

Seroprevalence of Foot and Mouth Disease and Peste des Petits Ruminants in Small Ruminants in Northern Zambia on the Border to Tanzania

Searching for common traits among seropositive herds



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SUMMARY

Foot and mouth disease (FMD) and peste des petits ruminants (PPR) are highly contagious viral diseases affecting sheep and goats all over the world. Small ruminants are often owned by poor small-scale farmers in the developing world, and several studies have identified them to be among the most important livestock species for the poor. Controlling, or even eradicating, FMD and PPR is important for alleviating poverty. More than half of Zambia's population was living below the national poverty line in 2015. FMD is today considered to be endemic in Zambia, the most recent outbreak was in March 2018. No clinical cases of PPR have been found within the country so far but PPR is highly present in Zambia's bordering countries in the north and Zambia is therefore under constant risk of the incursion of PPR.

This study was carried out in the districts Nakonde and Mbala in northeastern Zambia on the border to Tanzania. The purpose was to investigate the seroprevalence of FMD and PPR in sheep and goats and to identify possible associations between animal characteristics, management of herds or trade and seropositivity. Serum samples were collected from 480 small ruminants from 160 herds in 40 randomly selected villages. Details on species, breed, origin, sex, age and history of disease were noted for each sampled individual. The owner of each herd was also questioned about management of the herd and trade. Serum samples were analyzed for presence of antibodies to foot-and-mouth-disease virus and peste-des-petits ruminants virus. Potential risk factors were analyzed with Fisher's Exact Test to see if there were any significant correlations with seropositivity for FMD or PPR.

The results showed that the dominating species in the villages was goat, and the dominating gender was female. The majority of the sampled animals were born at the farm where they were sampled, only two were bought from another country. The herd size varied between two and 34 animals. The majority of the farmers let their herds graze freely in the dry season and had them tethered during the rainy season. Seventy-one percent of the herds met other herds of sheep and/or goats on at least a weekly basis, and the proportion that met cattle as often was only 21%. Four percent of the herds met wild ruminants on at least a weekly basis. Most farmers never bought goats or sheep from other countries. The true herd seroprevalence in the Nakonde and Mbala districts was approximately 3.2 percent (95% CI 1.1; 7.4) for FMD and 0.03 percent (95% CI 0;3) for PPR. No risk factors for FMD or PPR seropositivity could be identified.

The study design was considered to have good external and internal validity. The low seroprevalences of both FMD and PPR, relatively to what was expected, are most likely representative of the true seroprevalences in the target population. This study found serological evidence of PPR in a goat in Zambia, but this result needs to be confirmed with other methods since there is a high risk of the result being falsely positive. It could be interesting to do further research on whether there are any protective factors keeping the small ruminants from encountering FMDV and PPRV in this area since the seroprevalence was lower than expected for both diseases.

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INTRODUCTION

Foot and mouth disease (FMD) and peste des petits ruminants (PPR) are two highly contagious viral diseases affecting sheep and goats all over the world with impact on economy and human and animal welfare. They are categorized as transboundary diseases meaning that they are epidemic diseases that can spread extremely rapidly regardless of national borders. Both diseases have been identified by the Food and Agricultural Organization (FAO), the World Animal Health Organization (OIE), the World Bank and the International Livestock Research Institute (ILRI) to have a large impact on the poor, costing billions of USD each year (OIE, 2018a; OIE and FAO, 2015; Perry *et al.*, 2002; Rushton and Knight-Jones, 2012; The World Bank and TAFS Forum, 2011). To be able to control, or even eradicate, these diseases is important for alleviating poverty in many developing countries. Poverty is increasing the fastest in sub-Saharan Africa (Perry *et al.*, 2002), and in 2015, 54.4% of Zambia's population were living below the national poverty line (The World Bank, 2018).

Sheep and goats are often owned by poor and vulnerable small-scale farmers who depend on them for food and other products such as wool, skin and manure as well as income and as an insurance when they are struck by drought or low crop yields (de Haan *et al.*, 2015; Herrero *et al.*, 2013; OIE, 2018a). Women are often the ones taking care of the small ruminants (Animal Production and Health Division, 2013; de Haan *et al.*, 2015; Herrero *et al.*, 2013) which empowers them since it allows them to decide when to sell, how to use the income and thereby provides a sense of security (de Haan *et al.*, 2015). The income from small ruminants is important to see to that children get adequate nutrition and can attend school (Animal Production and Health Division, 2013; de Haan *et al.*, 2015; Herrero *et al.*, 2013). Poor livestock keepers generally experience a higher risk of incursion of animal disease because of confounding factors such as bad housing and poor nutrition (Perry *et al.*, 2002). Animal disease decreases livestock and farm productivity, reduces market opportunity and impairs human welfare (Animal Production and Health Division, 2013) and the bare risk of disease causes high costs when attempting to prevent disease (Gall and Leboucq, 2004; Perry *et al.*, 2002). Disease can also change management in some regions e.g. that farmers choose to not hold one type of livestock because of the high risk of a certain disease affecting that species in that area.

FMD is today endemic in Zambia while no clinical cases of PPR have been found within the country so far (OIE, 2018b). PPR is, however, highly present in Zambia's bordering countries in the north, i.e. Tanzania and the Democratic Republic of Congo. Zambia is therefore under a constant threat of the incursion of PPR.

The purpose of this study was to determine the seroprevalence for the transboundary diseases FMD and PPR in small ruminants in the Nakonde and Mbala districts in Zambia on the border to Tanzania. The study also aimed to identify possible associations between animal characteristics, management of herds or trade and seropositivity.

LITERATURE REVIEW

Why is research on small ruminant diseases important?

In 2002, the International Livestock Research Institute (ILRI) in Kenya carried out a study with the purpose to identify research opportunities within the field of animal health that could have significant impact on poverty reduction (Perry *et al.*, 2002). The first part of the study consisted of mapping the distribution and extent of poverty in Asia and Africa, determining which livestock species that were most important to the poor in these regions and in different production systems, to identify diseases with impact on these species in these regions and finally ranking the diseases based on impact on the poor. Poverty was increasing the fastest in sub-Saharan Africa, and sheep and goats were found to be the most, or the second most, important livestock for the livelihood of the rural poor in Eastern, Central and Southern Africa (ECSA), regardless of the type of production system. A survey conducted by the World Bank, where veterinary officials from 22 African countries answered a questionnaire regarding poverty and animal disease, showed a similar picture (Gall and Leboucq, 2004). The majority stated poultry as the main source of income for the rural poor and thereafter small ruminants.

Goats and sheep contribute to household assets in many ways. They provide income through the sales of milk, meat, hides and animals; they are a source of food and manure for the owners and they are important for social networking (de Haan *et al.*, 2015; Gall and Leboucq, 2004; OIE, 2018a; Perry *et al.*, 2002). Small ruminants are also easy to keep since they can survive under many different conditions, they require minimal management and they feed on diets that do not compete with food production for humans (de Haan *et al.*, 2015; Perry *et al.*, 2002). Most poor livestock keepers, however, depend on common grazing areas and common water points for their animals which favors disease spreading, they live in countries where there is usually a lack of a structured veterinary organization and they have little political impact with a limited access to public resources which halts small ruminant development (de Haan *et al.*, 2015; Rushton and Knight-Jones, 2012). Small ruminants also suffer from many diseases in the tropic and sub-tropic areas for which there are a lack of control strategies and for which very little research has been carried out so far (Perry *et al.*, 2002). ILRI identified small ruminants as an important species to do further research on, as one step in reducing poverty.

