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Seroprevalence of CCPP, FMDV, BTV and BVDV among small ruminants in Tanzania

A minor field study with focus on differential diagnoses of peste des petits ruminants virus



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Seroprevalens av CCPP, FMD, BTV och BVDV hos små idisslare i Tanzania

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SUMMARY

This degree project has been performed as a Minor Field Study (MFS) in Tanzania, investigating the seroprevalence in goats and sheep of three diseases causing similar signs as peste des petits ruminants virus (PPRV): foot and mouth disease (FMD), bluetongue (BT) and contagious caprine pleuropneumonia (CCPP), as well as bovine viral diarrhoea (BVD) and border disease (BDV) which is believed to affect an outbreak of PPR due to immunosuppression. Sheep and goats play a key role in national food and nutritional security, income security and livelihood resilience in the least economically developed countries across the world and because of the complicated epidemiology and socio-economic impact of these diseases it is of great interest to further evaluate the prevalence of PPRV and its differential diagnosis.

In this study blood samples were collected from 483 goats and sheep. Samples from a previous MFS done in 2014 were also analysed. The sampled animals came from three different regions in south-eastern Tanzania and the seroprevalence of FMD, BT, CCPP and BVDV was investigated by tracing antibodies in serum by competitive ELISA. The seroprevalence differed highly between the diseases. BTV had the highest seroprevalence with a total seroprevalence of 67.0%, while BVDV had the lowest with a total seroprevalence of 2.9%. The total seroprevalence of FMD was 26,5% and CCPP 19.0%.

The results in this study show that FMDV, BTV, BVDV and CCPP are serologically present in southern Tanzania. These diseases are likely circulating and endemic in the area but the importance of the different diseases varies because of the differing levels of severity and consequences for farmers. FMD and CCPP are relevant differential diagnoses to PPR and should be taken into consideration when working to prevent, and in longer term, eradicate PPRV. The risk of mistaking PPR for BT and vice versa appears to be very low at the moment considering these diseases do not appear to cause similar clinical signs as PPR in Tanzanian sheep and goats.

SAMMANFATTNING

Detta examensarbete har genomförts som en Minor Field Study (MFS) i Tanzania med målet att undersöka seroprevalensen av tre utvalda differentialdiagnoser till PPR hos får och getter: mul och klövsjuka (FMD), bluetongue (BT) och smittsam pleuropneumoni (CCPP). Även bovin virusdiarré (BVD) och border disease ingick i studien då denna sjukdom sannolikt kan påverka utbrott av PPR genom immunosuppression. Får och getter har en mycket viktig roll i att säkra mattillgång, ekonomisk stabilitet och möjlighet till uppehälle i många av världens minst ekonomiskt utvecklade länder. PPR och vissa av sjukdomens differentialdiagnoser kan ha stora konsekvenser och orsaka stort ekonomiskt lidande och av den anledningen är det av intresse att fortsätta undersöka dessa sjukdomars epidemiologi och socio-ekonomiska påverkan.

I denna studie samlades blod från 483 får och getter från tre olika regioner i sydöstra Tanzania. Även prover från tidigare Minor Field Studies (Wensman et al., 2015) analyserades. Seroprevalensen av FMD, BT, CCPP och BVD undersöktes i serum genom att använda kompetitiv ELISA. Seroprevalensen av de olika sjukdomarna varierade kraftigt. Den högsta seroprevalensen var av BT med en total seroprevalens på 67.0% medan BVD hade den lägsta totala seroprevalensen på 2.9%. Den totala seroprevalensen av FMD och BVD var 26.5% respektive 19.0%.

Resultaten i denna studie påvisar att antikroppar mot FMDV, BTV, BVDV och *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) finns hos får och getter i sydöstra Tanzania där tama och vilda djur har kontakt. Sjukdomarna cirkulerar bland får och getter och är troligtvis endemiska. Betydelsen av de olika sjukdomarna varierar beroende på hur allvarliga konsekvenser insjuknande av djur har för djurägaren. CCPP och FMDV kan anses vara relevanta differentialdiagnoser till PPR och bär tas i beaktande när man arbetar för att förebygga och bekämpa PPR. Risker att BT misstas för PPR eller vice versa kan anses vara liten eftersom BT inte verkar orsaka likartade symptom som PPR hos får och getter i Tanzania.

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INTRODUCTION

Aims

The purpose of this study was to evaluate the seroprevalence of three diseases belonging to the differential diagnosis to Peste des Petits ruminants virus (PPR): foot and mouth disease (FMD), bluetongue (BT) and contagious caprine pleuropneumonia (CCPP). In addition, sero-prevalence of bovine viral diarrhoea (BVD) and border disease (BD), that likely can affect an outbreak of PPR due to immunosuppression, was investigated.

Peste des petits ruminant is a contagious disease that affects small ruminants like sheep and goats in Africa, the Middle East and parts of Asia. The disease is caused by the morbillivirus Peste des petits ruminants virus (PPRV). PPRV is closely related to Rinderpest virus, which today is eradicated from the world. PPRV has been identified as one of the next targets for eradication, but there are still some knowledge gaps to be filled before successful eradication schemes can be implemented (FAO, 2013). For example, the role of wildlife in the epidemiology and virus transmission needs to be clarified (Munir, 2013).

The importance of the exchange of PPR and some of the diseases which cause similar signs between livestock and wildlife in Tanzania is today uncertain. Wildlife could play an important role in the epidemiology of these pathogens since wildlife and livestock often live side by side. The prevalence of PPR and its differential diagnoses like foot and mouth disease (FMD), bluetongue (BT) and contagious caprine pleuropneumonia (CCPP) is not determined, therefore it is of interest to do a serologic screening of small ruminants in this area.

The pathogens selected for this study could present clinical signs similar to those of PPR, for example FMD where the most common feature of both infections is lesions in the mouth (Munir et al., 2013). FMD lesions are very small and do not occlude the oral cavity. Neither do these lesions cause a foul smell in the affected animals, whereas in PPR the oral lesions are prominent and can create hindrance in feed intake. Another similar disease is BT, which also causes pyrexia and lesions in the mouth. CCPP and PPR share some clinical signs, such as difficult breathing and coughing, but oral lesions and diarrhea are not present in CCPP (Munir et al., 2013). Co-infections with multiple pathogens could affect the severity of clinical signs and clinical outcome of a PPR outbreak. PPR, FMD, BT and CCPP cause major economic losses in affected countries. First of all because the high lethality rate among small ruminants leads to a decrease in the amount of food, but also because the diseases obstruct trade and transportation of animals. Most of the countries having problems with these diseases are low- or middle-income countries. Since small ruminants are the only income for many families in these countries, the diseases above inhibit the development in a wider perspective and consequently prevent the fight against poverty in the world. This study 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 work done in this study will contribute 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 45 million people on 945 203 km². The country is famous for its rich nature and wildlife. Agriculture is the most important sector and is estimated to be 25 % of the gross domestic products (National Bureau of Statistics, Tanzania Ministry of Finance, 2014). The sampling for this study was done in three districts in the Morogoro region. The region is located in south eastern Tanzania and has an average temperature that differs between 18.6°C- 30°C and the average annual precipitation is 935 mm (Climatedata, 2015). Morogoro is the centre for agricultural sciences in the country. The three sampled districts were Kilombero, Ulanga and Mvomero. Agro-pastoral farming is practiced in all of these areas. In Ulanga district the sampling was done during two different field studies, one in 2014 (Wensman et al., 2015) and this one in 2015. In 2014 it was mainly the villages Tanga, Lupunga and Mwaya who were sampled, while in 2015 it was the villages Mbuyuni, Kivukoni, Ipera Asikia, Lukande and Mbuga. Because the two samplings in Ulanga were done during different years and with slightly different groups of animals being targeted, the samples from 2015 will be referred to as Ulanga (2015) and the samples from 2104 as Ulanga (2014) in this paper. These specific study areas were chosen for several reasons. First of all, to get a broad perspective of the current seroprevalence of PPRV (as part of another degree project by Nils Roos to be published in 2016), FMDV, BTV, BVDV and CCPV in southern Tanzania. Secondly, all of these regions practice pastoral farming and have a large number of small ruminants. Thirdly, to be able to sample small domestic ruminants living in close contact with wild animals.

