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Seroepidemiology of Peste des Petits Ruminants in central Tanzania alongside an evaluation of filter paper as transport medium

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Seroepidemiology of Peste des Petits Ruminants in central Tanzania alongside an evaluation of filter paper as transport medium

Seroepidemiologi av Peste des Petits Ruminants i centrala Tanzania med samtidig utvärdering av filterpapper som transportmedium

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Abstract

Peste des Petits Ruminants (PPR) is a disease of increased attention since the eradication of Rinderpest virus (RPV) in 2011. PPR is an acute and highly contagious viral disease of small ruminants caused by a *Morbillivirus* named Peste des petits ruminants virus (PPRV). The devastating effects of PPR are mostly present in developing countries, where herding of small ruminants like goat and sheep is a major source of income for millions of farmers. Therefore, PPR is indubitably a disease of major socioeconomic impact. The FAO recently announced a program to eradicate PPR by 2030.

The purpose of this study is to evaluate the diagnostic use of filter paper as sample medium in serology analysis for evidence of PPR in sheep and goat in field conditions. The evaluation is performed alongside an epidemiologic screening of PPR antibodies in domestic small ruminants with and without wildlife contact in the Morogoro region, central Tanzania.

Present study show that filter paper like Nobuto's and Sartorius 3MM filter paper soaked with whole blood can be a viable alternative to serum samples, and successive serology through cELISA, in areas where keeping a sustained cold chain is problematic. This study also presents arguments showing how the interpretation of filter paper results could refine and supplement the identification of animals having antibodies against PPRV. Raising the cutoff value in the interpretation of positive individuals from serologic analysis of filter paper samples can improve the accuracy of this method. The serologic screening show that PPRV infection is present in three out of three areas adjacent to the wildlife preservation area Selous Game Reserve.

It is of great interest to further evaluate the use of filter paper as an alternative to serum samples in PPR serology to assist the study of PPR in remote areas.

Keywords: Seroepidemiology, Seroprevalence, Filter paper, PPRV, Tanzania.

Sammanfattning

Peste des petits ruminants (PPR) är en sjukdom som uppmärksammats mer och mer sedan boskapspest utrotades 2011. PPR är en akut och mycket smittsam sjukdom hos små idisslare som orsakas av ett virus av genus *Morbillivirus* vid namn Peste des petits ruminants virus (PPRV). Den förödande effekten av PPR är mest påtaglig i utvecklingsländer där får- och getbesättningar är vanligt förekommande och huvudinkomsten för miljontals bönder. Därför är PPR onekligen en sjukdom som medför stora socioekonomiska effekter. FAO gick nyligen ut med ett officiellt program för att utrota PPR till år 2030.

Syftet med denna studie var att utvärdera filterpapper som alternativt transportmedium för att påvisa antikroppar mot PPR hos får och getter. Utvärderingen utfördes parallellt med en epidemiologisk screening av antikroppar mot PPR hos små domesticerade idisslare med eller utan kontakt med vilda djur i Morogoro, en region i centrala Tanzania.

Serologi på blod från filterpapper kan vara ett bra alternativ till serumprover i områden där det är problematiskt att upprätthålla kontinuerliga kylkedjor. Genom analys och statistiska uträkningar visade denna studie hur resultat från filterpapper bör tolkas för att upptäcka djur med antikroppar mot PPRV. Genom att höja gränsvärdet i tolkningen av den serologiska analysen kan säkerheten i denna metod förbättras. Screeningen visar att infektion med PPRV förekommer inom tre av tre områden i anslutning till reservatet Selous Game Reserve.

Det är av yttersta vikt att fortsätta utvärdera filterpapper som en del i diagnostiken av PPR och som ett alternativ till serumprover.

Nyckelord: Seroepidemiologi, Seroprevalens, Filterpapper, PPRV, Tanzania

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1 Introduction

This master thesis is a small part of an extensive three-year collaboration project between Sweden, Pakistan, Tanzania, Botswana, France and the UK, funded by the Swedish Research Council (VR Developmental research grant Swedish Research Link). The aim of this larger project is to study the prevalence of Peste des petits ruminants virus (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 contributes to the larger project by collecting data, performing lab work, data analysis, statistical analysis, and improving diagnostic methods. Funding of this master thesis was contributed by Swedish International Development Cooperation Agency (SIDA) and the Swedish University of Agricultural Sciences (SLU).

1.1 Aims

One of the goals with this smaller study was to evaluate the use of filter paper for serological diagnostics of infection with PPRV. This evaluation was performed alongside a screening of the seroprevalence of PPR antibodies in domestic small ruminants with or without wildlife contact in Tanzania. Thus, there were two study topics in this master thesis:

1. The evaluation of filter paper for serological diagnostics of antibodies against PPRV in domestic small ruminants.
2. Seroepidemiology of PPR in domestic small ruminants with or without wildlife contact.

The filter paper evaluation will use current scientific advances as groundwork to improve the transportation and analysis of whole blood samples. This possible improvement is performed through applying the method of ELISA analysis of filter paper samples compared to serum samples. By fine-tuning the interpretation of these results, the goal is to suggest a practical and functional alternative to serum samples as transport medium.

By participating in this project I was hoping to learn more about epidemiology in general and particularly the epidemiology of PPR, a disease that is not currently present in Sweden. As a benefit for me and for my future professional life as veterinarian, my laboratory skills were

improved through this project. By travelling to Tanzania I also broadened my way of thinking and learned more about disease control on a global scale. Through living and working in an unfamiliar country, I learned the importance of cultural and religious differences. This type of understanding in general is of utter importance for international networking and is fundamental to trans-national epidemiologic research.

2 Literature review

2.1 Peste des petits ruminants virus

PPRV is currently considered one of the main animal transboundary diseases that constitutes a threat to livestock production in many parts of the world, particularly developing countries across Africa, the Middle East and south Asia (Banyard *et al.*, 2010). PPRV is currently present in West, East and Central Africa, Arabia, the Middle East and southern Asia (Karimuribo, 2011; Nanda *et al.*, 1996; Shaila *et al.*, 1996). According to FAO, roughly 80% of the world's total population of sheep and goats is considered to be at risk of infection with PPRV (FAO, 2015b).

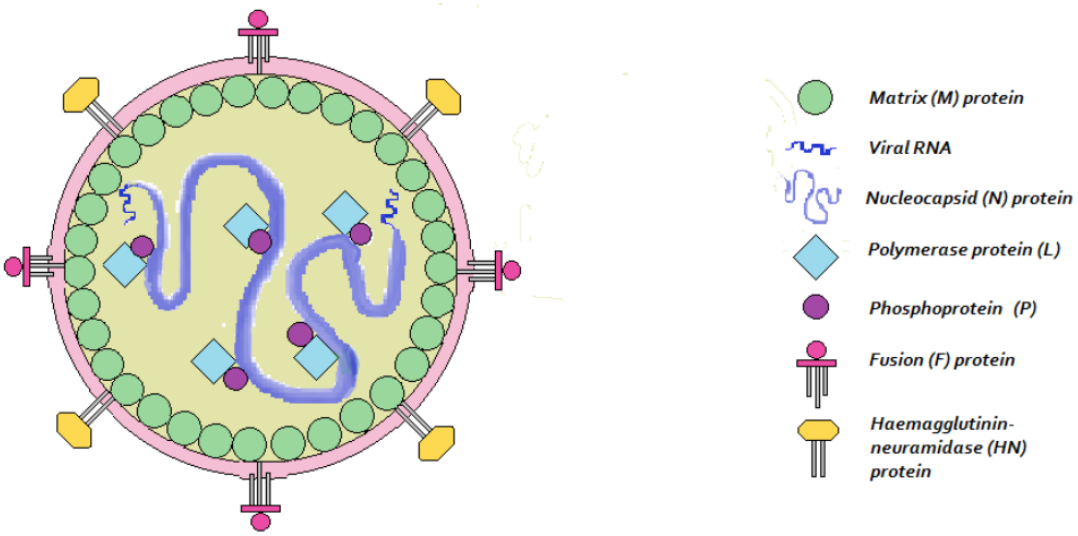

Figure 1, Schematic structure of the PPR virion (Herbe 2015)

2.1.1 Genetic properties and taxonomy

PPRV is a negative single strand RNA virus belonging to the genus *Morbillivirus*, sub-family Paramyxovirinae and family Paramyxoviridae. It is closely related to Measles virus (MV) (Brown *et al.*), Rinderpest virus (RPV) and Canine distemper virus (CDV) amongst others.