Why is research on foot and mouth disease and peste des petits ruminants important?

FMD is endemic in nearly all countries of the developing world (Rushton and Knight-Jones, 2012). Approximately 77% of the global livestock population is estimated to be exposed to FMD. Since FMD affects many species and is highly contagious, all livestock owners in contact with an infected population either geographically or through trade are affected. FMD is estimated to have an annual economic impact of five billion USD due to production losses and vaccination campaigns. According to the World Livestock Disease Atlas, Zambia was the country most affected economically by FMD in the world between 2006 and 2009, mainly due to loss of cattle (The World Bank and TAFS Forum, 2011). FMD was also listed among the top ten diseases causing the highest loss of wild animals in the world.

PPR affects 30 million animals across the globe every year (OIE, 2018a). The economic loss from PPR in the world is estimated to 1.4-2.1 billion USD yearly. The large economic loss is mainly due to animal deaths, reduced production and expenses from fighting the disease (de Haan *et al.*, 2015; OIE, 2018a). FAO states that the eradication of PPR is an important step towards fighting poverty and has together with OIE set up a global strategy to eradicate PPR by the year of 2030 (OIE and FAO, 2015). Halting the spread of PPR into countries at immediate risk today, such as Zambia, is key for protecting small scale farmers that depend on small ruminants for income and food (Animal Production and Health Division, 2013). Control of PPR would increase farm productivity, food security, income and social empowerment (OIE, 2018a).

ILRI listed the 20 diseases assessed to have the largest impact on the poor globally and ranked them according to their impact (Perry *et al.*, 2002). The disease would get a high impact score, and thus have a high ranking, if it had economic impact both at the poor farmer level and national level, if it occurred in species that are important to the poor, if it occurred in multiple species and if it occurred in regions or production systems with a large population of rural poor. FMD and PPR were two of the twenty diseases listed. FMD was ranked among the top ten diseases with the highest impact on the poor in a global perspective, but received lower rankings for the impact on goats and sheep, as well as its impact on the poor in ECSA. PPR received the highest ranking of impact when assessed for the species goats and sheep, but it received a lower ranking for its impact on pastoral and agro-pastoral production systems and for the region ECSA in general. When looking at the top 20 diseases that have the largest impact on the poor in ECSA, neither FMD nor PPR was included. On the other hand, they were both part of the corresponding list for West Africa. In the previously mentioned survey carried out by the World Bank, FMD was identified as one of the diseases with a large impact on poverty, disease control costs, market and trade, and with a high public expenditure for disease control (Gall and Leboucq, 2004). Notably, though, is that only one third of the 22 participants answered this part of the questionnaire. In the World Livestock Disease Atlas, both FMD and PPR were listed among the top ten diseases in sheep and goats that caused the highest loss of livestock units in the world between 2006 and 2009 (The World Bank and TAFS Forum, 2011). PPR was identified as the third most important disease of goat and sheep diseases in the same publication.

In the second part of the ILRI study, the purpose was to assess what animal health research should focus on to achieve the greatest impact on poverty reduction. A lack of basic data on epidemiology and impact of many diseases that affect the rural poor was found and it was concluded that it is very important to ask livestock owners directly about their perceived problems, to involve them in the research and to give them feedback afterwards (Perry *et al.*, 2002). A participatory epidemiology approach to animal disease research would probably give more accurate data compared to conventional methods (Fischer *et al.*, 2016). Including the animal owners in the process of research would also empower them and make them more prone to commit to e.g. a disease control program.

ILRI also saw a need for better information on disease distribution, dynamics and impact to be able to identify risk factors for disease (Perry *et al.*, 2002). ILRI acknowledged FMD and PPR

as important areas of research for alleviating poverty with, among others, a focus on estimating the incidence, impact and transmission dynamics in different production systems.

Overview of diseases

Foot and mouth disease

Foot and mouth disease (FMD) is also a highly contagious viral disease enzootic in many parts of the world including Africa (OIE, 2013). Europe, Australia, New Zealand, Japan, Central- and North America and the Pacific Islands are considered free from FMD. All cloven-hoofed animals including cattle, pigs, sheep, goats and buffalo are susceptible to the disease.

The foot-and-mouth disease virus (FMDV) belongs to the genus *Aphthovirus* and family *Picornaviridae* (Smith, 2014b). It has seven immunologically different serotypes: serotype A, O, C, SAT1, SAT2, SAT3 and Asia1. Infection with one serotype does not yield cross-immunity to other serotypes and within these serotypes there are at least 60 subtypes. Vaccination against one subtype does not necessarily provide immunity for another subtype.

The incubation time for FMD is 2-8 (up to 14) days (OIE, 2013). The clinical presentation of FMD in sheep and goats includes pyrexia, vesicle formation on the buccal and nasal mucous membranes and/or between the claws and coronary band and agalactia in milking sheep and goats (Alexandersen *et al.*, 2003; OIE, 2013). The vesicles ruptures after about 24 hours and leave erosions. The oral and foot lesions often pass by undetected with only mild lameness. The affected animal usually recovers after 8-15 days except for young animals that often die due to viral myocarditis without preceding clinical signs. The clinical presentation of FMD in sheep and goats is indistinguishable from vesicular stomatitis.

FMDV survives drying and may persist for weeks in organic matter when moist and cool (OIE, 2013). Under the right conditions it can survive in the environment for up to one month. It is preserved when refrigerated or frozen but inactivated when heated to a temperature of 70°C for a minimum of 30 minutes. It is also inactivated by sunlight, pH below six or above nine and many disinfectants.

The virus is transmitted through aerosols, airborne droplets, direct contact, indirect contact, consumption of contaminated meat, milk or fodder, artificial insemination with contaminated semen or through humans that can carry the virus in their respiratory tract for 24-48 hours after contact with infected animals (Alexandersen *et al.*, 2003; OIE, 2013). The virus is excreted in all body fluids of infected animals and can also be found in meat products where pH has remained above 6. Animals that have recovered from FMD can carry the virus for a few months up to years depending on the species. African buffalo are the major maintenance host of the SAT serotypes and an individual buffalo can carry the virus for at least five years (Condy *et al.*, 1985). There is evidence of the virus circulating in an isolated herd of buffaloes for at least 24 years over several generations. Sheep can carry the virus for up to five months after exposure according to a study by Burrows in 1968 (Burrows 1968, as cited in Anderson *et al.*, 1976). The role small ruminants play in maintaining FMDV is uncertain (Barnett and Cox, 1999). In a study, investigating whether goats or sheep living in an area where FMD is enzootic in cattle

become carriers of FMDV, showed an almost complete absence of virus carriers in small ruminants (Anderson *et al.*, 1976). In the same study, some goats were also inoculated with FMDV in the coronary band, and one out of 14 goats still carried the virus after three weeks. The most important factor for spreading of FMD within regions endemic for FMD is movement of infected animals (Rweyemamu *et al.*, 2008).

FMD is confirmed through either virus identification or serology (OIE, 2013). Virus can be identified through isolation or PCR on epithelial samples from vesicles or esophageal-pharyngeal fluid.