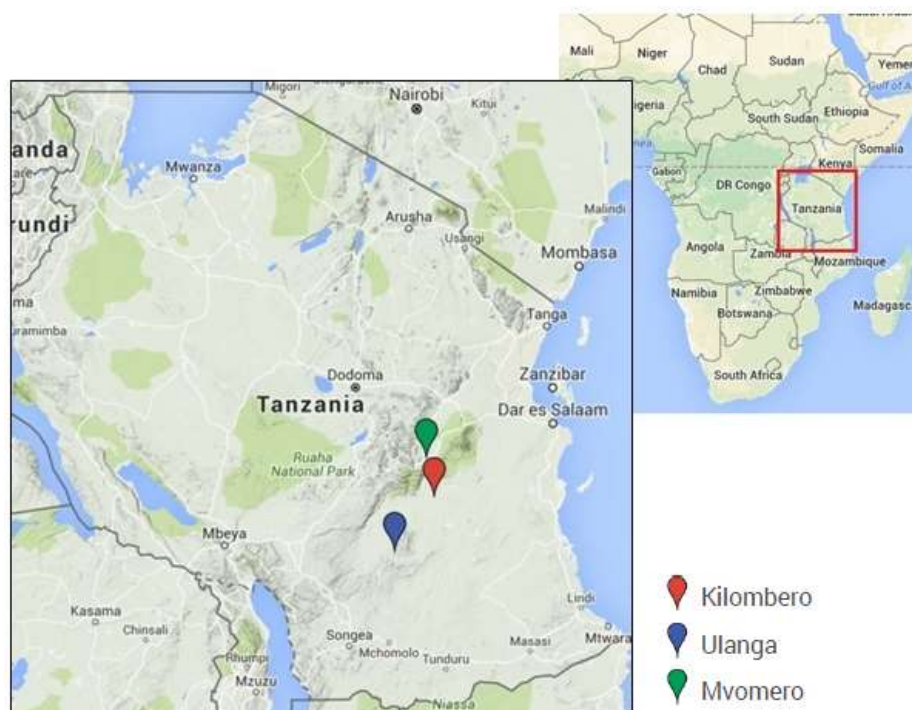


Figure 1. The coloured dots show the three study areas.





Figure 2. Examples of animal husbandry in Kilombero and Ulanga.

LITERATURE REVIEW

CCPP

Contagious caprine pleuropneumonia is a severe infectious disease that affects goats, sheep and wild ruminants. It occurs in many countries in Africa and Asia and is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) (OIE 2015). The disease was first reported in Algeria in 1873, as reviewed by Thiaucourt & Bölske (1996) but the importance and distribution of this disease

remain largely unknown. The causal agent of CCPP was first isolated in 1976 (MacOwan et al., 1976), but it was not given its species name *M. capricolum* subsp. *Capripneumoniae* (Mccp) until 1993 (Leach et al., 1993). There have been very few declarations of outbreaks of the disease to the OIE in the last 15 years, perhaps due to a lack of awareness of the disease and confusion with other diseases, such as “Peste des petits ruminants” (PPR) or *Pasteurella* infections (Peyraud et al., 2014).

Etiology

Mycoplasma capricolum subsp. *capripneumoniae* (Mccp) belongs to the so called *Mycoplasma mycoides* cluster, which is a group of mycoplasma of particular importance in veterinary medicine. Members of this cluster are biochemically and antigenically closely related which makes it difficult to diagnose the diseases caused by them (OIE, 2015). Pleuropneumonia in goats is caused by three different mycoplasmas; *Mycoplasma mycoides* subsp. *capri*, *Mycoplasma mycoides* subsp. *Mycoides* LC and *Mycoplasma capricolum* subsp. *capripneumoniae*. However, only Mccp causes the classical contagious caprine pleuropneumonia (OIE, 2015).

Epidemiology

Goats were thought to be the only susceptible host for Mccp (Litamoi et al., 1990) but it has been reported that sheep can be infected and seroconvert after mixing with affected goats. Mccp has also been isolated from healthy sheep and they may function as a reservoir (Thiaucourt and Bölske, 1996, Houshaymi et al., 2002). There have been confirmed cases from Qatar of CCPP in wild captive ungulates including wild goat, Nubian ibex, Larisstan mouflon and gerenuk with significant morbidity and mortality in these species (Arif et al., 2007).

Outbreaks of CCPP follow the introduction of an infected animal or animals into a susceptible herd. The mycoplasma is transmitted over short distances through the expulsion of infected droplets during coughing. The disease is very readily contagious, and only brief periods of contact are necessary for successful transmission (Bölske et al., 1996).

No evidence of indirect transmission has been shown as the mycoplasma is highly fragile in the environment. As with many mycoplasma diseases such as contagious bovine pleuropneumonia, the disease is introduced into a region by asymptomatic carrier animals (OIE, 2015).

Clinical signs and diagnostics

Goats of all ages and sex can be affected with CCPP (Thiaucourt and Bölske, 1996). The acute disease is more noticeable in naive populations in newly affected areas with high mortality and morbidity rates. The disease is characterized by cough, severe respiratory distress, pyrexia (40.5–41.5 °C), nasal discharge, which is catarrhal at the beginning and becomes muco-purulent in the later stage of disease. In chronic cases, the nasal discharges become thick and pasted on the nostrils. At this stage, animals show sporadic coughing, emaciation and diarrhea (Radostitis et al. 2006). In the terminal stages, the goats are unable to move and death follows quickly. In subacute or chronic forms, signs are milder with coughing usually noticeable only following exercise. High mortality can be seen in kids as a result of septicaemia (Nicholas and Churchward, 2012). Typical findings at post-mortem examination are fibrinous pleuropneumonia with massive lung hepatisation and pleurisy, accompanied by accumulation of straw-coloured pleural fluid (Thiaucourt and Bölske, 1996).

Definite diagnosis of *M. capricolum* subsp. *capripneumoniae* infection requires culture of the causative organism from lung tissue samples and/or pleural fluid taken at post-mortem, although high mortality and typical early thoracic lesions in goats are highly indicative of the disease. Isolates can be identified by several biochemical, immunological and molecular tests (OIE, 2014). Polymerase chain reaction based tests can be applied directly to clinical material such as lung and pleural fluid and have been shown to be a specific and sensitive test (Bashiruddin et al., 1994, Hotzel et al., 1996).

Serology has not been widely applied to identify the cause of outbreaks of pleuropneumoniae in goats and sheep. This is due to the fact that acute cases caused by Mccp rarely show positive titres before death and occurrence of false positive results. For this reason, serologic tests are best used on a herd basis rather than for diagnosis in individual animals. The serologic tests that are currently available according to OIE are complement fixation, passive hemagglutination, and ELISA. The latex agglutination test can be done in the field directly on whole blood as well as on serum samples in the laboratory. Serologic cross-reactions may occur with other members of the *Mycoplasma mycoides* cluster (OIE, 2014).

Foot and mouth disease

History

Foot and mouth disease (FMD) is a highly contagious viral disease of domestic and wild cloven-hoofed (even-toed) animals. It is characterized by fever and vesicles on skin and mucous membranes, usually in the mouth and on the muzzle, hooves and teats (Radostitis et al. 2006). The first documentation of FMD in Tanzania was in 1927 (Kivaria 2003) and the disease is considered endemic in the country. Since the first documentation many outbreaks have occurred in Tanzania, affecting almost every region. The first virus type isolation was done in 1957 and out of the seven known serotypes of FMD, four have been found in Tanzania (type O, type A, SAT-1 and SAT-2) (Kasanga et al., 2012).

The clinical signs of FMD are less severe in small ruminants compared to cattle and intensively reared pigs and therefore the disease is often ignored or misdiagnosed in small ruminants (Radostitis et al., 2006). Goats and sheep are often not vaccinated against FMD which makes it possible for them to serve as reservoirs for further infection and spread of the disease. However, since FMD has previously been controlled in certain areas without vaccinating small ruminants, it is unlikely that small ruminants play a large role to the epidemiology of the disease (Anderson et al., 1976, Casas 1984).

The virus

Foot and mouth disease virus (FMDV) is classified within the Aphthovirus genus as a member of the Picornaviridae family (Belsham 1993), being a non-enveloped, icosahedral virus, 26 nm in diameter, containing positive sense RNA of around 8.4 kb. During intracellular, cytoplasmic replication, viral RNA is translated into a polyprotein that is proteolytically cleaved into 12 structural and non-structural proteins. The complete viral capsid consists of 60 copies of each of the four structural proteins VP1-4, with many critical determinants for infection and immunity inherent in the molecular constituents of the VP1 protein (Alexandersen et al., 2003). There are seven immunologically distinct serotypes of FMDV: A, O, C, SAT1, SAT2, SAT3, and Asia1 which do not confer cross immunity. New FMD variants are generated by mutation from error-prone RNA replication, recombination, and host selection. (Davies 2002, OIE 2014)

Epidemiology

FMDV has many features, such as a wide host range, an ability to infect in small doses, a high level of viral excretion and multiple modes of transmission, which make it a difficult and expensive disease to control and eradicate (OIE 2014).