Although PPRV is sometimes referred to as an emerging virus, having only been recognized as a distinct viral entity since 1979 (Gibbs *et al.*, 1979). The PPRV branch from the supposed common predecessor of the morbilliviruses is at least as long as those presenting the evolutionary distance of MV and RPV from their point of separation (Gibbs *et al.*, 1979). Given that MV is believed to have separated from this common ancestor at least 1,000 years ago (Furuse *et al.*, 2010), it would seem that PPRV has been with us for many hundreds of years, unrecognized as a virus until the development of molecular methods to distinguish it from RPV.

2.1.2 Life cycle and survival

The PPRV enters the and attaches to host cell membrane receptors. This binding is mediated by a haemagglutininneuramidase (HN) protein and the sialic acid on the host cell membrane anchored receptors (Munir *et al.*, 2013). The interaction proceeds with the fusion protein (F) allowing fusion of the virion envelope with cellular membranes (Chauhan *et al.*, 2009). The virus becomes uncoated and the nucleocapsid (N) is eventually released into the cytosol of the infected cell through endocytosis. Replication of the viral RNA takes place in the cytosol and results in full-length, positive stranded anti-genomes as well as a number of mRNAs. The full-length anti-genomes are transcribed into copies of the negative-stranded virus genome. Furthermore, translation of viral proteins takes place in the endoplasmic reticulum and the cytoplasm. All structural and non-structural proteins self-assemble with the genomes near the cell surface whereas the budding process begins, resulting in the release of functional virions (Munir *et al.*, 2013).

2.1.3 Lineages

There are one serotype of PPRV, nonetheless there are clear lineages based on phylogenetic analysis of the virus genes, more specifically the N or F genes (Baron *et al.*, 2011). PPRV can be divided into four different lineages: I, II, III and IV. Historically, viruses of lineages I and II have been identified in West Africa. Lineage III have been found in East Africa and Arabia, in countries as Sudan, Yemen and Oman. Lineage III has also been identified in southern India, though only at one occasion. Historically, lineage IV has been found in the Middle East and the Asian subcontinent (Banyard *et al.*, 2010; 'Dhar *et al.*, 2002). However, lineage IV has been found circulating all across the PPR endemic areas (Parida *et al.*, 2015; Libeau *et al.*, 2014). The utility of lineage identification lies in the information it provides regarding the probable origin of the virus causing the outbreak. PPRV lineage identification showed for example that an outbreak in Morocco in 2008, which was the first time the virus had been seen in North Africa, was a lineage IV virus. Since the virus was of this lineage it therefore had not come from West Africa, in which all viruses are of lineages I or II. Instead this virus was likely to have been introduced from the Middle East, where PPRV of lineage IV circulates (Baron *et al.*, 2011). This type of genetic data is routinely used to construct phylogenetic trees for PPR and attribute different isolates to each of the four lineages (Banyard *et al.*, 2010; 'Dhar *et al.*, 2002). Though the characterization of viruses into lineages is important to epidemiological studies, it appears unlikely that this lineage differentiation has a relationship to virulence of isolates. The differentiation is rather a result of geographical speciation (Munir, 2015).

Phylogenic analysis of nucleoprotein and fusion genes have been made on samples isolated from wild ungulates. The result of these analyses indicates that strains identified from PPRV infection in wild ungulates have historically been exclusively from lineage IV (Munir, 2014). This was the case until recently, when lineage II was identified in wild ruminants near domestic livestock in northern Tanzania (Mahapatra *et al.*, 2015).

2.1.4 Occurrence and history

PPR was first described 1942 on the Ivory Coast as a disease of its own. As a sickness of sheep and goat it had formerly been recorded as Bluetongue amongst other diseases (Gargadennec & Lalanne, 1942). Moreover, because of the clinical similarities and the classical course of the disease, PPRV was long thought to be a variant of RPV that had become adapted to small ruminants and lost its virulence for cattle (Banyard *et al.*, 2010; Gibbs *et al.*, 1979). It is likely that early cases of RP described in sheep and goat actually were PPR (Baron *et al.*, 2011). Because of this common misconception we do not know the true origin of PPRV or how long it has been circulating in different parts of the world. PPR has only been recognized as a completely distinct disease in the past 40 years when Gibbs *et al.*, proved in 1979 the PPRV to be a member of the genus *Morbillivirus.*

Since its first discovery, PPR has spread through sub-Saharan Africa, the Middle East, Turkey as well as the Indian subcontinent. Until 1979 PPR was described mostly in West African countries after which it emerged in Eastern and Northern Africa as well. In the last few years, the first outbreaks of PPR have occurred in China, in East Africa, and in North Africa (Munir *et al.*, 2012). The transboundary spread of PPR is suggested to be exacerbated by the development of trade relations, tourism, transport and migration of wild animals (Kaukarbayevich, 2009). The disease is currently present in Central, Eastern and Western Africa, Asia, and the Middle East (Banyard *et al.*, 2010). Today, a total of 76 countries have officially confirmed PPR within their borders. The regions in which these countries are situated is the home to approximately 1.7 billion heads, roughly 80 percent, of the worlds total population of sheep and goat (FAO, 2015b).

2.1.5 Hosts and transmission

The main hosts for PPRV are goats and sheep of both sexes and all ages. Other ruminants like cattle, buffaloes and wild small ungulates, as well as camels and pigs, may be infected (Abraham *et al.*, 2005). In poor conditions cattle can show signs of infection similar to RP when infected with PPRV (Kwiatek *et al.*, 2011; Diallo *et al.*, 2007). Though other species than small ruminants like cattle might get infected, there is at this time no evidence that unusual hosts like cattle, buffaloes or camels have a significant role in the transmission of PPRV (OIE, 2015a). A study from 2008 suggests that the prevalence of PPRV infection is higher in animals older than two year relative to younger animals. Furthermore, there is a correlation between gender and infection with PPRV where females tend to be infected to a greater extent than male sheep and goat (Khan *et al.*, 2008). Additionaly, findings following experimental infection with PPRV suggest that sheep display milder clinical disease compared to goats (Truong *et al.*, 2014).

Transmission of PPRV from infected animals occurs via discharges from eyes, nose and mouth, as well as diarrhea or loose faeces. All of these excretions contain high titers of virus and transmission usually occurs through an aerosol over very short distances. Moreover, except for close contact, which remains the most frequent means of disease transmission, additional routes of transmission may be contaminated water, feed troughs and bedding. Virus does not survive for long outside the host, therefore most transmission occurs during the febrile, acute clinical stage of disease (Braide, 1981).

Wildlife

Wild ruminants may play an important epidemiological role as source for PPRV in domestic small ruminants (Kinne *et al.*, 2010). Antelope and other small wild ruminant species can be severely affected (Adel *et al.*, 2004). Clinical disease has been reported in wildlife resulting in deaths of gazelles and Sindh ibex (Abubakar *et al.*, 2011). The American white tailed deer can be infected experimentally with possible fatal consequences (Hamdy & Dardiri, 1976), though natural infection with PPRV have not been observed on the American continent.

It is crucial to further evaluate the role of wildlife in the epidemiology of PPR. Since domestic and wild ruminants intermingle in many areas, inter-species transmission of PPRV is possible. This might interfere with current disease surveillance and eradication programs but might as well be a serious threat to the survival of endangered wildlife species (Munir, 2014). One hypothesis is that PPRV infection in wild animals is not self-sustained and therefore cannot survive within the wild population itself. Instead it is suggested that infection among wildlife is the result of PPRV spread by domestic small ruminants neighboring natural parks (spill-over) (Couacy-Hymann *et al.*, 2005). To conclude, the wildlife host range of PPRV needs additional research and is not yet fully understood. There is at this time no reason to believe that PPRV circulates in wild animals and thereby acts as a possible source of virus for domestic species (Munir, 2014).

2.1.6 Pathogenesis

At experimental infection through intranasal route it is suggested that immune cells within the respiratory mucosa absorb the PPR virus. The immune cells then transport virus to lymphoid tissue, from where primary virus replication occurs. Virus subsequently enters the circulation for continued systemic infection. It is hence believed that the initial reproduction of virus instead of being performed within the epithelial cells of the respiratory mucosa, is in the nearest draining lymphoid tissue (Pope *et al.*, 2013). Moreover, an evaluation of tissue tropism at PPRV infection showed that lymph nodes, lymphoid tissue and digestive tract organs are the predominant sites of PPRV replication (Truong et al., 2014). As infection progresses, leucopoenia, lymphopoenia and a suppressed antibody response may be observed. It also results in an increased risk of activating latent infections, for example intestinal and blood parasites, which increase the perceived mortality rate of PPR (Taylor, 2005). Upon infection with PPRV, both sheep and goat develop clinical signs though sheep tend to display a milder clinical presentation compared to goats (Truong *et al.*, 2014). Some clinical signs presented at infection, particularly respiratory disease, derive from secondary infection as a result of the immunosuppressive effect from PPRV (Baron *et al.*, 2011).