Peste des petits ruminants

Peste des petits ruminants (PPR) is a highly contagious viral disease affecting sheep and goats in many parts of the world including western and southern Africa, the Middle East, Turkey, central Asia, the Arabic Peninsula, India and Bangladesh (OIE, 2009). The disease is caused by peste-des-petits ruminants virus (PPRV: species *Small ruminant morbillivirus*), classified in the family *Paramyxoviridae* and genus *Morbillivirus* (Amarasinghe *et al.*, 2018). The virus enters through the respiratory mucosa and infects lymphoid tissue in lymph nodes, Peyer's patches, tonsils, splenic corpuscles and cecal lymphoid tissue, where it destroys the germinal centers resulting in the animal being immunosuppressed (Khan *et al.*, 2018; Smith, 2014a). It also destroys the alimentary mucosa. PPRV is very similar to rinderpest virus with the same antigenic properties and causing similar clinical signs (Khan *et al.*, 2018; OIE, 2009). The incubation time is 3-10 days and the first signs of disease are fever, depression, loss of appetite and a serous nasal discharge. The nasal discharge eventually becomes mucopurulent and sometimes can become a profuse catarrhal exudate that occludes the nostrils causing dyspnea. Bronchopneumonia is also a common feature. A few days after the fever debuts, the animals develop erosive lesions in the oral mucosa leading to hypersalivation and halitosis. Most animals also get severe, watery diarrhea that may be bloody. The diarrhea in combination with inappetence, due to the painful oral lesions, results in severe dehydration and cachexia with an often-fatal outcome. PPR can sometimes also cause abortion and conjunctivitis. Goats are in general more severely affected than sheep (Abubakar *et al.*, 2009; Muse *et al.*, 2012; OIE, 2009).

PPRV is spread through airborne droplets and secretions from infected animals, through direct contact and through contaminated fomites (Smith, 2014a). Recovered animals only carry the virus for a few months. Wild ungulates can get infected by PPRV and outbreaks of PPR in wild ungulates have had a large negative impact on the population numbers of some species (Aziz-Ul-Rahman *et al.*, 2018). It is not yet clear whether transmission from wild ungulates and domestic small ruminants can occur (OIE, 2009). It has recently been discovered that experimentally infected pigs and wild boars can transmit PPRV to goats and sheep making them potential sources of infection (Schulz *et al.*, 2018). Cattle can get infected but do not get sick and do not spread the virus further (OIE, 2009). When cattle and small ruminants co-exist, spillover of PPR from sheep and goats to cattle is likely and cattle can therefore be used as indicators of PPRV circulation (Lembo *et al.*, 2013). The virus can remain viable for one year

in frozen tissue, but is inactivated by two hours of sunlight and is sensitive to most disinfectants (Smith, 2014a).

Morbidity rates in susceptible populations are often 90-100% and mortality rates can reach 50-100% in severe instances (Smith, 2014a). Morbidity and mortality are both lower in areas endemic to PPR and in adult animals compared to young animals (OIE, 2009). Outbreaks in areas that are not endemic to PPR can e.g. be controlled through quarantine and slaughter of infected and contact animals. Sanitizing the environment is also important (Smith, 2014a).

PPR is diagnosed through virus identification or serology. Virus can be identified through PCR on discharge, mucosal swabs, whole blood or tissue samples (OIE, 2009).

Risk factors for disease

Foot and mouth disease

In a study carried out on cattle in Ethiopia, large herd size and increasing age were identified to be risk factors for FMD (Bayissa *et al.*, 2011). In another study, also in Ethiopia, associations between herd size, age of animals, contact with wild ungulates and contact with other herds/animals and seropositivity for FMD in cattle, sheep and goats were found (Beyene *et al.*, 2015). Contradicting these results, a study in Israel on FMD in small ruminants, found large herd size and grazing herds to be protective factors (Elnekave *et al.*, 2016). The authors discuss whether it was because these larger and grazing herds were under more intensive management and better biosafety.

Torsson *et al.* (2017) conducted a study in Tanzania during 2014 and 2015 where they investigated the seroprevalence of PPR and some selected differential diagnoses (FMD, bluetongue, bovine viral diarrhea and contagious caprine pleuropneumonia) and searched for risk factors associated with these diseases. They found that interaction with other domestic herds was a significant risk factor for being seropositive for FMD and bluetongue but did not find any evidence that interaction with wildlife was a risk factor for any of the diseases.

Peste des petits ruminants

PPRV seroprevalence has been found to be higher in goats than in sheep and in females compared to males regardless of species (Al-Majali *et al.*, 2008; Aziz-Ul-Rahman *et al.*, 2015; Kardjadj *et al.*, 2015). That might be confounded by the fact that farmers in general keep females for a longer period, increasing the likelihood of them encountering PPRV. Studies on PPR seroprevalence and risk factors carried out in Algeria, Ethiopia and Jordan found that seropositivity for PPRV was significantly higher in herds mixed with sheep and goats compared to only sheep herds and in herds that have had contact with other flocks (Al-Majali *et al.*, 2008; Kardjadj *et al.*, 2015; Megersa *et al.*, 2011). Another study conducted in Pakistan found a higher seroprevalence in sheep than in goats (Abubakar *et al.*, 2009). The authors discuss that it might be due to the fact that sheep more often survive PPR compared to goats.

In the study in Jordan, large herd size, visiting live animal markets and a lack of veterinary services were also identified as risk factors for an animal being seropositive for PPRV (Al-

Majali *et al.*, 2008). In a study in Tanzania, communal grazing and introduction of new animals into the herd were found to be risk factors for PPR (Mbyuzi *et al.*, 2014)

Disease control

Foot and mouth disease

When developing strategies for FMD control, the specific characteristics of the different serotypes must be taken into consideration. Even areas endemic to FMD can suffer from outbreaks if new virus strains enter the country with globalization and increasing trade of animals (Rweyemamu *et al.*, 2008). Disease control aims to minimize direct losses from disease, e.g. losses in animal and herd productivity, but the cost of disease control is an indirect loss from the disease. From an economical perspective, it is therefore important that the losses avoided through the control program are greater than the costs of control (Rushton and Knight-Jones, 2012).

Individual livestock keepers cannot control FMD by themselves. There is always a risk that they will make unequal efforts to control the disease which can result in persisting reservoirs of infection that can re-infect areas where FMD control has been accomplished (Rushton and Knight-Jones, 2012). If an outbreak of FMD would occur in a previously FMD-free country, the FAO and OIE recommendation is to apply stamping out on infected, recovered and susceptible contact animals, if the outbreak is discovered at an early stage (Geering and Lubroth, 2002; OIE, 2013). There should also be a backup vaccination plan in case that the spread of FMD is not contained by stamping out. Ring vaccination, targeted blanket vaccination or suppressive vaccination can be done in selected areas to halt the spread (Geering and Lubroth, 2002). FAO acknowledges that large-scale stamping out is not a possibility for many countries and emphasizes the importance of vaccination in those cases. In countries endemic to FMD, FAO suggests a step-by-step vaccination of different regions in order to successively create FMD-free zones, supported by strong disease surveillance and control of livestock movements.

In Zambia, vaccination campaigns in cattle for FMD are carried out twice per year in areas considered endemic to FMD, i.e. Mbala, Kafue Flats and Southern Province (Musso Munyeme, personal communication, 2018). It has previously been voluntary for farmers to participate in the vaccination campaigns and therefore the vaccination coverage has not always been satisfactory. This year, however, the government has instituted a new law making farmers obliged to take part in vaccination campaigns against all major diseases of national economic importance, including FMD. There is no routine vaccination carried out against FMDV on sheep or goats in Zambia today.

In the case of an outbreak of FMD in Zambia, the primary measure applied is ring vaccination (Musso Munyeme, personal communication, 2018). In some cases, when there is an outbreak in e.g. non-endemic areas and the impact of a potential spread would be devastating, stamping out is applied.