Transmission of FMD can occur by direct contact between infected and susceptible animals, direct contact of susceptible animals with contaminated inanimate objects (hands, footwear, clothing, vehicles, etc.), feeding of untreated contaminated meat products, ingestion of contaminated milk by calves, artificial insemination with contaminated semen, inhalation of infectious aerosols and airborne (OIE 2014).

It has long been debated if FMDV after the acute stage of infection can cause a prolonged, symptomless, persistent infection in ruminants. Carriers (defined as an animal from which the virus can be recovered 28 days or more after infection) have been recorded in cattle (Van Bekkum et al 1959), African buffalo (Hedger and Condy 1985), sheep (Burrows 1968), and goats but not in pigs. It occurs with all serotypes and has been identified in both experimentally and naturally infected animals (Van Bekkum et al 1959, Hedger 1968). The carrier period appears to vary between species, being in excess of 12 months in cattle, up to 9 months in sheep and goats and at least 5 years in African Buffalo (Condy et al., 1985). Although there is substantial evidence of carrier animals it is unclear if these carriers can transmit the disease to other animals (OIE 2014).

Clinical signs and diagnostics

The incubation period for FMD is 2–14 days, depending on the infecting dose, susceptibility of the host and strain of virus. The clinical picture differs between species and is generally more severe in cattle and pigs who develop fever up to 41°C, anorexia, vesicles in the mouth and on the feet as well as causing significant drop in milk yield from the herd (OIE, 2014). In sheep and goat the symptoms are milder and can be easy to miss as sudden onset of severe lameness may be the only clinical sign of disease in a herd. Vesicles are most commonly seen on the coronary band and the interdigital cleft of the feet and only sometimes in the mouth (Radostitis et al. 2006). The mortality of FMD is generally less than 5%, however, there is a higher mortality in young lambs, kids, calves and piglets due to virus-induced damage to the developing cells of the myocardium (Radostitis et al., 2006, OIE 2014).

Diagnosis is done by analyzing fluid samples from vesicular lesions. Virus can be detected using antigen-ELISA, cultivation or RT-PCR. “Pen-side” for diagnostics in the field are under development but have not yet been approved by OIE. Serology can be used for later stages of the infection and for surveillance of the disease (Alexandersen et al., 2003)

Bluetongue virus

History

Bluetongue (BT) is an insect-borne virus disease of ruminants characterised by fever, hyperaemia and oedema of the oral region and muzzle, and rawness and reddening of the coronary band. The clinical signs vary from inapparent or mild to acute (Howell, 1963; Erasmus, 1975; Hourrigan and Klingsporn, 1975).

BT was first recorded at the end of the 19th century in South Africa (Spreull, 1905). Since then Bluetongue virus (BTV) has been isolated from ruminants and/or vector insects from all continents except Antarctica (Gibbs E.P. & Greiner E.C., 1994).

The virus

Bluetongue virus is a member of the *Orbivirus* genus in the *Reoviridae* family. It is similar in morphology to other orbiviruses, such as African horse sickness and epizootic hemorrhagic disease virus. So far 27 BTV serotypes have been identified world-wide (Roy and Noad, 2006, Schwartz-Cornil et al., 2008, Jenckel et al. 2015). BTV is a non-enveloped virus, 90 nm in diameter, with a triplelayered icosahedral protein capsid (Roy 2008). Its genome consists of ten double-stranded RNA segments coding for seven structural proteins (VP1-VP7) and four non-structural proteins (NS1-NS3 and NS3A) (Kar et al., 2007; Roy, 2008). BTV serotypes 1, 2, 3, 4, 6, and 10 have a high pathogenic index and high epidemic potential (Dungu, Gerdes T, Smit T, 2004). However, a high genetic diversity of BTV exists that is a consequence of both drift (i.e., point mutations) and shift (i.e., reassortment of individual BTV gene segments) so pathogenicity even within a serotype may be highly variable (Saegerman C, Hubaux M, Urbain B, Lengelé L, Berkvens D, 2007).

Epidemiology

Bluetongue virus is transmitted between animals by haematophagous insects of the genus *Culicoides*. BTV multiplies in the ruminant host and is found in the blood and blood cells. On biting the host, the *Culicoides* vector ingests infected blood. The virus penetrates the gut wall of the midge and multiplies in the tissues. After 7 days the midge can infect a new host when taking a blood meal (Sellers 1984). *Culicoides* midges are found in many places in the world but only around 50 out of the 1500 known species of *Culicoides* can transmit the virus to ruminants. The global distribution of BTV is therefore restricted to those regions where these vector species of *Culicoides* occur and its transmission period is limited to the times when adult vectors are active. Activity is positively correlated with temperature and the optimal temperature for spread of the disease is between 28°C and 30°C (Wilson and Mellor, 2009).

Due to the seasonality of the vector's presence, transmission of the virus was believed to be limited to the warmer months of the year. However, recent evidence from California indicated the possibility that the virus can survive through the winter in long-living *C. soronensis* female midges, which had been infected during the previous flying activity period (Mayo et al., 2014). BTV can infect a broad spectrum of domestic and wild ruminants but serious clinical signs have been observed only in certain breeds of sheep (improved breeds) and a few deer species (MacLachlan 1994, Taylor 1986). Because cattle and goats usually only develop subclinical infections, they may serve as important and concealed viral reservoirs for sheep (MacLachlan 1994). However, some serotypes such as serotype 8, which has caused infection in northern Europe, exhibit a more important virulence in cattle (Guyot et al, 2007, Thiry et al., 2006) with serious socioeconomic consequences (Saegerman et al., 2007).

Wild ruminants are considered to function as a potentially important reservoir for virus and vector maintenance and a new hypothesis suggesting that two disease cycles exists, one prevalent in wild and one in domestic ruminants has been recently proposed (Ruiz-Fons et al., 2014).

Clinical signs and diagnostics

Bluetongue virus infects domesticated or wild ruminant species. Clinical manifestations of bluetongue vary widely between different animal species, as well as virus strains. Often, the infection remains

subclinical. The most serious symptoms are seen in breeds of improved sheep (Kahn and Line, 2010). Animals may exhibit disease with initial high fever and depression, followed by hyperaemia of the buccal and nasal mucosa, increased salivation, oral discharge and nasal discharge. These signs may be accompanied by oedema of the tongue, lips, face, eyelids and ears. Lameness due to hyperaemia of the coronary bands and haemorrhages around the hoofs is common. Additionally, pregnant animals may abort. Severe swelling of the tongue, which can become cyanotic ('blue tongue'), has given name to the disease but is not a common symptom. In acute cases of disease, animals may die within ten days of infection, mainly due to pulmonary oedema (Verwoerd and Erasmus, 2004 and Darpel et al., 2007).

A preliminary diagnosis of BT can be made based on typical clinical signs and post mortem findings, especially in areas where the disease is endemic (Afshar, 1994). Laboratory confirmation of BT is based on identification of viral RNA by PCR or on virus isolation in embryonated chicken eggs or mammalian and insect cell cultures. The identity of isolates may be confirmed by immunofluorescence, immunoperoxidase, serotype-specific virus neutralization tests, serotype-specific PCR, or hybridization with complementary gene sequences of group- or serotype-specific genes, the group-specific antigen-capture ELISA, group-specific PCR, (Katz et al., 1994; MacLachlan et al., 1994). A serologic response in ruminants can be detected 7–14 days after infection and is generally lifelong after a field infection. Current recommended serologic techniques for detection of BTV antibody include agar gel immunodiffusion and competitive ELISA. ELISA is the test of choice and does not detect cross-reacting antibody to other orbiviruses, especially anti-EHDV (epizootic hemorrhagic disease virus) antibody (Koumbati et al., 1999).

Bovine viral diarrhea virus

History

Bovine viral diarrhea virus (BVDV) and the very similar border disease virus (BDV) are single stranded RNA viruses belonging to the *Pestivirus* genus of the family *Flaviviridae*. BVDV causes bovine viral diarrhea (BVD), which was first reported as a transmissible disease in 1946 (Olafson et al., 1946). Since then, BVD has been reported worldwide and can have large economic consequences for farmers because of its effect on production and reproduction (OIE, 2008). BVDV virus can lead to a variety of clinical outcomes that range from subclinical infections to the more severe presentations including abortion, infertility, and the fatal mucosal disease. The condition is highly immunosuppressive and secondary respiratory and enteric complications often occur. There are two genotypes, BVDV type 1 and BVDV type 2, which are further classified as cytopathogenic (cp) or noncytopathogenic (ncp) based on in vitro cell culture characteristics (Brownlie J, 1990).