2.1.7 Clinical picture and pathology

The clinical presentation of PPR is divided into three categories: Acute, peracute and subacute form. As the acute form often presents sudden pyrexia and a gradual exacerbation of clinical signs that may result in death, the peracute form presents a severe pyrexia, depression and higher mortality (OIE, 2016). The subacute form may result in inconsistent clinical signs for 10-15 days. Incubation time for PPR is usually 4–6 days, but can range between 3 and 10 days. The clinical phase of the acute form presents pyrexia up to 41°C that can last for 3–5 days (OIE, 2016).

Figure 2, Sheep with sings of mucopurulent nasal discharge in the Mahenge area. (Photo: Nils Roos)

Affected animals become anorectic, depressed, and develop a dry muzzle. Oculonasal discharges gradually become mucopurulent (Figure 2) and persist for up to 14 days, though death may already have ensued by then. Gums eventually become hyperemic, and erosive lesions with or without necrosis develop in the oral cavity combined with excessive salivation. Diarrhea is common in the later stage, often watery and bloodstained (Abubakar *et al.*, 2008). Pneumonia, coughing, and abdominal breathing also occur. The morbidity rate can reach up to 100% with a high case fatality rate in severe cases. Morbidity and case fatality may however be low in mild outbreaks and the disease may therefore be overlooked (OIE, 2015a). While case fatality rates in naïve herds have reportedly reached up to 90%, in endemic areas the fatality may drop as low as 20% (Chauhan *et al.*, 2009).

At necropsy, findings include crusty scabs along the outer lips and severe interstitial pneumonia is frequently presented (Chauhan *et al.*, 2009). Erosive lesions may extend from the mouth via esophagus to the reticulorumen junction. Erosive or hemorrhagic enteritis is generally present and the ileo-caecal junction is often involved. Peyer's patches can also be necrotic. Enlarged

lymph nodes and necrotic lesions may be observed in the spleen and liver (OIE, 2015a). Findings suggest that infection with PPRV also is associated with abortion in goat (Abubakar *et al.*, 2008).

2.1.8 Diagnosis

Acute infection with PPRV can often be diagnosed through its clinical signs. A number of diseases is clinically similar but may be excluded in the case of PPR from its significant differences. Some of these diseases are foot and mouth disease, bluetongue, sheep-/goat pox, contagious caprine pleuropneumonia and pneumonic pasturellosis. These are all diseases that may be found circulating in areas with PPR and have similar presentations. Though differential diagnoses often can be excluded it may however be difficult to distinguish them from PPR if the clinical presentation is mild or secondary infections have occurred (Baron *et al.*, 2011). When diagnosing mild PPR, it is therefore necessary to use laboratory methods to confirm the disease. The two general approaches to this confirmation are firstly to search for the PPR virus traces like antibodies directed against the nucleocapsid (N) protein through immunocapture ELISA or virus nucleic acids through PCR. Both of these methods are used to detect acute infection. Secondly, another approach is to search for antibodies induced by PPRV infection through competitive ELISA (c-ELISA), a method commonly used in surveillance studies to map out the spread of the disease (Libeau *et al.*, 1994; Libeau & Lefevre, 1990).

2.1.9 Impact on poverty and economics

The economic incentive for PPR control and/or eradication has been a major subject for discussion in the past. Current epidemiology, low individual economic value of hosts (i.e. sheep and goat) and high turnover in herds has made accurate cost/benefit-calculations difficult to achieve (Baron *et al.*, 2011). Since few recent studies have been able to estimate the economic losses caused by PPR, the complete and comprehensive economic impact of PPR on small ruminants is currently not known (Munir, 2015). Though there is a discussion of the exact severity of the economical impact of the disease, there is no doubt that losses caused by PPR strike at the heart of vulnerable livelihoods. Countries have experienced accumulative yearly losses varying from tens to hundreds of millions of US dollars (*The Global Strategy for the Control and Eradication of PPR*, 2015).

Though the economical calculations are problematic, there are a number of studies on the estimated cost of PPRV infection (Kaukarbayevich, 2009; Hussain *et al.*, 2008; Opasina & Putt, 1985), some of which suggest that the lowest costing preventive measure of controlling PPR outbreaks could be timely vaccinations (Thombare & Sinha, 2009).

2.1.10 Eradication strategy

In the eradication strategy of PPR there are a number of aspects that needs to be addressed. Often when eradication of PPR is discussed, parallels are drawn to the strategic eradication of RPV (Munir, 2015). Since the two pathogens have fundamental antigenic and immunological similarities, this is appropriate. A lot can be learned from the way RPV was eradicated but there

is many factors in which PPR differ from RP. This needs to be considered. For instance, species infected by PPRV like sheep and goat have a considerable higher reproduction rate and turnover compared to species generally infected by RPV like cattle (Munir, 2015). This makes the infection rate faster and complicates control of the disease status of a herd. Also, the duration of immunity following an infection with RPV is lifelong contrary to infection with PPRV which is 3 years (Munir, 2015). Furthermore, while the host spectrum with ability to carry RPV for further infection was limited to domesticated animals, spread of PPRV through for example wildlife is still uncertain (Baron *et al.*, 2011). Additionally, with the eradication of RPV and vaccination against this disease have ceased, infection with PPRV is found to increase as a result. This is not only because of the cross-protective effect of RPV vaccination against PPRV, but also because many disease cases, which before were discarded as variations of RPV infection, are now correctly labeled as PPR. Hence the disease might appear to be emerging in greater extent than it actually is (Munir, 2015; Baron *et al.*, 2011). Also, the absence of RPV implicates that small ruminants no longer can get infected and thereby gain cross-immunity against PPRV. The infection rate of PPRV may thereby increase (Baron *et al.*, 2011).

FAO and the OIE convened in the spring of 2015 and are together mobilizing the international community around the fight against PPR. At the conference, FAO and OIE launched the global campaign to eradicate the disease by 2030. The campaign concentrates on areas in Asia, the Middle East and Africa affected by the disease. The two organizations hope to lead and coordinate global efforts of governments, regional organizations, research institutions, funding partners and livestock owners to eradicate PPR (FAO, 2015b). The conditions to achieve this global control and eradication by 2030 is to adequately distribute and coordinate resources at all levels. Furthermore it is important that this strategy is supported by all cooperating partners (*The Global Strategy for the Control and Eradication of PPR*, 2015).

2.1.11 Vaccines

Live attenuated homologous non thermo-stable PPR vaccines have been available on the market for some time. They contain a range of PPRV isolates, for example Nigeria 75/1 and Shungri/96, which have been attenuated through serial passages in Vero cells (Saravanan *et al.*, 2010). The vaccines have been proven to be highly effective and are suggested to provide lifelong immunity in both sheep and goats. These successful vaccines are used in many PPRV endemic countries (Munir *et al.*, 2012). Though there are at this point no working PPRV vaccine making it possible to serologically distinguish vaccinated animals from infected ones, a so-called DIVA vaccine (Munir, 2015).

2.2 Tanzania

Tanzania is a country in East Africa with 26 different regions and a population of almost 51 million people. On an area of 945 203 km^2 (*Landguiden*, 2015; FAO, 2015a), the country is famous for its rich nature and diverse wildlife. While tourism is calculated to generate 22.9% of the annual income, agriculture is the most important sector and is estimated to be 26% of the gross domestic products. Morogoro, which is the name of the city as well as the region, is also

the centre for agricultural sciences in Tanzania (*Landguiden*, 2015). Most of research around PPR in Tanzania is performed at the Sokoine University of Agriculture (SUA) in Morogoro.

2.2.1 Peste des petits ruminants in Tanzania

PPR has been found in several regions of Tanzania over the last decade (Muse *et al.*, 2012; Swai *et al.*, 2009). The first outbreak of PPR in Tanzania was confirmed in the northern region in 2008. It was supposedly introduced from neighboring countries in the north (Swai *et al.*, 2009). A study made in the southern part of Tanzania on the occurrence of PPR infection in sheep and goat confirmed the spread of PPR since 2009 and showed a seroprevalence of 31% in tested animals (Muse *et al.*, 2012).

There are suspicions that PPR outbreaks occurred in Tanzania long before 2008. A study based on reports from livestock field officers and District Veterinary Officer suggest that PPR was present in northern Tanzania at least four years before the official confirmation (Karimuribo, 2011). PPRV was yet again detected in the south of Tanzania in 2010 (Muse et al., 2012). Samples collected in 2012-2013 from the northern and eastern parts of Tanzania also confirmed active PPRV-infections (Kgotlele *et al.*, 2014). This indicates that there is an on-going spread of the disease. There is since 2009 evidence of natural transmission of PPR and circulation of the virus within domestic herds (Swai *et al.*, 2009). There has been an apparent risk that PPR may continue to spread southward across and beyond the Tanzanian border (Muse *et al.*, 2012). The reality of this risk is emphasized as Karimuribo *et. al.*, point out how the governmental apparatus in Tanzania is somewhat limited in surveillance, reporting and control of transboundary diseases (Karimuribo, 2011). Today, we know that PPR is continuing to spread across the Tanzanian border and that subclinical infection with PPRV has been discovered in Zambia (OIE, 2015b).