Peste des petits ruminants

OIE and FAO have together set up a global strategy to eradicate PPR by the year of 2030 (Animal Production and Health Division, 2013). PPRV has many traits that favors the prospect of eradicating it globally: i) the virus only has one serotype, ii) there is no carrier state, iii) there is no known reservoir of PPRV outside the small ruminant population, iv) there are vaccines available which are safe, relatively cheap and provide long life immunity from a single dose, and v) there are diagnostic tests available to detect the virus and monitor vaccination programs. There is increasing support for elimination of PPRV among politicians, and the public still remembers the success of rinderpest eradication (Animal Production and Health Division, 2013). One of the challenges with eradicating PPRV is that the small ruminant production has a short reproduction cycle and mainly is run by poor people, making them less prone to be able to invest in animal health such as vaccination. In summary, many technical aspects are in favor of eradicating PPR. The main challenge is to understand small ruminant production locally so that a targeted approach in trying to combat the disease can be made. It is also important with early detection of disease outbreaks and to take action quickly, otherwise it is probable that the outbreaks will be larger, and morbidity and mortality higher, which in turn results in higher costs for disease control and higher impact on livelihoods (de Haan *et al.*, 2015).

FMD in southern Africa

Africa has the greatest diversity of FMD serotypes. Six of the seven serotypes of FMD (O, A, C, SAT-1, SAT-2, SAT-3) have been found there (Rweyemamu *et al.*, 2008). Botswana, Namibia, Swaziland, Lesotho and South Africa have met the conditions of the OIE for zonal or country freedom from FMD without vaccination. There are wildlife areas where FMDV circulates among African buffaloes in some of these countries. In Zimbabwe, Zambia, Mozambique, Malawi and southern Tanzania, FMD spreading was halted during the 1970s and 1980s through restrictions of animal movements and vaccination programs. However, in 2000, there was a spread of FMD from a game park in Zimbabwe where livestock had been exposed to buffaloes because of weakening of the game fencing. Zimbabwe's veterinary services could not handle the outbreak and FMD spread within Zimbabwe and to neighboring countries. Outbreaks of SAT-1 and/or SAT-2 in Mozambique, Malawi and Zambia during 2002-2004 were either due to spread from other countries or contact between cattle and buffalo within the country. Movement of infected animals is the most important factor for spreading FMD.

FMD is present in the DRC, Angola and Tanzania (OIE, 2018b). The latest report of a large FMD outbreak in the DRC was in 2017 with 816 clinically diseased cattle and 36 deaths. In 2016, FMD was reported to be present in Angola but limited to one or more zones in the country (OIE, 2018b). No information about new outbreaks in Angola has since then been reported to the WAHIS. Cases of FMD have been reported in Tanzania each year for the last twelve years (2005-2017); information for 2018 is not yet available (OIE, 2018b). In 2006, the density of FMD outbreaks in Tanzania was greatest along the border with Zambia and on the coastline (Picado *et al.*, 2011). A study of the seroprevalence of FMD in sheep and goats was carried out in 2014 and 2015 in four districts in Tanzania (Ngorongoro, Ulanga, Kilombero and Mvomero) (Torsson *et al.*, 2017). The seroprevalence was 39.0% and 14.1% in 2014 and 2015, respectively.

FMD in Zambia

FMD has two main epidemiological spreading patterns in Zambia. One is related to the serotypes O, SAT1 and SAT2, which are maintained by livestock movement along the border to Tanzania (Sinkala *et al.*, 2014). The other one is related to serotypes SAT1 and SAT2, maintained and spread by African buffalo and through movement of domestic livestock mainly in the Kafue flats and Zambezi Basin.

When looking at previous outbreaks of FMD in Zambia, three high risk areas can be identified: the Southern and Western provinces adjacent to Namibia and Botswana, the Central and Southern provinces on the Kafue flood plains and in the North Eastern provinces on the border to Tanzania (Hamoonga *et al.*, 2014; Overby and Zyambo, 1983). Northern Zambia is under constant threat of FMD spread from southern Tanzania (Rweyemamu *et al.*, 2008). The closest area to Mbala where African buffalo and other wild animals susceptible for FMD can be found is in a game park in Tanzania, about 600 km away (Banda *et al.*, 2014). Therefore, the possibility of interaction between livestock in Mbala and wild mammals is relatively low. However, there is evidence of such interaction between Tanzanian livestock and wild animals on the border of the game park, and villagers in Mbala states that Tanzanian traders often travel to Mbala to sell cattle because the prices are higher in Zambia compared to Tanzania (Banda *et al.*, 2014). The traders then often spend a night or two in the local villages in Mbala on their way back to Tanzania, allowing their cattle to mingle with the local livestock. It is also quite common with intermarriage over the border and cattle are then often exchanged between the families.

Hamoonga *et al.* (2014) conducted a study in Zambia with the aim to quantify any correlation between FMD outbreaks and geographical factors. The study was carried out in cattle and the risk population was defined as all wards in Zambia where there were cattle. The study found a positive correlation between the intensity of FMD outbreaks in a ward and proximity to a large border crossing, proximity to a large road, a low wetness index in the ward and a decreasing median ward elevation above sea level. They could also see a higher frequency of outbreaks during the dry season. The results support the hypothesis that animals in dry areas are more prone to move to find water and to gather at communal drinking pools favoring the spread of FMD.

The most recent outbreak of FMD (serotype O) in Zambia was in March 2018 in the Chisamba and Chibombo districts of Central Province (OIE, 2018b; Musso Munyeme, personal comment.). It consisted of two outbreaks at five dairy farms with a total number of 113 diseased cattle. All the affected farms were quarantined, and movement restrictions were instituted in the area. The government also implemented surveillance within and outside the containment zone, screening of FMD, animal movement control inside the country and ring vaccination campaigns.

PPR in southern Africa

PPR was first discovered in the Ivory Coast in 1942 and has since then spread to over 70 countries in the world of which almost two thirds are in Africa (Animal Production and Health Division, 2013). There have been severe epidemics in sub-Saharan Africa, the Middle East and Asia since the end of the 1970s. The disease has continued its spread reaching Tanzania in 2008 (Swai *et al.*, 2009) and the Democratic Republic of Congo (DRC) in 2012. These countries now constitute the east-to-west southern border of the spread of PPR in Africa and they are both neighboring countries of Zambia. In 2012, there was a large outbreak of PPR in DRC which FAO stated as “the worst livestock epidemic” in the country in over a decade (Limited, 2012). PPR is since then stated as present but limited to one or more areas within the country, although there is so far no information for 2018 available (OIE, 2018b). PPR has also been reported in Angola in 2012, but it was only cases of seropositive sheep and goats that had been imported from the DRC. They were found during routine surveillance of high risk areas; no clinical cases were found. No cases of PPRV seropositive animals or PPR diseased animals in Angola have been reported to OIE since then but there is no information available in the World Animal Health Information Database (WAHIS) for 2017-2018.

PPR was first confirmed in Tanzania in 2008 but the virus was probably present in the country long before that (Swai *et al.*, 2009). It spread to the southern parts in 2009 (Mbyuzi *et al.*, 2014; Muse *et al.*, 2012) and is now considered to be endemic in Tanzania (OIE, 2018b). In a retrospective study of the seroprevalence of PPR in the Mtwara region of southern Tanzania, the seroconversion rate was 35.7% in goats and 28.8% in sheep in 2009 (Mbyuzi *et al.*, 2014). The samples used were collected after an outbreak of suspected PPR with high mortality, which means that the result was probably false low. In another study carried out in the districts Ngorongoro, Ulanga, Kilombero and Mvero in Tanzania, the seroprevalence for PPR was 49.3% and 10% in 2014 and 2015, respectively (Torsson *et al.*, 2017).