Epidemiology

Serologic surveys conducted throughout the world suggest that BVDV is endemic in the cattle population of most cattle-producing countries and it is considered one of the most economically important diseases of cattle in some parts of the world (Kampa et al., 2008). BVDV has been demonstrated in a number of species other than cattle such as sheep, goats and alpacas (Jewett et al., 1990, Bachofen et al., 2013, Goyal et al., 2002). Persistent infection with BVDV is documented in some of these species (Scherer et al., 2001, Bedenice et al., 2011, Bachofen et al., 2013) and the fact BVDV can infect a variety of other species that are in natural contact with cattle poses a risk for reinfection of pestivirus-susceptible cattle populations (Krametter-Froetscher et al., 2010).

Persistently infected cattle serve as a natural reservoir for virus. This form of the disease develops when noncytopathic BVDV is transmitted transplacentally during the first 4 months of fetal development. The calf is born infected with the virus and remains infected for life. These persistently infected animals are also usually immunotolerant to the resident noncytopathic virus. If transplacental infection occurs later in gestation it can result in abortion, congenital malformations, or birth of healthy calves that have antibody against BVDV (Houe 1999). The prevalence of persistent infection varies among countries and between regions within a country. According to OIE, the estimated mean animal prevalence of persistent infection with BVDV is ~1%–2% but may approach 4% on dairy farms with endemic BVDV infection (OIE 2008). Since persistently infected cattle can shed large amounts of BVDV in their secretions and excretions, they can easily transmit virus to susceptible herd mates and clinical disease and reproductive failure is often seen when healthy cattle come in contact with persistently infected animals. Although persistently infected cattle are important in transmission of BVDV, the virus may also spread by biting insects, fomites, semen and biologic products (OIE, 2008)(Radostitis et al. 2006).

Clinical signs and diagnostics

Although the name of the disease is bovine viral diarrhoea, diarrhoea is not a major clinical sign of BVDV. The clinical presentation can manifest in a variety of ways ranging from subclinical disease to the fatal mucosal disease. Virulence factors related to genotype and strain are partially responsible for these variations, together with host factors (Brownlie 1985).

Goats and sheep

Similar to BVD in cattle, pestivirus infections in small ruminants can cause a variety of clinical syndromes including reproductive failure, abortion, still birth, respiratory disease, poor growth rate, diarrhoea, nervous signs and muscular tremor. Acute infection in immunocompetent animals usually causes transient mild disease followed by seroconversion, whereas infection of fetus before development of immune system leads to birth of persistently infected (PI) animals, which are the main source of transmission (Nettleton et al.1998). Historically, all pestivirus isolates from sheep and goats were referred as BDV. But it is now known that sheep and goats can be infected with BVDV-1, BVDV-2 and BDV producing similar clinical signs (Sullivan et al. 1997; Pratelli et al. 2001; Kim et al. 2006; Valdazo-Gonzalez et al. 2006).

Acute infection

Infection of immunocompetent, susceptible animals with either noncytopathic or cytopathic BVDV is called acute or transient BVD. The most common form of infection in the field is inapparent or subclinical infection without any clinical signs that is followed by seroconversion (Ames 1986). Typical signs of acute clinical BVD are fever, depression, decreased milk production, transient inappetence, rapid respiration, excessive nasal secretion, excessive lacrimation, and diarrhoea. The clinical signs are usually seen 6–12 days after infection and last 1–3 days. Lymphoid tissue is a primary target for replication of BVDV, which may lead to immunosuppression and enhanced severity of intercurrent infections (Brownlie 1985).

In pregnant cattle, BVDV may cross the placental barrier and infect the fetus. The consequences of fetal infection usually are seen several weeks to months after infection of the dam and depend on the stage of foetal development and on the strain of BVDV. If infection becomes established at the time of insemination, conception rates may be reduced, and early embryonic death is increased when the virus is introduced at a slightly later stage (Carlsson et al., 1989, Mc Gowan et al., 1993). Foetal infection in

the first trimester (50-100 days) may also result in death, although expulsion of the foetus often does not occur until several months later. Congenital defects can arise from transplacental infection between days 100 and 150. This is caused by an inappropriate inflammatory response mounted to BVDV by the immune system, which is undergoing the final phase of development at this stage (Duffell 1985).

Persistent infection

Fetal infection with a non-cytopathic BVDV before 120 days of gestation may result in the birth of persistently infected animals. At this stage in gestation, the immune system is partially competent and recognises the BVDV antigen as self, meaning that there is no immune response. The animal becomes tolerant to the virus, which persists into neonatal life (Brownlie et al., 1984). Persistently infected animals can be identified at birth as being antigen-positive but seronegative (Bolin et al., 1985). Persistently infected animals continuously shed large amounts of virus throughout their lives, providing a major source of infection for naive cattle (Houe 1999). Persistent infection with BVDV is the prerequisite for developing mucosal disease (Brownlie et al., 1984).

Mucosal disease

Mucosal disease is an uncommon but highly fatal form of BVD that occurs in persistently infected cattle between 6 and 18 months of age and can have an acute or chronic presentation (Brownlie, 1990). Mucosal disease is induced when persistently infected cattle become superinfected with cytopathic BVDV. The origin of the cytopathic BVDV is usually internal, resulting from a mutation of the resident persistent, noncytopathic BVDV (Brownlie et al., 1984). Acute mucosal disease is characterized by fever, leukopenia, dysenteric diarrhea, inappetence, dehydration, erosive lesions of the nose and mouth and death within a few days of onset. Mucosal disease has not been observed in sheep or goats (Brownlie, 1985).

Diagnostics

BVDV and BDV antibodies can be detected in both blood and milk samples and the tests can be carried out on individual animals or groups of animals. The diagnosis of BVDV infection depends on the identification of a) virus using virus isolation, antigen ELISA, polymerase chain reaction (PCR) or b) evidence of exposure to virus by antibody ELISA (Brownlie et al., 2000). Antibody tests are useful in assessing the status of a group of animals or a whole herd prior to, or as a part of, a disease control programme. Tests for BVDV identify those animals that are persistently infected and should be used on a whole herd basis for virus eradication programmes. The only certain way of identifying a PI animal is by the demonstration of persisting virus. As the viraemia following acute infection (that is can be detected with RT-PCR) usually lasts no longer than 10 to 14 days, any animal that has a positive viraemia on first sampling and also at a second sampling performed a minimum of three weeks later, can be considered persistently viraemic. These animals usually have a low level or total absence of specific BVDV antibodies in both samples. (Brownlie et al., 2000)

MATERIAL AND METHODS

Animals and sampling

Blood samples and nasal swabs were collected from 238 goats and 238 sheep among 46 different herds in three different districts in Tanzania, during two separate field trips between June and July 2015. The blood was collected from the animal's jugular vein using a syringe, vacutainer, and blood collection tubes. One serum tube and two different nasal swabs (for PCR analysis) were collected from each individual. From animals that showed symptoms of PPR or its differential diagnoses (nasal/ocular discharge, oral lesions, diarrhea, fever or lethargia) an EDTA tube (for PCR analysis) and a nasal swab for bacterial analysis were also collected.

Individuals between 3 months and one year of age were targeted, although in certain herds the small number of young animals made it necessary to sample older animals. The age was estimated by asking the owners how old the animal was. When possible, equal proportions of sheep and goats in each herd were sampled. The sample size was decided by calculating how many animals were necessary to sample to get a representative sample of each herd while at the same time sampling as many different herds as possible. We chose to collect samples from approximately 12-15 animals in each herd. However, the size of the herds varied between a couple of animals and several hundred which also had an impact on the number of sampled animals in each herd. During the field trips blood samples were stored in a plastic coolbox with cold clamps, maintaining a temperature around 5°C in the box. The serum was separated in the evenings and was thereafter contained in cryotubes that were also stored in a plastic coolbox with cold clamps. Due to field conditions and time constraints, some of the samples were not separated until the next day. After returning to Sokoine University all the cryotubes were stored in -45 °C until serum was used for detection of antibodies.

The areas that were sampled were chosen together with professor Gerald Misinzo from SUA. We chose villages in or on the borders of Selous Game Reserve (with the theory that these animals would have contact with wild animals) as well as villages outside Selous where animals should have less contact with wild animals. When this selection had been decided, a randomized selection of villages in these areas was done. The goal was to have two groups (with/without contact with wildlife) of a similar size. If a village was not possible to sample, we would instead sample the village that was closest geographically. We also changed our plans slightly to be able to sample some villages in Ulanga that had had reported outbreaks of PPR recently and that had not been sampled before.