2.2.2 Prevention and vaccination

Tanzania has been the southern border of PPR and Chazya *et al.* concluded in 2014 that the overall risk of PPRV spreading to northern Zambia could be considered as high (Chazya *et al.*, 2014; Muse *et al.*, 2012). As a result of this risk, an emergency vaccination program were launched and implemented in the northern half of Tanzania in 2010 (Munir *et al.*, 2012). In September of 2011, a vaccination campaign with a focus on small ruminants along livestock marketing routes was implemented (OIE, 2012). Furthermore, due to mandatory vaccination campaigns most herds in the area around Mikumi National park (central and eastern part of Tanzania) were vaccinated in late spring of 2013 (Herbe, 2015). Despite the ambition of the vaccination program, the effect varied. For example, coverage of the vaccinations was only 32% in northern parts of Tanzania. This suggests continued prevalence of the disease in certain areas (Karimuribo, 2011). In May 2015 antibodies against PPRV was found in Zambia on four separate occasions (OIE, 2015b).

2.3 Filter paper as sample transport medium

In many developing countries, the diagnosis and genotyping of PPRV may be hampered by inadequate infrastructures and by lack of proper laboratory facilities. Samples with potential fragile virions risk being spoiled during transport with an insufficient cold chain from the field to laboratories (Bhuiyan *et al.*, 2014). This suggests that a sample medium that sustains the PPR virion or its corresponding antibodies without the need for a cold chain thereby improves the method of detecting PPRV infections in areas with insufficient infrastructures. Filter paper samples may be a potential solution to this issue (Punnarugsa & Mungmee, 1991).

2.3.1 Nobuto filter paper strips

Several studies have been made where Nobuto filter paper (FP) has been used as a transport medium with both serum and whole blood for detection of an agent or antibodies from infection. Already in 1974 a study by Wolff and Hudson investigated the value of Nobutos' FP in serological studies. Here the method showed difficulties of detecting samples with low titers. In spite of this, the advantages of FP was considered and said to counterbalance this issue. Some of the advantages was said to be the simple method of sample collection and handling, minimal personnel training, and the low amount of blood required (Wolff & Hudson, 1974).

In 2011 Curry *et al*., showed how detection of *Brucella* spp. in caribou through c-ELISA could be used to compare blood-saturated Nobuto FP strips to serum samples. To evaluate FP performance, serum sample results were used as gold standard. The result showed a difference in prevalence from *Brucella* spp. with FP at a sensitivity of 89% (95% CI: 82-95%) and specificity of 99% (95% CI: 97-100%) compared to the serum sample gold standard. This trial also showed how dried FP blood samples from caribou stored for two months in room temperature were comparable with serum for use in *Brucella* spp. c-ELISA (Curry *et al.*, 2011). The Nobuto FP was furthermore evaluated by Curry *et al*., in a study published 2014 where whole blood eluded FP was compared to serum for detecting antibodies from a number of pathogens in reindeer and caribou. Two of these pathogens, *Neospora caninum* and West Nile virus (WNV), were analyzed through c-ELISA. The FP analysis resulted in a specificity of 92% (95% CI: 61.5-99.8) for both pathogens and a sensitivity of 98% (95% CI: 91.3-100.0) for *Neospora caninum* and 95% (95% CI: 80.7-100.0) for WNV. This trial also showed that in case of insufficient sensitivity from FP samples, lowering the threshold to raise sensitivity could compensate this issue without thereby lowering the specificity notably (Curry *et al.*, 2014).

The Nobuto FP was also evaluated 2011 in a trial by Dusek *et al*., investigating the detection of avian influenza antibodies in mallards (*Anas platyrhynchos*). The mallards were experimentally infected with low pathogenic avian influenza viruses and later on bled with subsequent serological analysis of both serum and FP. FP samples proved to reliably detect positive and negative samples, which prompted the verdict: “Nobuto strips are a convenient and sensitive alternative to the collection of serum samples when maintaining appropriate storage temperatures is difficult” (Dusek *et al*., 2011).

2.3.2 Sartorius 3MM filter paper sheets

It has been shown in previous studies that Whatman® 3MM FP (GE Healthcare, France) preserves nucleic acid sufficiently for subsequent diagnostic analysis. The nucleic acid of PPRV has been shown to be preserved in FP for at least 3 months at 32°C (Bhuiyan *et al.*, 2014).

The 3MM FP is a chromatography paper made of 100% cotton linters with an alpha-cellulose content of more than 98%. This FP from Sartorius is delivered in sheets ready to be cut in suitable shapes and sizes. The use of this type of paper are not only limited to chromatography but can also be used for a wide range of absorption applications (Sartorius).

In a trial from 2014 by Randriamparany *et al.*, the Sartorius 3MM FP was used to detect antibodies against African Swine Fever (ASF). Blood samples on FPs and serum samples were drawn from experimentally infected pigs, farm pigs in Madagascar and from Côte d´Ivoire. The study showed that the Sartorius FP was successful in preserving antibodies against African Swine Fever virus (ASFV) from blood-dried samples through ELISA analysis (Randriamparany *et al.*, 2014). The sensitivity was very close to conventional analysis on serum samples and can therefore be considered as being an alternative to serum in this type of study (Randriamparany *et al.*, 2014).

Several studies have demonstrated that the use of Whatman FP and other types of 3MM FP as an alternative transport medium for blood samples with the purpose of detecting virus infections (Punnarugsa & Mungmee, 1991).

3 Material and methods

3.1 Study area and study design

The data collection was performed in three different areas in the Morogoro region. Two of these areas were in the Kilombero district and the third area was situated in the Ulanga district. Areas were chosen together with assistant supervisor Emeli Torsson, local supervisor Dr. Gerald Misinzo and local extension officers. Data collections took place during two field trips in June and July 2015 and were located to two districts in the Morogoro region. During the first field trip samples were collected in the Mikumi region, while during the second trip in the regions of Ulanga and Kilombero.

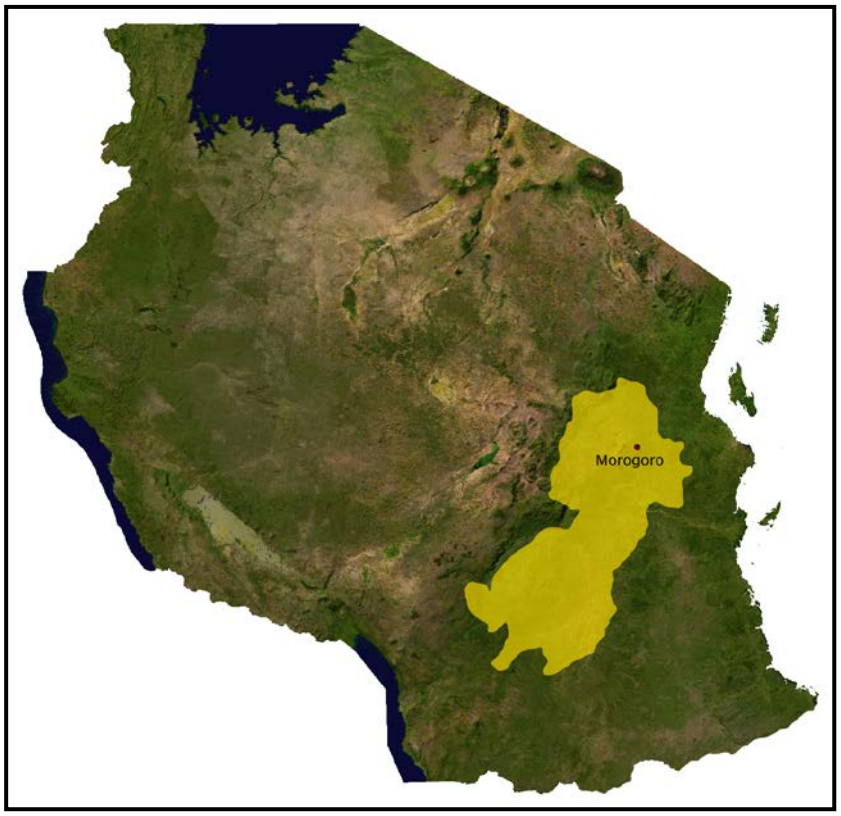

Figure 3. Tanzania, Morogoro district marked in Yellow. (Nils Roos)