Due to the proximity to Tanzania, there is a concern of PPR spreading to the border regions Mbala, Mpulunga and Nakonde in Zambia, because of frequent trade of animals across the border (Chazya *et al.*, 2014). The yearly risk of introducing PPR from Tanzania into Northern Zambia through importation of live goats has been qualitatively assessed to be high (Chazya *et al.*, 2014). In both countries, the factors assessed to determine the risk were: veterinary structure organization, presence and capability of diagnostic facilities, epidemiologic surveillance, status of disease, animal populations and animal movement. The probability that a goat being exported to Zambia was infected with PPRV was moderate. It was due to relatively high seroprevalence of PPR in Tanzania, a lack of diagnostic facilities in Zambia, short transport time between Tanzania and Zambia allowing good viability of PPRV when arriving in Zambia, and that the majority of animals are moved illegally/informal from Tanzania to Zambia and therefore did not go through quarantine before being exported. The probability of exposure to PPRV for susceptible animals was high due to lack of post transit quarantine facilities in Zambia, that goats in Zambia are free-grazing and therefore can spread disease efficiently and that there was no vaccination program for PPRV in northern Zambia. The naivety for PPRV of the goat population in northern Zambia and the fact that PPRV is highly contagious also contributed to the risk. Altogether it was concluded that the risk for introduction of PPRV from Tanzania into

northern Zambia through import of live goats was high and it was recommended that all import should be forbidden until measures have been taken to reduce the risk and shown to be effective.

According to Dr Musso Munyeme, Head of Department of Disease Control at School of Veterinary Medicine at University of Zambia (2018), there were no actions taken after this report by Chazya *et al.* (2014).

PPR in Zambia

In May 2015, OIE received an immediate notification from the Department of Veterinary Services at the Ministry of Fisheries and Livestock in Zambia regarding that goats had been found seropositive for PPRV in four areas in Zambia (see Figure 1) (OIE, 2018b).



Figure 1. Locations where goats seropositive for PPR were found in 2015 are indicated with a blue ring (OIE, 2018b).

The affected areas were all close to the borders of infected or high risk PPR countries, one of the affected areas was Mbala in Northern Province. No clinical cases were found at the time. The discovery initiated a clinical surveillance and laboratory screening of affected areas and high-risk areas thereafter. No new cases of seropositive animals were found, and no clinical cases were identified, so the event was classified as “resolved” and there was no scientific evidence to justify a vaccination program for PPR in Zambia. Researchers were discussing if the documented seropositive goats could be goats that had been illegally imported from bordering countries where they had previously been vaccinated against PPR.

There has not been any reports of PPR in Zambia since then and as of 2018 the only measure made against PPR in Zambia is movement control of cattle, goats and sheep within the country (OIE, 2018b).

MATERIALS AND METHODS

Study design

The study was carried out in the Nakonde and Mbala districts in the northeastern part of Zambia on the border to Tanzania. Forty villages, 30 in Nakonde and ten in Mbala, were randomly selected. In each village, four households keeping goats and/or sheep were selected through snowball sampling which means that after the first household had been identified, the farmer in that household provided information about other households in the village (Lewis-Beck *et al.*, 2004). The aim was to have at least: one household with less than five goats/sheep, one household with 5-15 goats/sheep and one household with over 15 goats/sheep. In the case that there were no goats or sheep in one of the randomly selected villages, another village close by was sampled instead. In the case that there were less than four households with small ruminants in one village, additional households were sampled in other villages as substitution. If there were less than three goats or sheep older than four months in one household, additional goats or sheep were sampled in another household, when possible, in the same village.

The sample size was calculated by using the tool “Sample size to estimate a true prevalence with an imperfect test” on the website Epitools (<http://www.epitools.ausvet.com.au>) that is based on the method described in Humphry *et al.*, (2004). The inputs that were used are specified in Table 1. Sensitivity and specificity for the PPR ELISA were used since they were lower than for the FMD ELISA. The assumed true prevalence was set to 0.5 because that generates the largest sample size. The calculation resulted in a sample size of 444 animals. To make room for any errors during sampling and lab work, the sample size was increased to 480 samples.

Table 1. *Inputs for calculating sample size on Epitools' website*

Assumed true prevalence	0.5
Sensitivity	0.94
Specificity	0.99
Desired precision	0.05
Confidence interval	0.95

Collection of samples

All the samples were collected during six weeks of field work in September-November 2018. Within each household, blood samples were collected from three animals (goats and/or sheep). When the herd consisted of both goats and sheep, the samples were distributed among goats and sheep proportion wise. Only animals more than four months old were sampled to minimize the risk of interference with maternal antibodies and to increase the chance of them having encountered the pathogens of interest. The blood was collected from the animal's jugular vein (see Figure 2) using a syringe, vacutainer, and a blood collection tube without additives (BD vacutainer, Plymouth, United Kingdom). One serum tube was collected from each individual and labeled accordingly to allow identification of each animal sampled. The samples were kept in a cooler box during the day where they were left in a vertical position allowing them to

coagulate and separate. The serum was transferred to 1.5-ml cryotubes within 24 hours of sampling and after transfer, the samples were stored in a freezer. The samples taken in Mbala were centrifuged before transfer of sera. The breed, age, sex, origin, if the sheep or goat had been sick within the last year and if there were any signs of disease present at sampling were noted for each sampled individual. The GPS-coordinates were noted for each household. The study was approved by an ethical committee (ILRI - IREC2018-04).



Figure 2. Collecting a blood sample from a goat. Photo: Owen Malambo, 2018.

Questionnaire

One person responsible for the animals in every household answered a questionnaire (see Appendix 1) regarding management of the herd and trade. This questionnaire was translated by an interpreter to the owner's first language: Namwanga in Nakonde and Mambwe in Mbala. In Nakonde, a local veterinary assistant and other local veterinary staff did the interpretation. In Mbala, the local livestock officer and the district veterinary officer did the interpretation.

ELISA

The samples were analyzed with commercial competitive enzyme-linked immunosorbent assay (ELISA) kits to identify presence of antibodies to FMDV and PPRV. *ID Screen FMD NSP Competition* ELISA, with a sensitivity of 100% and specificity of 99.4% (ID Vet, 2018), that detects anti-FMDV non-structural protein (NSP) antibodies were used. *ID Screen PPR Competition* ELISA, with a sensitivity of 94.5% and a specificity of 99.4% (Libeau *et al.*, 1995), that detects anti-PPRV nucleoprotein antibodies was used. The *ID Screen FMD NSP Competition* ELISA test can differentiate vaccinated animals from infected animals (ID Vet, 2018) while the *ID Screen PPR Competition* ELISA cannot. The kits were used and interpreted according to the manufacturer's instruction.

Statistical analysis

The true prevalence, taking the sensitivity and specificity of the tests in consideration, was calculated through using the tool “*Estimated true prevalence and predictive values from survey testing*” on the website Epitools (<http://www.epitools.ausvet.com.au>). Herd results were investigated for possible risk factors for seropositivity: sex, species, age, contact with other sheep and/or goat herds at least once per week, contact with other cattle herds at least once per week, contact with wild ruminants at least once per week, grazing system, buying animals from other countries and herd size with Fisher’s Exact Test. The “*Easy Fisher Exact Test Calculator*” on the website Social Statistics (<https://www.socsistatistics.com>) was used for the calculations. The level of significance in the analyses was five percent.