Because of the limited number of samples that could be analysed with the resources at hand, a selection had to be made from the sampled individuals. This was done by using a randomizer. The analysed samples came from two different field studies, the one done during the summer of 2015 and the one done by master students Nica Wachtmeister, Ida Herbe and Lovisa Levin in the autumn of 2014 (Wensman et al., 2015). The aims of the field studies were slightly different and therefore the selection of animals differ. During the 2014 sampling the focus was on animals of all ages, while we



during 2015 wanted to sample animals between 3 months and 1 year of age.

ELISA

Enzyme-linked immunosorbent assays (ELISAs) were performed at the Genome Science Center, Sokoine University of Agriculture in Morogoro, to examine the presence of antibodies towards FMDV, BTV, BVDV and CCPV using four different ELISA kits. The number of samples that were analyzed varied for each disease, depending on the amount of plates available.

CCPP

The ELISA used for analysis of BTV was the IDEXX *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) Antibody Test Kit “for the detection of antibodies directed against *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) in individual caprine serum samples”.

The competitive ELISA was performed according to the instructions of the manufacturer and all the

Figure 3. Sampling in Ulanga. Photo: Nils Roos.

Figure 4. Laboratory work.

premixed with a specific monoclonal antibody detection solution (detection solution Mab) in a separate plate (“preplate”) and then transferred into the coated microplate. Any Mccp specific antibodies present in the sample will form an immune-complex with Mccp antigen coated on the microplate, competing with Mab in the detection solution for the specific epitopes. After washing away unbound material, an anti-mouse antibody enzyme conjugate that binds the Mab in the Detection solution is added. In the presence of immune-complexes between Mccp antigen and antibodies from the sample, the Mab cannot bind to the specific epitopes and the conjugate is therefore prevented from binding. Conversely in the absence of Mccp-antibodies in the test sample, the Mab can bind to its

specific epitopes and the conjugate is free to bind to it. Unbound conjugate is washed away and an enzyme substrate (TMB) is added. In presence of the enzyme, the substrate is oxidized and develops a blue compound becoming yellow after blocking. Subsequent color development is inversely proportional to the amount of anti-Mccp antibodies in the test sample. The result is expressed in “percentage of inhibition” by comparing the optical density in the test well with the optical densities in the Mab Control wells.

To control the validity of each plate, the conjugate control mean absorbance (CC_x) and Mab control mean absorbance ($MabC_x$) were calculated. The Percentage of Inhibition (S PI) for each sample and control was calculated by dividing ($S\ PI = 100x (MabC_x - S\ A(450)) / MabC_x - CC_x$). The plate was considered valid when the following criteria were met (PI = Percentage of inhibition, PC = Positive control, NC = Negative control):

$$0.500 \leq MabC_x \leq 2.000 \qquad CC_x < 0.300$$

$$Mean\ NC\ PI \leq 35\% \qquad 50\% \leq Mean\ PC\ PI \leq 80\% \qquad 60\% \leq Mean\ SPC\ PI \leq 90\%$$

If the sample value was less than 55%, the sample was considered negative. Samples with S PI% greater than or equal to 55% were considered positive.

Table 1: *Distribution of the 343 samples analysed by the CCPP-ELISA. The percentage shows the percentage of the sampled individuals that were analysed.*

	Goats <i>n</i>	Sheep <i>n</i>	Total analyzed <i>n</i>	Contact with wildlife <i>n</i>
Kilombero	183 (89%)	54 (78%)	237 (86%)	80 (100%)
Ulanga (2015)	40 (33%)	42 (50%)	82 (40%)	97 (100%)
Mvomero	13 (15%)	11 (13%)	24 (14%)	24 (14%)
Total analyzed	236 (49%)	107 (33%)	343 (43%)	177 (75%)

FMDV

The ELISA used was an ID. Vet Screen competitive ELISA for “the detection of anti-FMDV non-structural protein antibodies in serum and plasma from bovine, ovine, caprine, porcine and all susceptible species”. The competitive ELISA was performed according to the instructions of the manufacturer and all the samples were run once. Microwells are coated with the non-structural protein of the Foot and mouth disease virus (FMDV NSP). Samples to be tested and controls are added to the microwells. Anti-NSP antibodies, if present, form an antigen-antibody complex which masks the virus epitopes. An anti-NSP horseradish peroxidase (HRP) conjugate is added to the wells, which fixes the remaining free epitopes, forming an antigen-conjugate-HRP-complex. The excess conjugate is removed by washing, and the substrate solution (TMB) is added. The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested; in the absence of antibodies a blue solution appears which becomes yellow after addition of the stop solution, and in the presence of

antibodies no coloration appears (ID Screen® FMD NSP Competition manual, 2014). The plate is read at 450 nm.

To control the validity of each plate, the mean value of the two negative controls (OD_{NC}) was calculated and the plate was considered valid when $OD_{NC} > 0.7$ and the mean value of the two positive controls divided by OD_{NC} was < 0.3 . For each sample the competition percentage (S/N %) was calculated by dividing $(OD_{sample}/OD_{NC}) \times 100$. If the value was equal or less than 50 % the sample was considered positive while a value greater than 50 % was a negative result.

Table 2: *Distribution of the 805 samples analysed by the FMDV-ELISA. The percentage shows the percentage of the sampled individuals that were analysed.*

	Goats <i>n</i>	Sheep <i>n</i>	Total analyzed <i>n</i>	Contact with wildlife <i>n</i>
Kilombero	205 (100%)	69 (100%)	274 (100%)	80 (100%)
Ulanga (2015)	123 (100%)	84 (100%)	207 (100%)	97 (100%)
Mvomero	89 (100%)	87 (100%)	176 (100%)	176 (100%)
Ulanga (2014)	61 (100%)	87 (100%)	149 (100%)	149 (100%)
Total analyzed	478 (100%)	327 (100%)	805 (100%)	177 (100%)

BTv

The ELISA used for analysis of BTv was the IDEXX Bluetongue Virus (BTv) Antibody Test Kit “for the detection of antibodies directed against VP7 protein from Bluetongue Virus (BTv) in individual serum and plasma samples from bovine, ovine and caprine origin”. There are 27 known serotypes of BTv; among BTv viral proteins, VP7 is common to all known serotypes, strongly immunogen and specific to BTv.

The competitive ELISA was performed according to the instructions of the manufacturer and all the samples were run once. The wells of the microplate are coated with recombinant VP7 protein. Samples to be tested are diluted and incubated in the wells. Any antibody specific to VP7 present in the samples will form an antigen-antibody immune-complex. After incubation, an anti-VP7 antibody coupled to the peroxidase is added in the wells. If the sample contains specific VP7 antibodies, the VP7 sites are "masked" and the conjugate cannot bind on the corresponding epitope. After washing, the enzyme substrate (TMB) is added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of the colour is an inverse measure of the proportion of anti-VP7 antibodies present in the sample to test (IDEXX Bluetongue Competition manual, 2015). The plate is read at 450 nm.

To control the validity of each plate, the mean value of the two negative controls (OD_{NC}) was calculated and the plate was considered valid when $0.700 \geq OD_{NC} \leq 0.3000$ and the competition percentage (S/N %) of the positive control was $< 20\%$. For each sample the competition percentage (S/N %) was calculated by dividing $(OD_{sample}/OD_{NC}) \times 100$. If the value was equal or greater than 80%, the sample was considered negative. A value greater than 70% and less than 80% was considered a doubtful result. Samples with S/N% less than or equal to 70% were considered positive.

Table 3: *Distribution of the 455 samples analysed by the BTV-ELISA. The percentage shows the percentage of the sampled individuals that were analysed.*

	Goats <i>n</i>	Sheep <i>n</i>	Total analyzed <i>n</i>	Contact with wildlife <i>n</i>
Kilombero	155 (76%)	42 (61%)	197 (72%)	80 (100%)
Ulanga (2015)	103 (84%)	54 (64%)	157 (76%)	68 (70%)
Mvomero	28 (31%)	37 (43%)	65 (37%)	65 (37%)
Ulanga (2014)	13 (21%)	23 (26%)	36 (24%)	36 (24%)
Total analyzed	299 (63%)	156 (48%)	455 (57%)	249 (50%)

BVDV

The ELISA used for analysis of BVDV was the IDEXX BVDV p80 Ab Test Kit “for the detection of antibodies directed against p80 protein for diagnostic of BVDV and Mucosal disease (MD) in individual serum, plasma and milk samples and in pools of serum (maximum 10) and tank milk samples from bovine origin and for diagnostic of Border Disease (BD) in individual serum and plasma samples and pools of serum samples (maximum 5) from sheep”. It is based on the principle of competition between antibodies and a Peroxidase coupled monoclonal anti-p80-antibody “WB112”.