In these three areas, samples were obtained from goats and sheep belonging to both Maasai pastoralists and traditional farmers. Blood samples, nasal swabs, rectal temperature, observation of clinical signs and in some cases blood saturated FPs were collected from each animal. FP samples were collected only when allowed due to time, staff and workload issues. Goats and sheep of both sexes were sampled and the selection was focused to animals older than three months since younger animals could still hold maternal antibodies, and animals younger than one year were chosen to ensure correct vaccination status according to the owner. If possible, animals with clinical signs in concordance with PPR were selected. In total, 468 animals from 46 herds in 15 different villages were sampled. All areas had various amounts of interaction between wild grazing animals and domestic small ruminants. The farmers practiced communal grazing systems for their sheep and goats. In some areas, the animals more or less grazed side

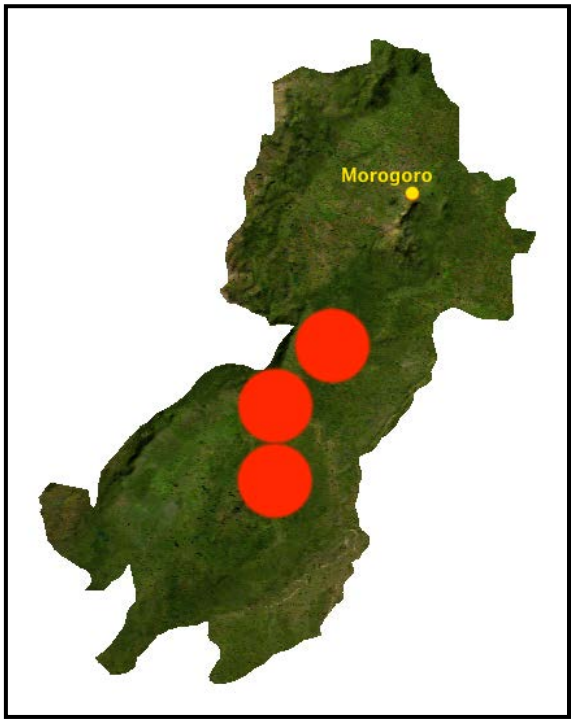

Figure 4. Morogoro district of Tanzania, sample areas in red. (Nils Roos)

by side with the wild population of ruminants. In some areas the domesticated animals came in contact with wild ruminants only during dry season.

3.1.1 Questionnaire

All herd owners were interviewed before sample collection, according to a premade questionnaire. The interview was performed with a translator, usually the assistant extension officer. The questionnaire was designed to focus on health, vaccination and deworming status, flock size, contact with other animals, sex and approximate age of each animal. There was also a section about the last presumed PPR outbreak, and what the detailed effects on the flock were in terms of affected animals, deaths and abortions (see table below). The questionnaire contained the following questions:

- How often do animals come in contact with other domestic herds?

- How often do animals come in contact with wildlife?
- Latest introduction of new animals to herd
- Last vaccination of herd against PPR…..… CCPP……. FMD…….
- Last de-worming treatment of herd…….. all animals treated?
- Last antibiotic treatment of herd……. all animals treated?
- Estimated date when first PPR case was observed at this farm
- Detail of animals
- Type of farming: [ ] Household, [ ] dairy production, [ ] meat production , [ ] individual seller at live animal market, [ ] others (please specify)
- Clinical signs at outbreak: [ ] Abortion, [ ] Diarrhea, [ ] Pneumonia, [ ] Oral mucosal lesions, [ ] Nasal and ocular discharges, [ ] High temperature
- Other signs or comments

Table 1. The *"details of animals"* part of the questionnaire given to the farmers

Age group	<1 year				>1 year		
	Total	Affected	Died	Aborted	Total	Affected	Died
Sheep							
Goat							

3.2 Data collection

Animals were bled from the jugular vein into serum tubes using a vacutainer system. Occasionally, when animals with signs of infection in concordance with PPR were encountered, EDTA blood tubes were also used. All tubes were placed in an upright position and in a cooler box before transported back to the laboratory. Later, all tubes were centrifuged to allow serum 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. Samples were transported to the laboratory at SUA, Morogoro for analysis. All samples were labeled accordingly so each animal and flock could be identified. Up to three different nasal swabs were taken of the sampled animals:

- A damp cotton-tipped swab to apply the content on FTA-cards for later PCR analysis.
- Copan Innovation's Advanced flocked swab was used on each animal for storage and later purification of RNA in the local laboratory.
- A nasal e-swab was occasionally used, mainly on individuals with clinical signs corresponding to PPR. These swabs were stored for later purification of DNA and transported to Brazil for analysis for presence of *Mycoplasma*.

Age, sex and breed of each animal were recorded. To determine the sample size in the seroprevalence study at least 270 samples from small ruminants would need to be included. This sample size would be enough to estimate the prevalence with a precision of 5% and a confidence interval of 95%.

3.3 Laboratory analysis

Serum samples were analyzed with a competitive enzyme-linked immunosorbent assay (cELISA) from ID.Vet Innovative Diagnostics, identifying levels of antibodies directed against the nucleoprotein of PPRV. The cELISA has a high diagnostic specificity (99.4%) and sensitivity (94.5%) for detection of PPRV antibodies in serum (Libeau *et al.*, 1995). All analyses were made from serum that had been decanted from serum tubes into cryotubes, frozen to -45°C and later thawed for analysis.

3.3.1 PPRV competitive ELISA

The microplates in the cELISA-kit had 96-wells coated with purified recombinant PPR nucleoprotein. To each plate, 92 serum samples were added together with two positive and two negative controls. With an added sample, existing antibodies in the sample formed a complex with the antigen thus masking the nucleoprotein epitopes. A peroxidase-conjugated anti-nucleoprotein antibody was then added to the wells, forming a complex with the remaining nucleoproteins. Subsequent washing and adding of the substrate solution caused a coloration to appear depending on the amount of antibodies in the original sample. The magnitude of the color yellow in each well indicated the correlating absence of antibodies in the original sample. No yellow coloration indicated the presence of antibodies in the original sample. Immediately after adding of the substrate solution the 96-well microplates were analyzed at 450 nm in the microplate reader. The result of this analysis was presented in an optical density of each well.

The validation of each microplate was performed through calculating the mean optical density of the negative and positive controls. If the mean value of the negative controls were greater than 0.7 and the mean value of the positive controls less than 30% of the optical density of the negative control, the analyses were valid. Each optical density from the 92 remaining wells was subsequently compared to the mean optical density of the negative controls with this mathematical formula:

S/N% = (Sample optical density / mean negative control optical density) x100

Samples with S/N% values less than or equal to 50% were considered positive (P) and samples with S/N% values greater than 60% were considered negative (N). Samples in between 50% and 60% were considered doubtful (D).

Filter paper use in blood sampling

Two kinds of FP were used in this study. The first type, Advantec Nobutos' FP strips is a precut blood sampling paper developed for serological reactions. The Sartorius FP was delivered in sheets of a thinner kind of paper that could be cut to fit the needs of the situation. The sheeted FP was cut to a similar size as the Nobuto strips, circa 10x50mm. In that way both types of FP could be placed in the “Nobuto filter strip drying rack” designed for Nobuto strips.

In the field, FP were used in parallel to vacutainers in order to collect blood samples from goats and sheep. Blood was absorbed to the filter strips through dipping the FP in blood-filled serum vacutainer tubes enough to saturate the narrow area of the Nobuto filter strips. To the second

type of FP, blood was absorbed to an area larger or equal to the narrow area of the Nobuto filter strips.

Both types of FP were placed in the drying rack. The filter papers were placed with enough distance to avoid for them to stick to each other. Blood-absorbed FPs were put in room temperature away from direct sunlight for at least 24 hours to dry. Dried FPs were stored in boxes, kept away from liquids and moist.

Figure 5. Filter paper, an alternative way to collect blood samples in field conditions. Photo Nils Roos

Filter paper use in laboratory analysis

Dried FP were cut with cleaned scissors into 75mm^2 pieces, which corresponds to half of the narrow area of the Nobuto filter strips. Each 75mm^2 piece was then cut into 5-8 smaller pieces and placed into a 1.5ml eppendorf tube. Each eppendorf tube was finger-flicked to make sure the pieces of FP rested in the bottom of the eppendorf tube.

The eppendorf tubes were marked with its corresponding sample ID number. Each tube was thereafter filled with 150µl Dilution Buffer 13 from IDvets cELISA PPR-kit. Filter papers were allowed to elude in room temperature for 60 minutes according to the Nobuto FP manufacturer's instructions. At the start and end of the incubation period each tube was vortexed for a few seconds to ensure that all pieces of FP came in contact with the solution and that the solution was homogenized. A volume of 50µl eluate was transferred to its corresponding test well in the

ELISA plate. The eluate was at this point diluted to approximately 2:15. This dilution was used as the only element in the ELISA wells prior to incubation.