RESULTS

Village distribution

In Nakonde, 32 villages were sampled. Out of the 30 villages on the original list, two had to be exchanged for a different village due to no, or too few, small ruminants in the village or owners not being available to give their consent. In two villages, there were too few households with small ruminants and therefore additional households were sampled in a village close by, that were not on the original list, as compensation. In total, 120 households were sampled in Nakonde (see Figure 3). The GPS coordinates could not be noted for the villages Mukuti, Mayembe and Nachisanga due to difficulties with the GPS.

In Mbala, eleven villages were sampled. Out of the eleven villages, two from the original list were exchanged for other villages close by due to one of them being inaccessible by car and the farmers in the other one not agreeing to catch their goats allowing us to sample them. One of the eleven villages had too few households with small ruminants and we therefore sampled additional households in a nearby village that was not on the original list. In total, 40 households were sampled in Mbala (see Figure 3).

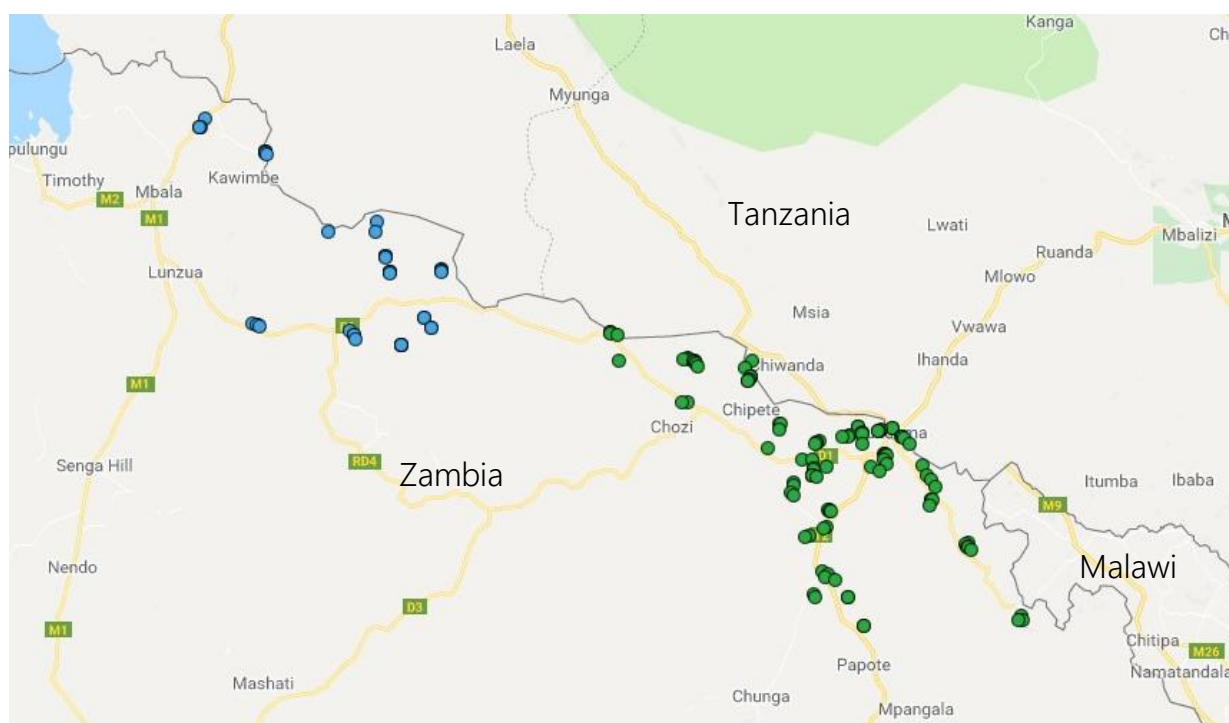


Figure 3. *Marked are the locations of households sampled. Green dots are households within the Nakonde district, blue households are within the Mbala district. Mukuti, Mayembe and Nachisanga village in Nakonde are not marked. Map created at <https://www.hamsternmap.com>.*

Sample distribution

The total number of animals that were sampled was 480. The 480 samples were distributed as can be seen in Table 2. The dominating species in the villages was goat (96%, $n=462$) and the dominating sex was female (75%, $n=360$). All sampled individuals were of local breed. Seventy-six percent ($n=359$) were born at the farm where they were sampled, two percent

(n=10) had unknown origin, eight were given from the government, two were bought from another country (Malawi), one was given through charity and the remaining came from within the village or from a village close by.

The largest herd consisted of 34 animals, the smallest of two. The mean herd size was 8.9 animals (95% CI 7.9;10.0) and the median was seven animals. Ninety-four percent (n=151) were herds consisting only of goats, 1.3 percent (n=2) were herds consisting of only sheep and four percent (n=7) were mixed herds.

Table 2. *Distribution of the 480 blood samples collected in Nakonde and Mbala*

	Herds <i>n</i>	Sheep <i>n</i>	Goats <i>n</i>	≥ 2 years <i>n</i>	Male <i>n</i>	Female <i>n</i>
Nakonde	120	15	345	222	90	269
Mbala	40	3	117	75	29	91
Total	160	18 (4%)	462 (96%)	293 (62%)	119 (25%)	360 (75%)

Questionnaire

Out of the 160 households that were sampled, all answered the questionnaire but some questions were not answered by all participants.

Grazing systems

During the dry period, 85% (n=134) of the farmers let their goats and/or sheep roam freely around their house (communal grazing) and six percent (n=9) out of them occasionally also used other grazing systems, such as tethering, herding or zero-grazing (see Figure 5). Nine percent (n=14) only herded their animals during the dry period and one household only used fenced grazing.

During the rain period, 73% (n=116) of the farmers had their goats and/or sheep tethered for grazing, but 31% (n=36) out of these also used other grazing systems such as herding or communal grazing occasionally (see Figure 4). Twenty-two percent (n=35) strictly herded them during the rainy season and the same household as during the dry season used fenced grazing also under the rain season.

Only 14 households used the same grazing system all year round. The reason for farmers to tether or herd their animals in the rain season was to prevent the animals from eating the crops that are grown in the fields during that period of the year.

Two households did not answer this question completely and they were therefore excluded from the analysis.

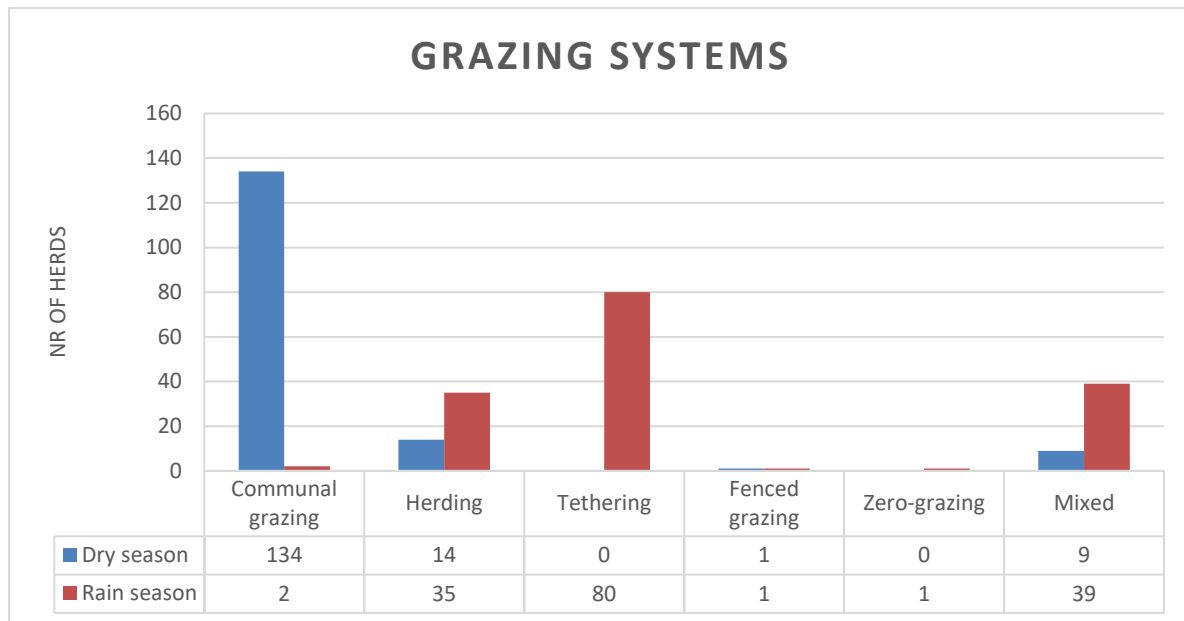


Figure 4. *Distribution of the different grazing systems that were utilized during rain and dry season.*