The competitive ELISA was performed according to the instructions of the manufacturer and all the samples were run once. The P80 protein is supplied coated on wells of the polystyrene microplate by means of a specific monoclonal antibody “WB103”. Samples to be tested are diluted and incubated in the wells. If specific antibodies are present in the sample, they form bovine (or ovine) antibody-P80 complexes, through which the P80 becomes "masked". After washing, a monoclonal antibody “WB112” (directed to another epitope of P80) coupled to the enzyme peroxidase is incubated in the wells. In presence of specific BVDV antibodies in the sample, the P80 protein sites are "masked", and the conjugate cannot bind on the corresponding epitope. After washing, the enzyme substrate (TMB) is added to the wells. If the conjugate is fixed in the wells, it transforms the substrate into a blue compound becoming yellow after the blocking. The intensity of the colour is an inverse measure of the rate of anti-p80 antibodies present in the sample to test (IDEXX BVDV p80 Ab manual, 2015). The plate is read at 450 nm.

To control the validity of each plate, the mean value of the two negative controls (OD_{NC}) was calculated and the plate was considered valid when $OD_{NC} \geq 0.8000$ and the competition percentage (S/N %) of the positive control was $< 20\%$. For each sample the competition percentage (S/N %) was calculated by dividing $(OD_{sample}/OD_{NC}) \times 100$. If the value was equal or greater than 50%, the sample was considered negative. A value greater than 40% and less than 50% was considered a doubtful result. Samples with S/N% less than or equal to 40% were considered positive.

Table 4: *Distribution of the 478 samples analysed by the BVDV-ELISA. The percentage shows the percentage of the sampled individuals that were analysed.*

	Goats <i>n</i>	Sheep <i>n</i>	Total analyzed <i>n</i>	Contact with wildlife <i>n</i>
Kilombero	160 (78%)	48 (70%)	208 (76%)	80 (100%)
Ulanga (2015)	99 (80%)	65 (77%)	164 (79%)	61 (63%)
Mvomero	33 (37%)	36 (41%)	70 (40%)	70 (40%)
Ulanga (2014)	13 (21%)	23 (26%)	36 (24%)	36 (24%)
Total analyzed	306 (64%)	172 (52.6)	478 (59.4)	141 (80%)

Questionnaire

At the sampling missions a sample submission form/questionnaire was used to gather basic epidemiological data of the herds. The questions were asked to each livestock keeper through an interpreter that most of the time was the local veterinarian or the assistant of the veterinarian. The questions were as following:

1. Amount of animal interaction with wildlife?
2. Latest introductions of new animals to herd?
3. Last vaccination of herd against PPR/CCPP/FMD?
4. Last de-worming of herd? 4b. all animals treated?
5. Last antibiotic treatment of herd? 5b. all animals treated?
6. Estimated date when first PPR case was observed at this farm?
7. Details of animals (number of sick and dead animals since the start of the outbreak, age of animals, number of goats/sheep)
8. Type of farming? (Household/Dairy production/Meat production/Individual seller at live animal market/Other)
9. Clinical signs of outbreak? (Abortion/Diarrhea/Pneumonia/Oral mucosal lesions/Nasal and ocular discharges/High temperature/Other)

RESULTS

Seroprevalence of CCPP

In total, 343 individuals from three different areas were screened for antibodies against CCPP. The animals came from 43 herds in the districts Kilombero, Ulanga (2015) and Mvomero. Out of the 43 herds, 17 herds had at least one seropositive animal. The herd-prevalence varied between 8%-100%. However, in some of the herds with 100% herd-prevalence only one animal was analysed. Of the 17 herds in Ulanga, 16 had been vaccinated against CCPP sometime between 2012 and 2014. No herds in Kilombero had been vaccinated. The owners in Mvomero were not asked if their animals had been vaccinated against CCPP. Two of the ELISA-plates were considered invalid and therefore the number of samples that were analysed were fewer than expected.

Table 5: Results from CCPP-ELISA showing the total seroprevalence and number of positive animals. In total 343 individuals were analysed.

	Goats % (n)	Sheep % (n)	Total % (n)	Contact with wildlife (n)
Kilombero	32.2 (59)	5.5 (3)	26.2 (62)	6.2 (5)
Ulanga (2015)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Mvomero	23.0 (3)	0.0 (0)	12.3 (3)	12.3 (3)
Total %	26.2 (62)	2.8 (3)	19.0 (65)	2.3 (8)

Of the 343 analysed samples totally 65 individuals were considered positive (Table 5). Using the equation $p = \pm 1,96 \sqrt{\frac{p(1-p)}{n}}$ to calculate a 95% confidence interval for the proportion gives that the total seroprevalence of CCPP can be said with 95% confidence to lie between 15% and 23%. Kilombero had the highest seroprevalence of the sampled areas with between 27% and 37% seroprevalence. The same area also had the highest seroprevalence among the herds. The seroprevalence was lowest in Ulanga district where none of the analysed samples were considered positive. As previously stated, the vast majority of the animals in Ulanga district were vaccinated against CCPP.

In Kilombero the seroprevalence in the herds who had contact with wildlife was with 95% confidence between 1,2% and 3,4% which is much lower compared to the total seroprevalence. The seroprevalence in goats was also than in sheep in both Kilombero and Mvomero. However, it is possible that this might be more related to the fact that the herds with high prevalence happened to consist of only goats.

Seroprevalence of FMDV

Serum from 805 individuals from four different areas was analysed for antibodies against FMDV. The animals came from 71 different herds and 40 of these herds had at least one seropositive animal. The herd-prevalence varied between 7%-91%. None of the herds had been vaccinated against FMD.

Table 6: Results from FMDV-ELISA showing the total seroprevalence and number of positive animals. In total 805 individuals were analysed.

	Goats % (n)	Sheep % (n)	Total % (n)	Contact with wildlife (n)
Kilombero	20.5 (42)	14.5 (10)	18.0 (52)	5.0 (4)
Ulanga (2015)	18.7 (23)	6.0 (5)	13.5 (28)	10.3 (10)
Mvomero	67.4 (60)	67.8 (59)	67.6 (119)	67.6 (119)

Ulanga (2014)	13.1 (8)	6.9 (6)	9.4 (14)	9.4 (14)
Total %	27.8 (133)	24.5 (80)	26.5 (213)	7.9 (14)

Of the 805 analysed samples totally 213 individuals were considered positive (Table 6). Using the equation $p = \pm 1,96 \sqrt{\frac{p(1-p)}{n}}$ to calculate a 95% confidence interval for the proportion gives that the total seroprevalence of FMDV can be said with 95% confidence to lie between 24% and 29%. Mvomero had the highest seroprevalence of the sampled districts with between 65% and 71% seroprevalence. The same area also had the highest seroprevalence among the herds, with one herd where 91% (10 out of 11 animals) of the sampled individuals were seropositive. The seroprevalence was lowest in Ulanga (2014) were between 7,2% and 11% of the animals were considered positive with 95% confidence.

The total seroprevalence among the herds with contact with wildlife was with 95% confidence between 6,0% and 9,7% which is lower than the total seroprevalence.

Seroprevalence of BTV

In total, 455 individuals from four different districts were screened for antibodies against BTV. The animals came from the same 71 herds that were analysed for FMDV. Out of these 71 herds, 69 herds had at least one seropositive animal. The herd-prevalence varied between 8%-100%. We did not ask the livestock keepers if they had vaccinated their animals against BTV.

Of the 455 analysed samples totally 305 individuals were considered positive (Table 7) and using the equation $p = \pm 1,96 \sqrt{\frac{p(1-p)}{n}}$ to calculate a 95% confidence interval for the proportion the total seroprevalence of BTV can be said with 95% confidence to lie between 63% and 71%.

Table 7: Results from BTV-ELISA showing the total seroprevalence and number of positive animals. In total 455 individuals were analysed.

	Goats % (n)	Sheep % (n)	Doubtful % (n)	Total % (n)	Contact with wildlife (n)
Kilombero	60.1 (94)	54.8 (23)	4.0 (8)	59.4 (117)	61.3 (49)
Ulanga (2015)	74.8 (77)	53.7 (29)	5.0 (8)	67.5 (106)	63,2 (43)
Mvomero	85.7 (24)	83.8 (31)	3.0 (2)	84.6 (55)	84.6 (55)
Mahenge	76.9 (10)	73.9 (17)	4.3 (3)	75.0 (27)	75.0 (27)
Total %	68.6 (205)	64.1 (100)	4.6 (21)	67.0 (305)	69.9 (174)

Mvomero had the highest seroprevalence of the sampled areas. With a confidential interval of 95% the seroprevalence in Mvomero was between 82% and 88%. The same area also had the highest

seroprevalence among the herds and in 6 of the 12 sampled herds all individuals were seropositive. The seroprevalence was lowest in Kilombero district where the total seroprevalence was between 55% and 64% with 95% confidential interval.