The cELISA was otherwise performed according to manufacturers instruction (see 3.3 Laboratory analysis), resulting in each sample ID being analyzed up to three times (serum sample, Nobuto filter paper (FP) sample and Sartorius FP sample).

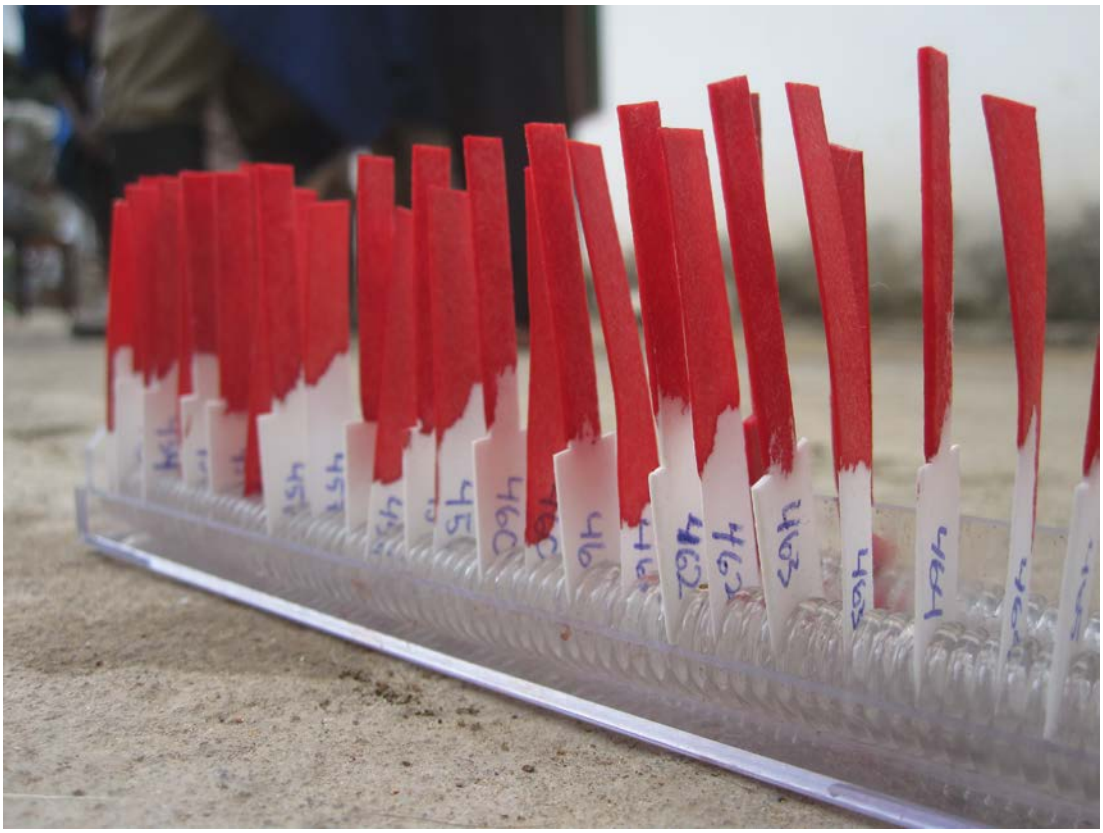

Figure 6. Filter paper drying and storage in the field. Photo Nils Roos

3.4 Calculating results, report writing and statistical analysis

Data collection and results from ELISA analyses were obtained on site in Tanzania. Back in Sweden the results were analyzed and evaluated to assess the critical points relevant to this project.

3.4.1 Statistical analysis

Results from the laboratory analysis were evaluated with different statistical methods depending on the data being analyzed. Statistical calculations were partly performed in the statistical software “Minitab” and partly performed by staff from the Unit of Applied Statistics and Mathematics, SLU. For the evaluation of PPR seroprevalence, a 95% confidence interval (CI) was

applied to the results. This resulted in a CI in which we could be 95% certain in the presence of antibodies from PPR infection in the sampled population.

The evaluation of FP through ELISA as a diagnostic technique was analyzed with the statistical methods “Cohen’s Kappa” and “Weighted Cohens Kappa” (Cohen, 1968). These methods evaluate the level of agreement between results from serum and FP analysis for each sample.

4 Results

4.1 Serological prevalence of PPRV

Table 2. *Frequency of PPRV seroprevalence in sheep and goats based on serum analysis with optical density from cELISA. The cutoffs used were 50% for positive, 60% for negative and doubtful for those in between.*

Species	Positive	Doubtful	Negative
Sheep (n=158)	8.2% (n=13)	6.3% (n=10)	85.4% (n=135)
Goat (n=323)	10.8% (n=35)	1.2% (n=4)	87.4% (n=284)
Total (n=481)	10.0% (n=48)	2.9% (n=14)	87.1% (n=419)

The table above shows the overall seroprevalence of PPRV in the sampled population. With a prevalence of 8.2% in sheep and 10.8% in goats, the total mean prevalence for both species comes down to 10.0%. A confidence interval (CI) of 95% for the total prevalence gives the span of 7.4% to 13%. This, though, cannot be considered as the true seroprevalence since vaccinated animals represent some of the positive results. Since there is no DIVA vaccine available and it thereby is impossible to distinguish a previously infected animal from a vaccinated one, this has to be taken into account. By re-doing the calculation and disregarding all vaccinated animals and animals older and younger than 3 to 12 months, we hoped to reach a seroprevalence closer to the truth.

Table 3. *Frequency of positive, doubtful and negative samples from non-vaccinated goats and sheep of ages 3-12 months based on cELISA serum analysis with optical density cutoffs at 50% for positive, 60% for, negative and doubtful in-between.*

Species	Positive	Doubtful	Negative
Sheep (n=93)	4.3% (n=4)	6.5% (n=6)	89.2% (n=83)
Goat (n=183)	2.7% (n=5)	1.1% (n=2)	95.6% (n=175)
Total (n=276)	3.3% (n=9)	2.9% (n=8)	93.5% (n=258)

From the table above we can see that disregarding animals with a positive or unknown vaccination status and at the same time only calculating the seroprevalence in animals 3-12 months of age gives a total seroprevalence of 3.3% (n=9; CI 3.2-6.1%).

To give insight into the possible aspect if the disease could spread to and/or from wildlife, one of the questions in the questionnaire given to the farmers regarded contact with wildlife. On the question “How often do animals come in contact with wildlife?” some farmers answered variations of “Every day” or “Yes” (n=16). While some answered variations of “No” (n=23), others did not answer at all (n=7). The absence of answers to this question might be lost in translation or be because the farmers themselves are uncertain on the herds contact with wildlife.

Table 4. *Frequency of positive, doubtful and negative samples from non-vaccinated animals of ages 3-12 months with differentiation between animals with and without wildlife contact, based on cELISA serum analysis with optical density cutoffs at 50% for positive, 60% for, negative and doubtful in-between.*

Wildlife contact	Positive	Doubtful	Negative
Yes (n=95)	1.1% (n=1)	2.1% (n=2)	95.8% (n=91)
No (n=181)	4.4% (n=8)	3.3% (n=6)	92.3% (n=167)

The seroprevalence in animals with wildlife contact, and stated criteria above, according to the questionnaire reached 1.1% (n=1; CI 0.03-5.73%). The seroprevalence in animals without wildlife contact, and stated criteria above, reached 4.4% (n=8; CI 1.9-8.5%).

4.2 Results from filter paper analysis

To measure the performance of the two types of filter paper, optical density (OD) results from each FP sample was compared to the result from its corresponding serum sample. Figure 7 illustrates to what extent the OD from positive FP samples follow the OD from corresponding serum samples. The specificity and sensitivity of the FP samples was also investigated through comparing them to their serum sample counterparts whilst changing the cutoff value for the positive, doubtful and negative labels.

4.2.1 Filter paper absorbance level depending on whole blood or serum sample

Positive samples on FP were compared between whole blood and serum in both types of filter paper. All positive FP samples were used in the comparison, a total of 21 samples.

