheep belonging to Maasai pastoralists. Blood samples and nasal swabs were collected from sheep and goats of both sexes and all ages. Collection of data took place in the northern part of Tanzania in the conservation area of Ngorongoro, Arusha region, in the south close to Mahenge, and in the area close to the Mikumi National Park, Morogoro region. In total, 39 herds from 15 villages were sampled. All areas have a various amount of interaction between the wild grazing animals and the domestic small ruminants, and the farmers practice communal grazing systems for their sheep and goats. In some areas, the herds more or less graze side by side with the wild population of ruminants.

Figure 4. *Map of Tanzania with the three main areas of sampling marked with yellow markers.*

Study design

The domestic animals (Table 1) were bled from the jugular vein into both serum and EDTA tubes, using a vacutainer system (Figure 5). Nasal swabs were taken from approximately 65% of the animals sampled. All samples were labelled accordingly so that each animal and flock could be identified. Age, sex and breed were recorded of each animal. The tubes were placed in an upright position and in a cooler bag before transported back to the laboratory. Later, all tubes were centrifuged to allow serum and plasma separation from clotted blood samples. The serum, buffy-coat and plasma were decanted and aliquoted into cryotubes marked with a number for each individual animal. The samples were transported to the laboratory at SUA, Morogoro for analysis.

Table 1. *Number of animals sampled in the three different areas*

Area	Sheep	Goat	Total
Mikumi	83	94	177
Ngorongoro	87	66	153
Mahenge	61	87	148
Total	236	242	478

Figure 5. *Sampling during field conditions. Photographer: Lovisa Levin.*

Questionnaire

All heads of herds were interviewed during the sample collections, according to a pre-designed questionnaire. The interviews focused on health and vaccination status, but also contained information about flock size, sex and approximate age of each animal. The questionnaire was divided in two parts. One part had main focus on PPRV and PPR outbreak status. The other part covered more general health aspects of the animals and the household.

The first part of the questionnaire contained the following questions:

- Estimated date when the first PPR case was observed.
- Detail of animals affected during the observed outbreak (see Table 2)
- Clinical signs at outbreak.
- Type of farming: Household, dairy production, meat production, individual seller at live animal market, others.
- Vaccination status for PPRV. Date when vaccinated.

The second part of the questionnaire contained the following questions:

- What kind of animals do you have?
- How many animals do you have?
- Have your animals had any injections or vaccinations? When?
- Have you bought animals during the last month?
- Which disease do you find most harmful for your livestock?
- Which clinical signs have caused the biggest problems in your livestock (for example diarrhoea, pneumonia, abortions)?

- Have you had an increased amount of malformed kids and lambs during the last year?
- If your livestock would get sick, how would that affect you and your family?
- Does your livestock have contact with wild animals? What kind of wild animals?
- Have you changed your habits around your livestock to prevent contamination?

Table 2. *Template for filling out details of animals affected during described outbreak*

Species	Age Group (0-1 year)				Age Group (> 1 years)		
	Total Animals	Affected	Died	Aborted	Total Animals	Affected	Died
Sheep							
Goat							

Laboratory analysis

PPRV cELISA

All sera from sheep and goats were analysed with cELISA (competitive enzyme-linked immunosorbent assay) from ID.Vet Innovative Diagnostics, identifying antibodies directed against the nucleoprotein of PPRV. All analyses were made from serum that had been centrifuged and decanted from serum tubes.

The microplates in the cELISA-kit had microwells coated with purified recombinant PPR nucleoprotein. When a sample was added, existing antibodies in the sample formed an antibody-antigen complex and masked the nucleoprotein epitopes. A peroxidase-conjugated anti-nucleoprotein antibody was then added to the wells, which formed a complex with the remaining nucleoproteins. After washing and adding the substrate solution, there was a coloration depending on the quantity of antibodies in the tested sample. The presence of yellow colour in the sample indicated absence of antibodies, while no coloration indicated presence of antibodies. The microplate was then read at 450 nm in the 96-well microplate reader. To each plate, 92 samples were added along with 2 positive and 2 negative controls.