Contact with sheep and/or goats from other herds

Seventy-one percent (n=115) of the herds met sheep and/or goats from other herds at least once a week. Two out of the 115 households did not specify if it was more frequent during any specific part of the year, but out of the 113 that did specify it, 84% (n=95) only had this contact during the dry season and 14% (n=16) had it all year round. Five percent (n=8) of the herds met sheep and/or goats from other herds more rarely than once per week and 23% (n=37) never met any sheep and/or goats from other herds.

Contact with cattle from other herds

Twenty-one percent (n=34) of the herds met cattle from other herds at least once per week, mostly during the dry period. Seventy-one percent (n=113) never met cattle from other herds at any time of the year and the remaining met cattle more rarely than once per week. One household did not answer this question.

Contact with wild ruminants

Four percent (n=6) met wild ruminants at least once per week when grazing and it was mainly all year round. Eighty-three percent (n=134) never met any wild ruminants.

International trade

Eight households claimed that they had bought sheep and/or goats from other countries. Out of these eight, three had bought animals from Malawi and the remaining from Tanzania.

Vaccinations

None of the sheep and/or goats in the herds that we sampled had been vaccinated against any diseases.

Risk factors

No association could be found between sex, species or age (≥ 2 years) and being seropositive for FMD or PPR. There was neither any significant association between being in a mixed herd, having at least weekly contact with other sheep and/or goats, having at least weekly contact with cattle from other herds, nor having at least weekly contact with wild ruminants and being seropositive for FMD or PPR.

Seroprevalence

The results from the ELISA tests are as described in Table 3. Out of the 480 collected blood samples, four samples in Nakonde and two samples in Mbala were seropositive for FMD. They were all goats, two years or older and female (see Table 3). All the seropositive goats came from different households and had no signs or history within the last year that corresponded to signs of FMD. They were all born at the farm where they were sampled except for one in Mbala that was bought from the neighboring village.

One individual was seropositive, and one was “doubtful” for PPR. The doubtful one is considered as negative in the analyses. The individual seropositive for PPR was a male goat, two years old that has had diarrhea, cough, runny eyes and a runny nose within the last twelve months, but no clinical signs were present when the blood was collected. The locations for the seropositive herds are specified in Figure 5.

The apparent seroprevalence was 1.3 percent (95 % CI 0.3;2.2) for FMD and 0.2 percent (95% CI 0;0.6) for PPR. The true seroprevalence was 1.3 percent (95 % CI 0.3;2.3) for FMD and 0 percent (95% CI 0;0.6) for PPR. The apparent herd seroprevalence was approximately 3.8 percent for FMD (95% CI 0.8;6.7) and 0.6 percent for PPR (95% CI 0;1.9). The true herd prevalence for FMD was approximately 3.2 percent (95% CI 1.1; 7.4) and for PPR 0.03 percent (95% CI 0;3).

Table 3. *Apparent seroprevalence of PPR and FMD*

	PPR			FMD		
	Goats% (n)	Sheep% (n)	Herds% (n)	Goats% (n)	Sheep% (n)	Herds% (n)
Nakonde	0.3 (1)	0 (0)	0.8 (1)	1.1 (4)	0 (0)	3.3% (4)
Mbala	0 (0)	0 (0)	0 (0)	1.7 (2)	0 (0)	5.0% (2)
Total	0.2 (1)	0 (0)	0.6 (1)	1.3 (6)	0 (0)	3.8% (6)

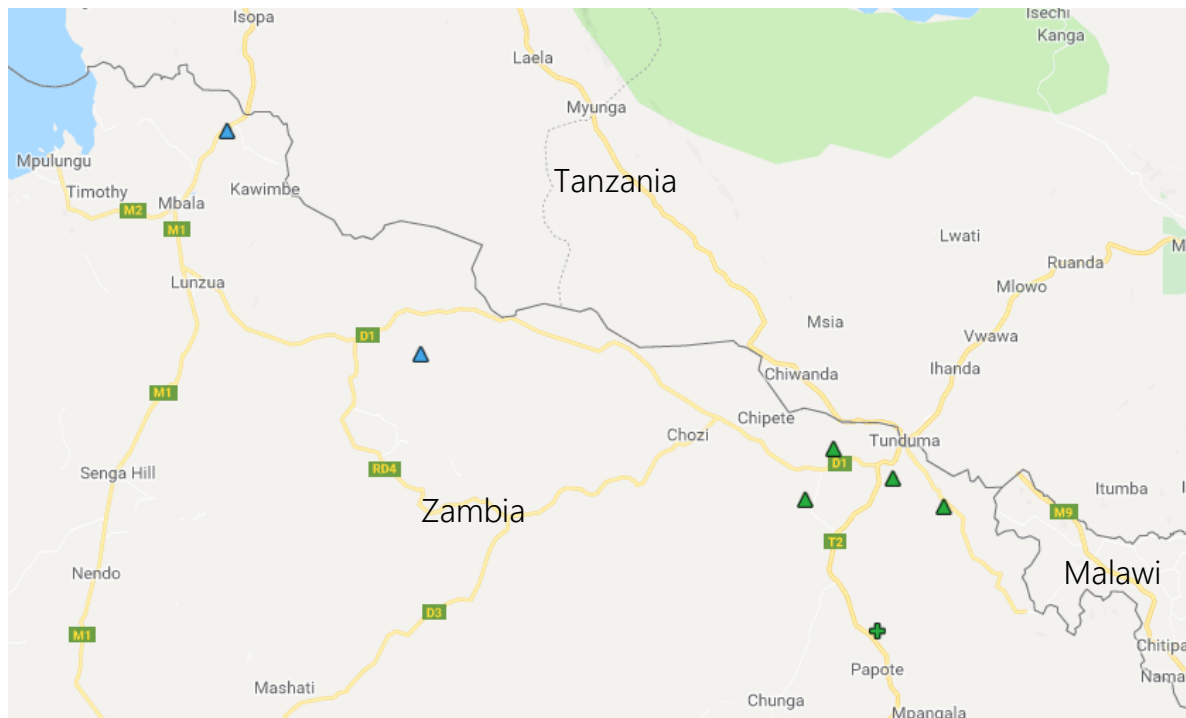


Figure 5. Marked are the locations of seropositive herds. Triangles represent herds seropositive for FMD. Plus represents herds with individuals that were seropositive for PPR. Green coloured markers represent herds in Nakonde district and blue markers represent herds in Mbala district. Map created at <https://www.hamstermap.com>.