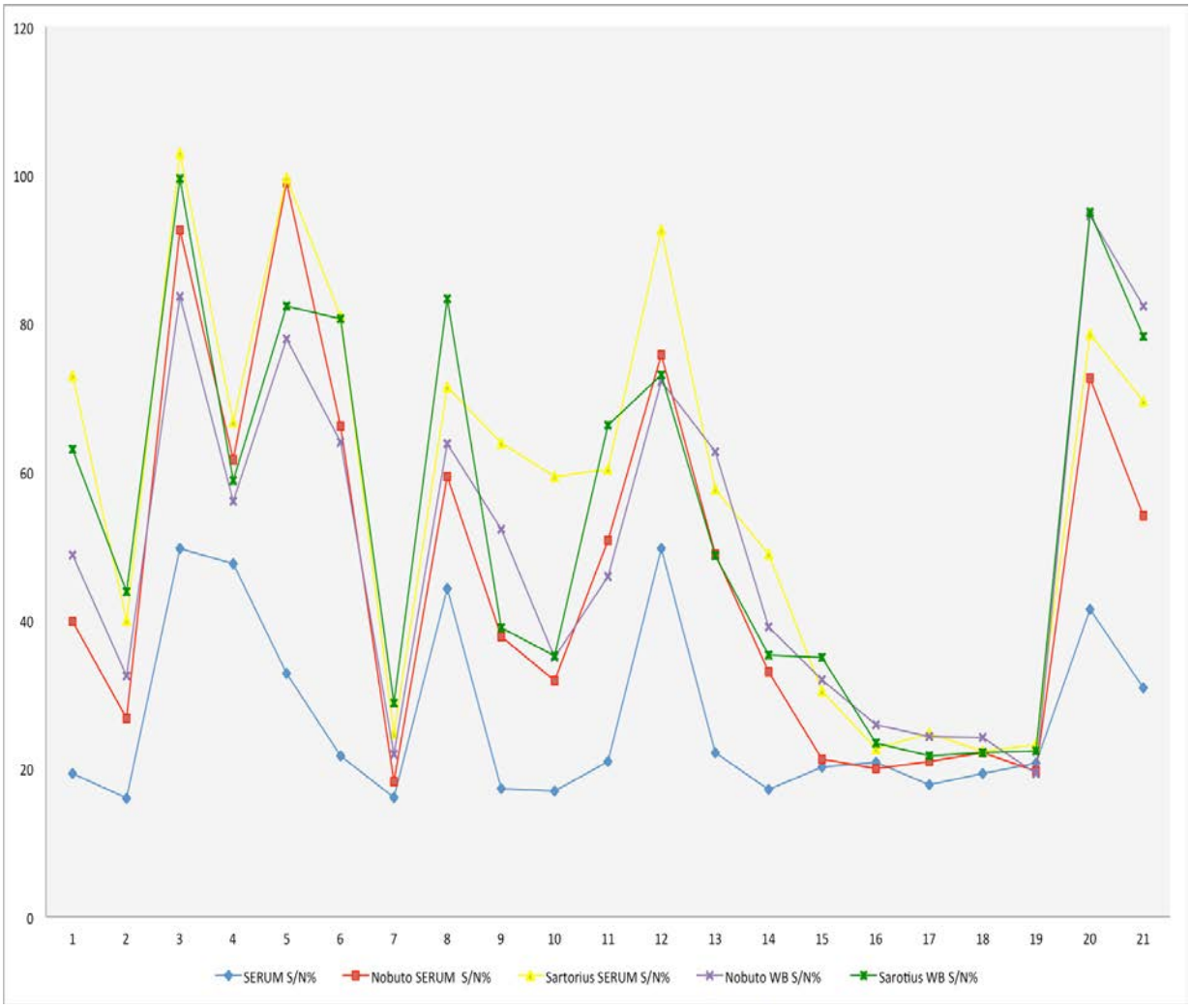

Figure 7. Serum samples positive for PPR compared to correlating filter paper samples: Nobuto and Sartorius filter paper eluate from either serum or whole blood (WB). Y-axis: OD percentage, X-axis: Sample ID.

The graph (Figure 7) shows the disparity between the optical densities from the serum sample relative to the FP equivalents. A number of aspects in this graph is worth noting: Firstly, as the optical density of the serum sample increases to 30-50%, the values of the FP differ even more compared to the samples where the optical density is lower, at 20%. Note the cluster at sample ID 7, 16, 17, 18 & 19 compared to the scattered values at sample ID 1, 3, 4, 5, 8, 12, 20 & 21. Secondly, another aspect worth noting is the difference in optical density between the Nobuto and Sartorius filter paper. In close to every sample the Nobuto FP samples are following the serum samples to greater extent compared to the Sartorius filter paper. Thus the crucial aspect in mimicking the result of the serum sample results might firstly be to use a FP of high quality rather than using serum instead of whole blood as the eluding agent. Moreover, this graph only shows the positive samples and accordingly we cannot yet say anything about the specificity of the different filter papers.

4.2.2 Nobuto filter paper results

Results from Nobuto FP OD compared to correlating serum sample OD. Sensitivity and specificity is monitored while cutoff values for FP OD is raised and thus changing the classification of some FP samples.

Table 5. *Correlation between results from serum sample analysis depending on different OD cutoff values regarding FP samples.*

Serum at	**Nobuto filter paper at 50/60% cutoff**			
50/60% cutoff	Positive	Doubtful	Negative	Total %
Positive (21)	11	2	8	10.5
Doubtful (0)	0	0	0	0.0
Negative (179)	0	1	178	89.5
Total %	5.5	1.5	93.0	100
		60/70% cutoff		
Positive (21)	13	3	5	10.5
Doubtful (0)	0	0	0	0.0
Negative (179)	1	0	178	89.5
Total %	7.0	1.5	91.5	100
		70/80% cutoff		
Positive (21)	16	2	3	10.5
Doubtful (0)	0	0	0	0.0
Negative (179)	1	1	177	89.5
Total %	8.5	1.5	90.0	100
		80/90% cutoff		
Positive (21)	18	2	1	10.5
Doubtful (0)	0	0	0	0.0
Negative (179)	2	9	168	89.5
Total %	10.0	5.5	84.5	100

The purpose of Table 5 is to show the number of true results from FP samples in relation to the serum sample result depending on the change of cutoff value for the different classifications positive, doubtful and negative. The table above shows that by raising the OD cutoff value for “positive” and “doubtful” from 50 and 60 respectively to 60 and 70%, the number of true positive results compared to the serum results increases from 11 to 13. The number of doubtful FP results, however, increased from 2 to 3. The number of false negative results from the FP decreased from 8 to 5, at the same time the number of false positive increased from 0 to 1. In this fashion the FP sample classification change in relation to serum samples as the cutoff value is successively raised.

4.2.3 Sartorius filter paper results

Results from Sartorius FP OD were compared to correlating serum sample OD. Sensitivity and specificity was monitored while cutoff values for FP OD was raised and thus changing the sample classification as positive, doubtful and negative for some FP samples.

Table 6. *Correlation between results from serum sample analysis depending on different OD cutoff values regarding FP samples*

Serum at	**Sartorius filter paper at 50/60% cutoff**			
50/60% cutoff	Positive	Doubtful	Negative	Total %
Positive (21)	11	1	9	10.6
Doubtful (1)	0	0	1	0.5
Negative (176)	0	1	175	88.9
Total %	5.6	1.0	93.4	100
		60/70% cutoff		
Positive (21)	12	2	7	10.6
Doubtful (1)	0	0	1	0.5
Negative (176)	1	0	175	88.9
Total %	6.6	1.0	92.4	100.0
		70/80% cutoff		
Positive (21)	14	2	5	10.6
Doubtful (1)	0	0	1	0.5
Negative (176)	1	0	175	88.9
Total %	7.6	1.0	91.4	100
		80/90% cutoff		
Positive (21)	16	3	2	10.6
Doubtful (1)	0	1	0	0.5
Negative (176)	1	7	168	88.9
Total %	8.6	5.6	85.9	100

The purpose of Table 6 is to show the number of true results from FP samples in relation to the serum sample result depending on the change of cutoff value for the different classification of positive, doubtful and negative. FP classifications change in relation to serum samples as the cutoff value is successively raised.

4.2.4 Evaluating cutoff values for Nobuto and Sartorius filter paper

To determine which cutoff value that is most suitable for each FP statistical calculations was made based on the disagreement between the serum results and the different filter papers. Two types of statistical methods were used: Cohen's kappa coefficient, calculating the disagreement between FP and serum result; and the similar method Weighted Cohen's kappa coefficient, with the addition of taking the level of disagreement into account. A 95% level confidence interval was included for both methods.

Table 7. Illustrating the correlation between serum sample results and different cutoff values for Nobuto FP and Sartorius FP with Cohen's kappa coefficient and Weighted Cohen's kappa coefficient with 95% confidence interval for both methods.

Cutoff value	**Cohen's kappa**	**95% CI**	**Weighted kappa**	**95% CI**
Nobuto 50/60%	0.66	0.49 – 0.83	0.71	0.54 – 0.88
Nobuto 60/70%	0.74	0.59 – 0.89	0.79	0.65 – 0.94
Nobuto 70/80%	0.81	0.68 – 0.94	0.86	0.75 – 0.98
Nobuto 80/90%	0.70	0.56 – 0.84	0.85	0.75 – 0.95
Sartorius 50/60%	0.63	0.45 – 0.81	0.68	0.49 – 0.86
Sartorius 60/70%	0.68	0.51 – 0.84	0.72	0.56 – 0.89
Sartorius 70/80%	0.75	0.60 – 0.90	0.80	0.66 – 0.94
Sartorius 80/90%	0.71	0.57 – 0.85	0.85	0.74 – 0.95

The table above shows how the highest level of agreement between serum sample results and Nobuto FP is reached with a cutoff value of 70/80% with both Cohen's kappa and Weighted Cohen's kappa (coefficient value of 0.81 vs. 0.86). At this cutoff the confidence interval spans from 0.68-0.94 for Cohen's kappa and from 0.75-0.98 for Weighted Cohen's kappa.