The results were calculated after microplate reading, thus the validity of each cELISA analysis were controlled. All analyses were valid after calculating the optical density of the negative (greater than 0.7) and positive (less than 30 % of the optical density of the negative control) controls. For each sample the competition percentage (S/N%) was calculated with this mathematical formula:

S/N% = (Optical density of the sample/optical density of the negative control) x100

Samples less than or equal to 50% were considered positive and samples greater than 60% were considered negative. The samples in between were considered doubtful.

Molecular epidemiology

RNA-extraction and FTA cards

Viral RNA was recovered from 365 buffy coat and 309 nasal swabs using Thermo Scientific Gene JET RNA Purification Kit from Thermo Scientific. Briefly, Proteinase K was added to all nasal swabs. All samples were lysed and homogenized using a lysis buffer. The lysate was then mixed with ethanol and loaded on a purification column, and washed with wash buffers. Purified RNA was then eluted under low ionic strength conditions with nuclease-free water. Extracted RNA was stored at -80ºC.

FTA cards were used for inactivating and shipping a selection of samples from Tanzania to Sweden for further analyses. Buffy coat and nasal swabs (approximately 125µl) were applied to FTA cards. The cards were dried at room temperature for at least 12 hours.

RT-PCR

Due to a number of unpredictable circumstances only a few RNA-extracted samples could be analysed with real-time RT-PCR (real-time reverse transcription polymerase chain reaction). A subset of the samples was selected according to seroprevalence and the clinical signs of the herds. All together, 68 samples from seven herds representing all three main sampling areas were analysed in Sweden, at the Swedish University of Agricultural Sciences in Uppsala. The samples were inactivated and shipped on FTA cards from Tanzania to Sweden. Nucleic acid elution was performed as follow. A small piece of each sample on the FTA card were cut out and placed in a RNA processing buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 800U/ml RNase Out, 2mMDTT) before incubated for 15 minutes at room temperature.

Samples were analysed using the AgPath-ID one-step RT-PCR kit from Applied Biosystems. PPRV specific primers were used; NPPRfTAQ (5'GAGTCTAGTCAAAACCCTCGTGAG-3'), NPPRrTAQ (5'-TCTCCCTCCTCCTGGTCCTC-3') and Taqman Probe NPPR (FAM-5'-CGGCTGAGGCACTCTTCAGGCTGC-3'-BHQ1), according to Kiwatek et al., (2010). The following thermo cycling conditions were applied according to the AgPath-ID™ One-Step RT-PCR Kit User Guide: Initial reverse transcription at 45ºC for 10 min, followed by reverse transcriptase inactivation and DNA polymerase activation at 95ºC for 15 min, and 40 cycles of amplification (15s at 95ºC and 60s at 60ºC).

RESULTS

Results from interviews

Mikumi

Region: Morogoro, district: Mvomero

In the area around Mikumi National Park, twelve herds were sampled and each head of herd were interviewed. Flock size varied between approximately 50 and 400 sheep and goats. Apart from sheep and goats, most households also had cattle and at least one dog. Animals were held for household, milk and meat production and selling meat and live animals. In spring 2013, there was a mandatory vaccination campaign in this part of the country.

Therefore, most of the herds were vaccinated against PPRV during late spring 2013 and only four of them reported suspected outbreaks of PPR. In one of the herds, all kids and lambs died (n=90) in a suspected outbreak in 2013, adjacent to being vaccinated to PPRV. Animals had shown one or several of the following clinical signs during a suspected outbreak: fever, diarrhoea, oral mucosal lesions, coughing, nasal discharge, pneumonia and abortions. The flocks were reported to have various amount of contact with the wild animals in the area. Other diseases that affected the animals were anaplasmosis, foot and mouth disease, brucellosis and contagious caprine pleuropneumonia (CCPP).

Ngorongoro

Region: Arusha, District: Ngorongoro

In Ngorongoro, animals from fourteen different herds were sampled. All herds had contact with wild animals, more species than reported in Mikumi. Some farmers also had dogs, cattle and poultry. Sheep and goat were kept for household mainly. None of the herds had been vaccinated against PPRV and suspected outbreaks were reported from all herds but one. Some herds had had an outbreak more than once. Two of the flocks were vaccinated against anthrax. In this area they have had some problem with anthrax in their livestock and also in some rare cases in humans. Flock size varied between approximately 50 and 500 animals. Other diseases reported were goat pox, heartwater, CCPP, tick borne diseases and parasites. Clinical signs at outbreaks were poor general condition, inappetence, diarrhoea, pneumonia, oral mucosal lesions, nasal discharge, abortions, and in some cases alopecia. In some villages, animals that had died of disease were burned to prevent contamination and disease transmission, and they also built a new “boma” (fenced area were animals are kept in the village) when they had had an outbreak of PPR.