The total seroprevalence in the herds who had contact with wildlife was only slightly higher (between 66% and 74%) compared to the total seroprevalence. In Mvomero and Ulanga (2014) all sampled animals had contact with wildlife and the total seroprevalence in these two regions was higher than in Kilombero and Ulanga.

Seroprevalence of BVDV

Serum samples from 478 individuals from four different areas were analysed for antibodies against BVDV. The animals came from 68 different herds and 7 of these herds had at least one seropositive animal. In one herd in Mvomero, 4 out of 4 sampled animals were seropositive. The herd-prevalence varied between 7%-100% but in most of the positive herds the herd-prevalence was around 20%. We did not ask the livestock keepers if they had vaccinated their animals against BVDV.

Of the 478 analysed samples totally 14 individuals were considered positive (Table 8). Using the equation $p = \pm 1,96 \sqrt{\frac{p(1-p)}{n}}$ to calculate a 95% confidence interval for the proportion gives that the total seroprevalence of BVDV can be said with 95% confidence to lie between 1,3% and 4,5%. Mvomero had the highest seroprevalence of the sampled areas. With 95% confidence the seroprevalence in Mvomero was between 4,7% and 9,5%. The same area also had the highest seroprevalence among the herds. The seroprevalence was lowest in Ulanga district where none of the analysed samples from 2014 were considered positive. It is worth noting that only a small number of samples (36) were analysed from Ulanga.

Table 8: Results from BVDV-ELISA showing the total seroprevalence and number of positive animals. In total 478 individuals were analysed.

	Goats % (n)	Sheep % (n)	Doubtful % (n)	Total % (n)	Contact with wildlife (n)
Kilombero	0.0 (0)	2.0 (1)	0.9 (2)	0.5 (1)	0.0 (0)
Ulanga (2015)	5.0 (5)	4.6 (3)	2.4 (4)	4.9 (8)	13.1 (8)
Mvomero	9.0 (3)	5.6 (2)	1.4 (1)	7.1 (5)	7.1 (5)
Ulanga (2014)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Total %	2.6 (8)	3.5 (6)	1,5 (7)	2.9 (14)	9.2 (8)

The total seroprevalence in the herds who had contact with wildlife, with 95% confidence between 6,6% and 11,8%, was higher than the total seroprevalence of all sampled animals.

DISCUSSION

The aim of this study was to do a serological screening of some of the diseases which can cause similar signs (FMD, BTV, BVDV and CCPP) of PPR among sheep and goats in southern Tanzania. The results show that all of these diseases are circulating and endemic in the area, assuming the results in the study represents the true prevalence in southern Tanzania. Therefore, these diseases are relevant in PPR differential diagnosis and need to be taken into consideration when planning the surveillance and control of PPR.

The seroprevalence differed highly between diseases. BTV had the highest seroprevalence with a total seroprevalence of between 63% and 71%, while BVDV had the lowest with a total seroprevalence of between 1,3% and 4,5%. The total seroprevalence of FMD and CCPP was between 24%-29% and 15%-23% respectively.

Although the aim was to get a representative sample of animals from the different districts, it is possible that the way animals were selected for sampling and analyses might have contributed to selection bias. The analysed samples came from two different field studies, the one done during the summer of 2015 and one done by master students Nica Wachtmeister, Ida Herbe and Lovisa Levin in the autumn of 2014. The aims of the two field studies were slightly different and therefore the selection of animals differs. During the 2014 sampling the focus was on older individuals while we during 2015 wanted targeted animals between 3 months and 1 year of age. For this reason, the sampled animals from Mvomero and Ulanga (2014) were generally older than the animals from Kilombero and Ulanga (2015). It is therefore possible that the higher seroprevalence of BTV in Mvomero and Ulanga (2014) can be attributed to the fact that the sampled individuals had had a longer time to possibly come in contact with the disease. In retrospect, I should perhaps have chosen to analyse samples from only the young individuals from Mvomero and Ulanga (2014) as well.

During the 2015 sampling we targeted herds in Ulanga that had had recently reported outbreaks of PPR to have a higher chance of finding individuals that were PCR-positive for PPRV. We were not told of any recent outbreaks in the other districts. We were also very dependent on our assistants in the different districts, who were the ones in contact with the farmers and who had a large impact on which herds were selected for sampling within the area. The sampling could in some instances be quite chaotic with a large number of people and animals involved, and therefore it is possible that some of

the information about the animals is not correct. While sampling animals, the owner were asked to give an approximate age of the animal and while some owners were very precise, it is likely that some of the ages are only a vague estimation. It is also difficult to know how much we can trust the information regarding vaccinations as some farmer were unsure about exactly when and against what their animals had been vaccinated.

The aim was to analyse individuals from as many herds as possible, as we thought that would be a better indication of prevalence rather than analysing a large number of individuals from the same herd. Because of unforeseen difficulties such as power breaks, some ELISA-plates were not usable and therefor the number of analysed samples in some cases were smaller than anticipated which resulted in only one individual being analysed in some herds.

CCPP

Of the 343 analysed samples totally 65 individuals were considered positive and the total seroprevalence was between 15% and 23% with 95% confidence. It is worth noting that the seroprevalence was 0.0% in Ulanga where 16 of the 17 sampled herds had been vaccinated against CCPP sometime between 2012 and 2014. The reason we found no seropositive animals, even though the herds had been vaccinated, might be that we targeted animals younger than 1 year, who would not have been alive during the recent vaccinations. Unfortunately, we do not know what vaccine was used and why the animals were vaccinated. According to OIE guidelines, CCPP vaccine efficacy should last at least 1 year and protect vaccinated animals from clinical disease. There are currently no CCPP vaccines permitting a DIVA (detection of infection in vaccinated animals) strategy. From this we can draw the conclusion that the vaccinations in this area seem to have protected the animals from outbreaks of CCPP. The latest vaccination we were told of occurred in 2014, and therefore it is also possible that the amount of antibodies had declined enough for the animals to be considered seronegative.

A previous study on seroprevalence of CCPP among goats and sheep in the two districts Lindi and Mtwara in southern Tanzania was done in 2014. The overall seroprevalences in the two districts were 35.5% in goats and 22.9% in sheep (Mbyuzi et al., 2014). In the study by Mbyuzi et al. there was also a great variation of the seroprevalence in sheep between studied districts, with the highest being 62.5% in Tandahimba and the lowest being 22.2% in Masasi, both in Mtwara region. It was further evident that seroconversion rates were significantly higher in the government farms than in traditional flocks. The authors explained that the high seropositivity of CCPP in government farms, as opposed to the observed low seroprevalence of CCPP in the traditional flocks, could possibly be because of the difference in available animal health services between the two systems of animal husbandry. Government farms often have better access to disease management which leads to more animals being treated when sick which in turn allows high recovery rates of affected animals as opposed to high case fatality rates observed in traditional flocks as a result of poor access to animal health services (Mbyuzi et al., 2014). Other authors (El Hassan et al., 1984, Thiaucourt and Bölske, 1996 and Wesonga et al., 2004) have reported high carrier status for animals recovering from CCPP following antibiotic treatment, which could be linked to the high seroprevalence of CCPP in government farms. In this study, the seroprevalence in the herds in Kilombero who had contact with wildlife was much lower compared to the total seroprevalence. This might be explained by the theory above, as the herds in contact with wildlife are mainly traditional flocks. The seroprevalence in goats was also much higher than in sheep in both Kilombero and Mvomero. It is possible that this is simply because the herds with high prevalence happened to consist of only goats and therefore no sheep were sampled. However, the

seroprevalence in goats was also higher compared to sheep in the 2014 study by Mbyuzi et al. and these results may support the theory that goats are more susceptible to CCPP than sheep.

Comparing my results to previous studies, I believe my results are credible. Because CCPP can cause similar signs in goats and sheep as PPRV and appears to be present in the study area, it is a relevant differential diagnosis of animals with suspected PPR in southern Tanzania.

FMDV

Of the 805 samples analysed for FMDV, totally 213 individuals were considered positive and the total seroprevalence was between 24% and 29% with 95% confidence. None of the analysed individuals had been reported as vaccinated which indicates that they have been exposed to natural infection of FMDV. The seroprevalence was slightly lower among the animals with contact with wild animals. It is difficult to say why this is since wildlife is considered a possible source of infection. It is possible that because the animals that have contact with wild wildlife were mainly livestock belonging to Maasai people, the fact that they are rarely brought to live animal markets and generally have a larger area to graze on make them less likely to contract the disease from domestic animals and that it is this factor rather than the contact with wildlife that results in a lower prevalence among these animals.