Simultaneously, the highest level of agreement between serum sample results and Sartorius FP is reached with a cutoff value of 70/80% using Cohen's kappa at a value of 0.75 (CI 0.60-0.90) and 80/90% using Weighted Cohen's kappa at a value of 0.85 (CI 0.74-0.95).

To assess the significance of mentioned values above, an internationally recognized scale of measurement is used. The scale is rated as follows: 0-0.2 rated as "Poor", 0.2-0.4 rated as "Fair", 0.4-0.6 rated as "Moderate", 0.6-0.8 rated as "Substantial", and 0.8-1 rated as "Good". Consequently, results from both Nobuto and Sartorius FP can be considered as "Good" at 70/80% and 80/90% cutoff respectively, using the method of Weighted Cohen's kappa coefficient. Using the confidence interval both FP methods ranges from being "Substantial" to "Good".

5 Discussion

Total PPRV seroprevalence in present study, measured for both sheep and goat of ages 3-12 months, results in a 3.3% (n=9; CI 3.2-6.1%) positivity for antibodies against PPRV in all three selected areas. This result is representative for animals between three months and one year of age. A seroprevalence of 3.3% is relatively low compared to earlier studies. Swai *et al*., showed a prevalence of 31% in southern Tanzania in 2009 (Swai *et al.,* 2009). Furthermore, Herbe showed in 2015 a total seroprevalence of 16% in central and northern Tanzania, in animals older than 3 months. In the Mikumi area, the seroprevalence was as high as 21% (Herbe, 2015). Since Herbes result was obtained in central Tanzania it is geographically comparable to the present study. Hence, this could implicate a drop in seroprevalence of PPRV in central Tanzania under the time period between late 2014 and mid 2015.

Though there are a number of additional recent epidemiological studies of PPRV in Tanzania, these studies focus on confirming ongoing spread of PPRV rather than its seroepidemiology (Munir, 2015; Kgotlele *et al.*, 2014). It is therefore problematic to compare the results of present study to other epidemiological studies of PPR in Tanzania regarding the quantitative extent of the disease.

The FP analysis shows that both Nobuto FP and Sartorius FP can be a viable alternative to serum samples in seroepidemiological screenings. It also shows good and substantial agreement between FP results, for both types of filter paper, and serum sample results when the cutoff value is raised. Though the present study concerns the previously not evaluated combination of PPRV as agent and FP as transport medium, there are a number of studies with a similar approach to the FP evaluation. Several studies establish that dried 3MM FP preserves antibodies, in serum or whole blood for later analysis, against a number of pathogens including Rubella virus and African swine fever virus (Randriamparany *et al*., 2014; Punnarugsa & Mungmee, 1991). These results are in agreement with the 3MM analysis in the present study. Moreover, the present study correlates with earlier research in establishing that Nobuto FP preserves antibodies in dried samples for later analysis. This is the case for analysis of antibodies against pathogens like *Brucella* spp., avian influenza, *Neospora caninum* and West Nile virus (Curry *et al*., 2014; Curry *et al.*, 2011; Dusek *et al.*, 2011). In concordance to the present study, Curry *et al*., also showed that adjusting the threshold of FP samples can lead to results comparable to serum samples (Curry *et al.*, 2014).

It can be argued that the chosen study areas from where samples were taken could be subject to biases in the way the areas were selected. Under optimal conditions, the areas would have been randomly selected and scattered equally over the targeted area. Attempts to randomly select these study areas were made but unfortunately discarded on site due to technical issues regarding randomization software. Instead areas were chosen together with our local supervisor with help from the extension officers assisting us in the field. While choosing areas in an un-randomized fashion, the risk of bias was considered at all times and the following data collection and subsequent analysis was performed with this risk in mind.

Seroprevalence of PPR antibodies in animals of age 3-12 months were lower in the group that according to farmers were in contact with wildlife compared to the group that did not have contact with wildlife. Though this result is not statistically significant, it is indeed interesting. The result might support a hypothesis that by having contact with wildlife, a sheep or goat suffer less risk of being infected with PPRV. This is not probable. Instead the lower seroprevalence in sheep and goat with wildlife contact is likely due to other factors, one of which is the different styles of livestock keeping.

In the section where FP absorbance level was evaluated (4.2.1) the importance of how raising the cutoff value includes false negative samples was illustrated in Figure 3. This figure demonstrates how FP results scatter in relation to the level of optical density. Low OD results in an aggregated cluster of results from both whole blood and serum in both types of filter paper, while high OD results in a much more scattered cluster. The figure also indicates a small but important difference in the OD results from FP saturated in whole blood compared to FP saturated in serum. The FP saturated in serum is consistently closer to the original serum sample OD result than corresponding whole blood sample. Even if the statistical confidence is missing in this observation, I believe that this difference between whole blood saturation and serum saturation must be taken in to account. Not doing so would make the FP results under-appreciated in relation to the serum sample results.

The use of Cohen's kappa and Weighted Cohen's kappa showed to what extent the results from the two different kinds of FP agreed with the serum sample results. A number of points regarding this analysis and its results can be discussed. The two coefficients implied that both filter papers reached the highest level of agreement to the serum sample results. According to the confidence intervals though, both the Nobuto and Sartorius FP result overlap the "good" to "substantial" classifications on the internationally recognized scale of measurement. This means that both FP might agree with the serum sample results only to the second highest classification. Ideally, the CI would have been a span narrow enough for the kappa result not to overlap between the "good" (0.6-0.8) and "substantial" (0.8-1) classification threshold. There are two ways to make the CI span more narrow: First, to lower the CI from 95% to 90%. This would result in a narrower confidence interval but at the cost of the assurance of it by 5%, making the result less accurate on the other end. Second, increasing the number of observations. This would result in a smaller confidence interval without being at a cost of anything else. This would be the ideal solution if it had been practically possible. Unfortunately, increasing the number of observations probably would have resulted in field related issues or budget and time limitations. With the current setup, an increased number of FP samples would most likely have resulted in a lower number of serum samples in this study. In hindsight it is difficult to say which of the two would have been better or worse for the result.

The difference between Cohen's kappa and Weighted Cohen's kappa should not be left untouched. As mentioned earlier, these methods evaluate the agreement between two different methods in the categorizing of data (Cohen, 1968). These two methods are variants of same principles but differ in how different levels of disagreement are valued. Cohen's kappa takes

agreements versus disagreements into account in a black and white fashion while Weighted Cohen's kappa values a disagreement different depending on its severity. In this case, a negative/positive situation is valued higher than a doubtful/negative or doubtful/positive situation. With this in mind, the Weighted Cohen's kappa is the most suited method of the two of them in this evaluation. With that being said, this study is unfortunately not free from the burden of inaccuracy. Therefore both of these statistical methods are used in parallel to complement the statistical evaluation.

Throughout the analysis of FP and serum samples I have at all times used the serum sample result as a gold standard. I did this since the cELISA on serum was the most accurate method at hand. Nevertheless, there could have been issues with this method that compromises some of these results. The reason to this FP evaluation is partly to enlighten the issues of keeping a continuous cool chain from when the sample is taken to analysis in parts of the world where infrastructure and new technology are faltering or missing. Now, it would be easy to assume that my team and me were exposed to these kinds of issues. Furthermore, could these issues have compromised the samples? And, in this case, how would this affect the samples in relation to the seroprevalence? A lower seroprevalence from serum sample results would give, not only a falsely low seroprevalence, but also giving the illusion of FP results being closer to the perceived true seroprevalence, hence giving them a false high result.

5.1 Conclusion

This study showed a PPRV antibody seroprevalence of 3.3% in sheep and goats of age 3-12 months in three areas adjacent to Selous Game Reserve in the region of Morogoro, Tanzania.

This study also showed that FP could be a viable alternative to serum samples and successive serology in areas where keeping a sustained cold chain is problematic. Furthermore, arguments' showing how the interpretation of FP results could refine and supplement the identification of samples containing antibodies against PPRV was presented.

It is of great interest to further evaluate the use of FP as an alternative to serum samples in PPR serology to assist the study of PPR in remote areas. A supplementary study with additional observations is suggested to be able to make an accurate suggestion to a change in cutoff value for a certain filter paper.

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