Mahenge

Region: Morogoro, District: Ulanga

Twelve herds were visited in the area around Mahenge. The flocks were overall smaller than in Mikumi and Ngorongoro. Animals were kept mostly for household purposes, but also for meat production and to sell. Apart from sheep and goat some farmers had cattle, poultry and dogs. They varied in size between just a handful of animals and 200 animals. Some of the villages were situated near a town and not all in rural environment as in Mikumi and Ngorongoro. These flocks had none or almost no contact with wild grazing animals. All herds but one had been vaccinated in 2013 against PPRV. Only three herds reported suspected outbreaks of PPR, but many farmers that did not report an outbreak among their livestock still had had clinical signs with diarrhoea, pneumonia, abortions and increased amounts of deaths. All rural herds had problems with diarrhoea and increased amounts of deaths during the rain season. The cause of this diarrhoea is thought to be parasite infection, and this was also the disease that farmers feared the most in their livestock.

All farmers in all three sampling areas said that diseased livestock and increased number of deaths among their animals would affect the family, the economy and the health situation. In all areas, the provided information on outbreaks showed that the mortality rate among animals

in the age group 0-1 year was higher, and in some cases considerably higher than among animals in the age group >1 year.

Serological prevalence of PPRV

The overall prevalence of PPRV antibodies in the selected sampled areas was 47% (Table 3). The highest percentage of seropositive animals was from the area near Mikumi National Park with 64.8%. The prevalence among goats was slightly higher in all areas than in sheep, in total 49.0% compared to 43.2%. The within-herd prevalence ranged from 0 to 100%. In the area around Mikumi National park all herds had both seropositive and seronegative animals, although the majority of herds had most seropositive animals. In Ngorongoro, there were one herd where all sampled animals were seronegative and one herd where all sampled animals were positive. Some other herds in this area only had one or a couple of seropositive animals and the rest were seronegative. In Mahenge, one herd was seronegative and another two herds had one doubtful animal. The remaining herds were seronegative.

Table 3. *Serological prevalence of PPRV in sampled areas*

Area	Species (number)	Positive	Negative	Doubtful
Mikumi	Sheep (83)	59.0%	38.6%	2.4%
	Goat (94)	67.0%	32.0%	1.0%
	Total (176)	64.8%	33.5%	1.7%
Ngorongoro	Sheep (66)	39.4%	60.6%	0.0%
	Goat (87)	40.0%	59.3%	0.7%
	Total (153)	39.5%	59.9%	0.6%
Mahenge	Sheep (87)	31.0%	63.2%	5.8%
	Goat (61)	34.4%	64.0%	1.6%
	Total (148)	33.8%	61.5%	4.7%
Total	Sheep (236)	43.2%	53.8%	3.0%
	Goat (242)	49.0%	49.8%	1.2%
	Total (478)	47.0%	50.7%	2.3%

In Table 4, the seroprevalence in animals less than a year of age and animals older than one year of age is shown. These animals are too young to have been vaccinated according to the interviews. The overall prevalence in animals less than a year old was 18.3 %. In this group, 19 out of totally 82 animals were three months or less than three months old, and five of them were seropositive. These might be seropositive due to maternal antibodies received at suckling. The percentage of seropositive animals older than three months and less than one year old was 16%. In this age group, all animals should have seroconverted due to natural infection.