FMD is considered endemic in Tanzania although the spatio-temporal distribution of FMD virus has not been clearly investigated. Previous studies of seroprevalence of FMDV in cattle in several districts have shown seroprevalence ranging between 81% (Bagamoyo district) and 15.4% (Temeke) (Joseph J. et al., 2014). I have not found any FMDV seroprevalence studies among small ruminants in Tanzania. However, a study in Uganda from 2009 show a seroprevalence of 14% in goats and 22% in sheep in one district, while some of the other districts included in the same study had seroprevalences between 0%-100% (Balinda et al., 2009). Studies in Morocco following an FMD outbreak in 1999 identified a prevalence of 13% among sheep (Blanco et al., 2002). In contrast, an earlier Kenyan study showed high antibody prevalences of 89% (type O) and 56% (SAT 2) in small ruminants (Anderson et al., 1976). Antibodies towards FMDV have been shown to decline faster in small ruminants than in cattle (Dellers and Hyde, 1964; Cunliffe, 1964; Garland, 1974) although limited earlier studies have shown that antibodies in sheep, despite a slight decrease after day 10, remain relatively high for at least 147 days (Dellers and Hyde, 1964). It is obvious that the seroprevalence is very varied among districts and even herds in the same district. Comparing my results to previous studies, I believe my results are credible as they are similar to those in previous studies. Because FMDV can cause similar signs in goats and sheep as PPRV and appears to be present in the study area, it is a relevant differential diagnosis of animals with suspected PPR in southern Tanzania.

BTV

Of the 455 samples screened for BTV, a total of 305 individuals were considered positive and the total seroprevalence was between 63% and 71% with 95% confidence. The total seroprevalence was higher in Mvomero and Ulanga (2014) than in Kilombero and Ulanga (2015). The seroprevalence in goats was higher than in sheep in all areas. Because the sampled animals from Mvomero and Ulanga were generally older than the animals from Kilombero and Ulanga (2015), it is possible that the higher seroprevalence of BTV in Mvomero and Ulanga (2014) can be attributed to the fact that the sampled individuals had had a longer time to possibly come in contact with the disease. However, this pattern could be seen in the seroprevalence of the other diseases in this study and the seroprevalence in Mvomero and Ulanga (2014) might actually be higher than in the other two regions. It is also possible that the disease is more prevalent in some areas compared to others due to climate, which might affect the amount of vectors in the area.

Bluetongue is considered endemic in Tanzania although very few serological studies on goats and sheep appear to have been done. In one study conducted in northern Tanzania in 1995, a seroprevalence of 74% in sheep and 77% in goats was demonstrated (Hyera and Lyary, 1995). It is interesting to note that this study showed a higher prevalence among older individuals and a slightly higher prevalence in goats compared to sheep. Studies done on wildlife showed that there was a high prevalence of antibodies against BT virus in buffalo, wildebeest kongoni, topi, waterbuck and impala which supports the suggestion that these species probably act as maintenance hosts (Hamblin et al., 1990).

A seroprevalence of 67% is similar to that in previous published studies (Hyera and Lyary, 1995). No vaccination programme against BT virus in sheep or goats is in practice in Tanzania and the antibodies in the sera of the sampled animals must have arisen as a consequence of either natural infection with the virus or of passive immunization with maternal antibodies. Clinical BT is generally seen amongst wool sheep and their crosses with native hair sheep (Hyera and Lyary, 1995). Hair sheep show evidence of challenge by BT but clinical manifestation of the disease has not been encountered. Almost 100% of the Tanzanian sheep population is native hair type. Consequently, clinical BT does not seem to occur in Tanzania (Hyera and Lyary, 1995).

It is not surprising that BTV infection occurs in Tanzania, but studies are needed to increase the knowledge about the epidemiology and pathogenesis of BTV among different animal populations, in particular domestic and wild ruminants in Tanzania. However, the chances of mistaking PPR for BT and vice versa appear very low at the moment considering that BT does not seem to cause any clinical signs in Tanzanian sheep and goat breeds.

BVDV

Of the 478 samples analysed for BVDV or BDV totally 14 individuals were considered positive and the total seroprevalence was between 1,3% and 4,5% with 95% confidence. This was by far the lowest total seroprevalence among the studied diseases. We did not ask the livestock keepers if their animals had been vaccinated against BVDV but it seems unlikely that the animals were vaccinated and therefore the antibodies in the sera of the sampled animals can be assumed to be because of natural infection with BVDV. The total seroprevalence was higher among the animals who had contact with wildlife compared to those who did not. The total seroprevalence in sheep was also slightly higher than in goats.

I have only been able to find one previous serological study of BVDV in goats and sheep in Tanzania, done by Hyera, Liess and Frey in 1991. This study showed that sheep and goats in northern Tanzania had seemingly been exposed to BVD virus. The study was done using a direct neutralising peroxidase-linked antibody (NPLA) assay and the seroprevalence was considered 32.1% in sheep and 24.9% in goats. The authors concluded that BVD virus possibly cycles within the cattle population in Tanzania and should be taken into consideration as a possible cause of death, abortion, weak and/or malformed offspring among and possibly also among sheep and goats (Hyera et al., 1991).

There has also been one study done on antibodies to BVDV in free-living buffalo in Tanzania. The number of seropositive buffalos varied considerably between different areas, with the highest seroprevalence being 71.9% in north Serengeti while no positive samples were found in several other areas (Hamblin et al., 1990).

It is difficult to draw any conclusions regarding my results from these previous studies. The seroprevalence in this study was much lower compared to the seroprevalence recorded by Hyera, Liess

and Frey in 1991. However, they used NPLA technique while I used a competitive ELISA technique for detection of antibodies. It is possible that their method was more sensitive. The study from 1991 was also done in northern Tanzania while this study was done in the south eastern part of the country. It is definitely possible that the prevalence varies between these regions. This observation together with the large difference in herd-prevalence possibly suggests the virus is only circulating within defined areas in Tanzania. Cattle, goats and sheep are often managed together in Tanzania and the first two species form the commonest group association. At night sheep and goats are kept together while cattle are held in separate enclosures. It is possible that an exchange of diseases such as BVDV occurs between cattle and sheep when the two species graze together. Goats on the contrary, prefer to browse on shrubs and bushes and are therefore less likely to be in close contact with cattle or sheep during the day and it is probable that they pick up BVDV mainly at night from infected sheep and at watering points where they come into close contact with infected cattle or sheep. This might explain the higher seroprevalence among sheep compared to goats in both this study and the study by Hyera, Liess and Frey in 1991.

BVDV is prevalent in buffalo, which suggest that wildlife is involved in the circulation and spread of the disease. To further understand the epidemiology and prevalence of BVDV in Tanzania, further specific virological and serological investigations should be made. Because BVDV can affect outbreaks of PPRV and other diseases due to immunosuppression and appears to be present in the study area, it is a relevant diagnosis to consider when investigating animals with suspected PPR in southern Tanzania.

Conclusions

This study has showed that FMD, BT, BVD and CCPV are serologically present in southern Tanzania where wild and domestic small ruminants intermingle. The importance of the different diseases varies because of the differing levels of severity and consequences for farmers. In my opinion, FMDV and CCPV are relevant differential diagnoses to PPR that should be taken into consideration when working to prevent, and in longer term, eradicate PPR. The risk of mistaking PPR for BT and vice versa appear very low at the moment considering these diseases do not appear to cause similar clinical signs to PPR in Tanzanian sheep and goats. As mentioned above, because BVDV can affect outbreaks of PPRV and other diseases due to immunosuppression and appears to be present in the study area, it is a relevant diagnosis to consider when investigating animals with suspected PPR in southern Tanzania.

The epidemiology, socio-economic impact and possible ways of preventing these diseases are very complicated and it is of great interest and importance to further evaluate the prevalence of PPRV and its differential diagnoses in different parts of Tanzania. By doing this the international community have a higher possibility of 1) stopping the rapid spread of disease to other regions 2) decreasing the economic, food security and livelihood impacts these diseases have because of the key role sheep and goats play in national food and nutritional security, income security and livelihood resilience in the least economically developed countries across the world and 3) sustaining the momentum created by the eradication of rinderpest that resulted in a growing interest among the international community to address PPR at a regional and global scale.

I hope that this thesis and the data collected during this project will contribute to the understanding and prevention of spread of PPR and the studied diseases, which in turn will hopefully enable prosperous livestock production and more effectively integrated conservation of wildlife, to the benefit of both animals and people.



Figure 5. *Maasai with their herd.*

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Photo: Emeli Torsson.

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