Table 4. *Seroprevalence of PPRV in animals younger and older than one year*

	Area	Age (years)	Total sampled	Number positive	Seroprevalence (%)
Goats	**Mikumi**	<1	9	3	33.3
		>1	85	61	71.7
	Ngorongoro	<1	28	8	28.6
		>1	59	26	44.0
	Mahenge	<1	10	0	0
		>1	51	21	41.2
Sheep	**Mikumi**	<1	5	1	20.0
		>1	78	50	64.0
	Ngorongoro	<1	6	1	16.6
		>1	60	25	41.6
	Mahenge	<1	24	2	8.3
		>1	63	26	41.2
Total		<1	82	15	18.3
		>1	394	209	53.0

Molecular prevalence of PPRV

The total number of 68 buffy coats and nasal swabs (eluated from FTA cards) were analysed with RT-PCR. The chosen samples came from animals in seven of the seropositive herds, selected according to seroprevalence and clinical signs. The results were negative for PPRV RNA in all samples analysed.

DISCUSSION

The seroprevalence was highest in animals from the area around Mikumi National Park with 64.8%. In this area, most herds were vaccinated in the late spring of 2013, due to mandatory vaccination campaigns in the area (Gerald Misinzo, Sokoine University of Agriculture, Tanzania, personal communication). Taking this into consideration, the results are not surprising. On the other hand, the seroprevalence among the young unvaccinated animals (less than a year of age) was the highest in Mikumi (33.3% in sheep, 20.0% in goats) compared to the other sampling areas. Out of the animals younger than a year, but without maternal antibody protection (animals younger than three months excluded, in agreement with Libeau *et al.,* (1992)), in total number 63, 16% were positive. They have according to the interviews not been vaccinated. This indicates that they have been exposed to natural infection with PPRV. In Mikumi area, this number was 21%. This shows that virus has been present in the Mikumi area during the last year, even though only three out of twelve farmers reported to having had an outbreak. Furthermore, it strengthens the already proven fact that the virus is present in the central and southern parts of Tanzania (Kgotlele *et al.,* 2014a, Muse *et al.,* 2012b).

In Ngorongoro, the seroprevalence was 39.9%. None of the sampled herds had been vaccinated against PPRV, and many farmers reported an outbreak history. This information is in contrary to the fact that an emergency vaccination programme was implemented in the northern half of Tanzania in 2010 (Munir *et al.,* 2013). The seroprevalence in this area of the country is considered due to natural infection and it is interesting that the total seroprevalence

was found higher in this unvaccinated area than in Mikumi (33.8%), where all flocks but one were reported vaccinated. It is interesting to find such a low seroprevalence in areas where most animals have been reported vaccinated. Antibodies in the bloodstream of a vaccinated animal should be detectable, since it is indicated that the vaccines provide lifelong immunity (Munir *et al.,* 2013). Maybe it has to do with the reliability of the interview results, or that the animals did not get vaccinated during the mandatory vaccination campaigns.

Also in the Mahenge sampling area, the young animals showed a low seroprevalence (8.3% in sheep, none in goats). This result indicates that the area has got a low activity of the disease and that it has been the situation for at least a year. The total seroprevalence in Mahenge was the lowest of the three compared areas (33.8 %). Goats had a slightly higher prevalence than sheep. Results from the interviews show that all herds but one were vaccinated against PPRV in 2013, and therefore the seroprevalence should be higher in this area.

One of the issues with the questionnaire was to get the communication to work. It is hard to get the information right with different interpreters and the farmers tending to tell you what they think you want to hear, and of course misunderstandings due to different languages. This is a bias that needs to be considered while evaluating the answers of the interviews. In Tanzania, it is almost rude to ask how many animals a farmer owns, since it is in direct correlation to a person's economic status. The sampling and the interviews were done under time pressure in most of the villages, to allow the livestock to go out grazing for the day, and therefore the answers may not be as reliable as desired. All these factors might have influenced the answers we got in the interviews. Since information and education are lacking in many cases in the rural villages, they have a natural scepticism against injections being done in their livestock. Some farmers think that vaccination of animals and children can cause damage, and during the mandatory vaccination campaigns, farmers sometimes hid their animals to avoid the injections (Anonymous 2014).

The age of some sampled animals is roughly estimated. Some farmers knew exactly the age of all his animals, while others were not as certain. In many cases the age was estimated by looking at the animals teeth. This method is not completely reliable; hence, the age of all animals may not be completely accurate.

Even though the age is estimated in some cases, most farmers were certain of the age of their young animals. Most of them could specify the age of lambs and kids in months. This is important to know when evaluating results from seropositive young animals. Animals younger than 3-4 months of age can be seropositive due to maternal antibodies received by suckling (Munir *et al.,* 2013, Libeau *et al.,* 1992), while animals older than that is more likely positive due to natural infection with PPRV, if they have not been vaccinated.

Most of the outbreaks described in the answers were diagnosed only according to clinical signs. Not all farmers had had their animals examined by a veterinarian, and all outbreaks of PPR reported in sampled areas were based on clinical signs only. Because PPR is hard to differ from other diseases (Baron *et al.,* 2011), the outbreaks described by many farmers might be due to other causes.

The goats showed a higher seroprevalence than the sheep in all sampled areas. This strengthens the theory that goats are more affected by PPRV than sheep (Muse *et al.,* 2012a). In contrary, none of the interviewed farmers have described their goats to have more severe clinical signs than sheep during the outbreaks. This is not according to earlier research in the subject (Troung *et al.,* 2014).

Animals younger than one year has been shown to be more susceptible to the virus than older animals (Muse *et al.,* 2012a). In the present study, the overall seroprevalence in animals younger than one year was 18.3 %. Since there have been vaccination campaigns in Tanzania in recent years, both in the central and northern parts, the seroprevalence among older animals can be due to vaccination. In contrary, animals younger than a year, but older than three months, are most certainly seropositive due to natural infection with PPRV. The results from the questionnaire on outbreak mortality in young animals compared to mortality in adult animals further strengthens the assertion that PPR is more severe in young animals (Munir *et al.,* 2013).

RT-PCR results were all negative. It is impossible to know if this is due to errors of some kind or if the samples truly were negative. Samples have been shipped to Sweden on FTA cards and RNA was eluated before the analysis. In Tanzania, the laboratory suffered from many long power cuts. Some of them lasted for more than 12 hours. Since the laboratory did not have a working generator to supply the freezers and fridges with electricity, the temperatures in which all samples, purified RNA and cELISA kits were stored, was not optimal. However, the temperature in the freezers did not rise above freezing at any point. Still this is considered as one of the sources of errors and should be considered while looking at the results.

The impact of wildlife on the epidemiology of PPR is uncertain (Munir, 2014), but it is a domain that should deserve more attention. PPR is progressing southward in Africa where wild ruminant density, as well as sheep and goat density, are high and the consequences of outbreaks can be severe (Albina et al., 2013).

It is well known that PPR has a great socioeconomic impact in affected countries and that sheep and goats are critical to food and income security for pastoral communities (FAO, 2010), but can PPR be the second animal disease and the third mammal disease (after smallpox in humans and RP in cattle) to be eradicated? In October 2014, FAO posted on their website the program on how to make the eradication of PPR possible. They say that the technical tools are already available for an eradication of the disease over a 15-year period. Difficulties are thought to be convincing the political leaders to commit the necessary financial and human resources, and to get a DIVA vaccine out on the market. (FAO, 2014)

Albina et al., (2013) suggests that a PPR control program should start in highly infected regions, at the beginning of the dry season in Africa in areas where intense animal contact occurs, for example large livestock markets or borders.

The original plan for this thesis was to sample and analyse both domestic and wild small ruminants in areas where they intermingle and graze together, in order to further evaluate the

role of wildlife in the virus epidemiology. Unfortunately, due to other research projects in Tanzania, our application for wildlife sampling was rejected. In the following years, the role of wildlife will hopefully be evaluated within the bigger three-year project.

Conclusions

In this study, I have showed that PPRV is serologically present in three different areas of Tanzania where wild and domestic small ruminants intermingle. The laboratory results and the results from interviews with farmers indicate that the disease is present and that the seroprevalence is to some extent due to natural infection. No virus was detected with RT-PCR, but only a subset of samples were analysed.

It is of great interest to further evaluate the prevalence of PPRV in different parts of Tanzania, especially where wild and domestic small ruminants intermingle. I therefore suggest that in the continuing work of this project, the analysis of samples collected during our field trips needs to be completed. Also, investigating the prevalence of PPRV among wild small ruminants in approximately the same areas would be very interesting.

I hope that this thesis and the data collected in this project will contribute to the understanding and prevention of spread of PPRV, and in longer term, the eradication of the virus in 2030, and thus contribute to the fight against poverty in the world.

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