DISCUSSION

The purpose of this study was to investigate the seroprevalence of the diseases FMD and PPR in small ruminants in the Nakonde and Mbala districts in Zambia on the border to Tanzania and to see if any predisposing factors could be identified. The true seroprevalence was 1.3 percent (95 % CI 0.3;2.3) for FMD and 0 percent (95 % CI 0;0.6) for PPR. The true herd prevalence for FMD was approximately 3.2 percent (95% CI 1.1; 7.4) and for PPR 0.3% (95% CI 0;3).

Study design

The source population in this study was randomly selected through a two-step selection. Villages were randomly selected within the districts and herds were selected through snowball sampling within the village. A stratified selection of herds within the village was not possible due to too few households with small ruminants in most villages, but small ruminants of different species, gender and age were sampled. Considering these aspects, the source population was most likely representative for the target population, i.e. the total population of small ruminants in Nakonde and Mbala districts. The external validity of this study was considered good.

The tests used to analyze the blood samples had high sensitivity and high specificity, and all the tests were validated. The internal validity of this study was therefore considered to be good. If the prevalence of a disease is low in a population, even a good diagnostic test will however have a low predictive value of a positive test (PVPT) which was the case in this study. The PVPT for the FMD ELISA test was 0.69 and for the PPR ELISA test 0.24, based on the apparent seroprevalence of 1.3 percent for FMD and 0.2 percent for PPR. In this study, the sampling was randomized, which can have contributed to the low seroprevalence. The PVPT could have been increased if the sampling had been directed towards animals that have or had had signs corresponding to FMD or PPR, or through sampling in areas that have suffered from FMD outbreaks previously, i.e. high-risk individuals. The source population would then not be representative for the entire population of small ruminants in Nakonde and Mbala.

Foot and mouth disease

The North Eastern provinces of Zambia have been identified to be high risk areas for FMD (Hamoonga *et al.*, 2014; Overby and Zyambo, 1983). Within the last ten years, there have been outbreaks of FMD in cattle in Mbala in 2010, 2012 and 2016 (OIE, 2018b). No reports regarding outbreaks of FMD in Nakonde have been made to the OIE within the last ten years, but according to the veterinary assistant in Nakonde Central, he has seen signs of FMD in cattle as recently as September 2018 during a vaccination campaign for Contagious Bovine Pleuropneumonia (CBPP) (Owen Malambo, personal communication, 2018.). One of the villages where there was an outbreak of FMD in 2016 in Mbala was Mayembe, which is one of the villages sampled in this study, but no seropositive individuals were found there. Seventy-five percent of the sheep and goats sampled in Mbala were two years or older, which means that the majority of the sheep and goats that were sampled were alive during the last outbreak of FMD in the area. Increasing age and contact with other domestic herds have been identified to be risk factors for FMD seropositivity (Bayissa *et al.*, 2011; Torsson *et al.*, 2017), and 21%

of all the sampled herds in Mbala and Nakonde had at least weekly contact with other sheep and/or goat herds. They were not identified to be risk factors in this study though. Considering these facts, the seroprevalence of FMD was expected to be higher. The information stated above, together with the fact that the PVPT for the FMD ELISA test in this study was 0.69, also indicate that the results are truly positive.

Why the seroprevalence of FMD in small ruminants in this area was lower than what was expected is hard to know. It could be because of errors in sample handling or during laboratory work although it is unlikely (see section Sample Collection and Lab Work below). To my knowledge, there is no study of the longevity of anti-NSP antibodies in sheep and/or goats to this date, but in a study of the longevity of anti-NSP antibodies in cattle naturally infected with FMDV serotype O, antibodies were still present after at least three years (Elnekave *et al.*, 2015). It is hard to know if this could be extrapolated to sheep and goats and for other serotypes of FMDV (the serotype that caused the outbreak in Mbala in 2016 is not reported in WAHIS), but it would be interesting to know since it probably would have large impact on the results of a study like this one. The most likely reason for the low seroprevalence is, however, that foot and mouth disease does not occur that frequent in small ruminants in Mbala and Nakonde today.

Contact with wild ungulates and large herd size have also been identified as risk factors for FMD seropositivity in other studies (Bayissa *et al.*, 2011; Beyene *et al.*, 2015), but no significant association could be found between these factors and FMD seropositivity in this study.

Peste des petits ruminants

In this study, one goat was found seropositive for PPR. It had also had signs within the last twelve months that resembled PPR (diarrhea, runny nose, runny eyes, cough), which makes it more likely that it is a true positive result. Could this then be the first identified case of a goat that had PPR in Zambia? Out of all the seronegative individuals, 32 had had diarrhea within the last twelve months, 37 had had a runny nose or been sneezing. Ten had had both diarrhea and a runny nose and/or been sneezing. No other goat or sheep had had the same range of signs as the one seropositive for PPR. The goat in question was born at the farm, so it could not be a previously vaccinated goat that had been imported from Tanzania as was expected in 2015, when other goats seropositive for PPR were found (OIE, 2018b). It could not be positive due to maternal immunity either since it was already two years old. The PVPT for the PPR ELISA test was however only 0.24 which means that the positive result is more likely to be false positive than true positive. Based on this information, it is therefore not likely that this goat has encountered PPRV. The result needs to be confirmed through a virus neutralization test before there can be any conclusions drawn about the presence of antibodies to PPRV in Zambia.

The seroprevalence of PPR was expected to be higher in the two regions because of the following factors: i) PPR is present in Tanzania (OIE, 2018b), ii) the risk of PPR spreading to Mbala and Nakonde from Tanzania has been assessed as high (Chazya *et al.*, 2014), iii) illegal movement of sheep and goats occur frequently across the border (Banda *et al.*, 2014; Chazya *et al.*, 2014) and iv) communal grazing, a lack of veterinary services and contact with other

sheep and/or goat herds have been identified as risk factors for PPR seropositivity (Al-Majali *et al.*, 2008; Mbyuzi *et al.*, 2014). In this study, 90% of the herds used communal grazing at least during the dry season and 72% had at least weekly contact with other sheep and/or goat herds. According to the study carried out by Chazya *et al.* (2014) and according to the veterinary assistant working in central Nakonde ward, there is a great understaffing of the veterinary wards in Nakonde and Mbala, and the existing veterinary staff has no access to vehicles making it hard for them to assist people in the rural areas (Chazya *et al.*, 2014; Owen Malambo, personal communication, 2018). There is at the moment a lot of transports going through Nakonde between Tanzania and the Democratic Republic of Congo (DRC), where PPR also is present (OIE, 2018b), because of the current war in the DRC forcing transports going different routes than usually (Owen Malambo, personal communication, 2018). This could compose a source of disease spreading, especially for FMD. Truck drivers also tend to buy live goats along the route that they bring to use as a source of food when on the road. They sometimes carry the goats alive for several kilometers before being slaughtered, which is an additional potential source of disease spread. In summary, these different aspects contributed to the expectation that there would be more individuals seropositive for PPR.

There are several possible reasons for the low result of PPR seropositivity: i) the trade of goats is mainly in the direction from Zambia to Tanzania due to a higher demand in Tanzania (Owen Malambo, personal communication, 2018), making spread from Tanzania less likely, ii) the great majority of the sampled animals were goats and goats are more severely affected than sheep with high mortality (Abubakar *et al.*, 2009; Muse *et al.*, 2012; OIE, 2009), i.e. the positive ones might be dead already and, most likely, iii) PPRV might not be present in Zambia since no clinical cases have been confirmed in the country yet (OIE, 2018b).

Questionnaire

The questionnaire was interpreted to the owner's first language and the answers were translated back to English by the interpreter and then written down in the questionnaire by me. Some information might therefore have been lost in translation.

When interviewing the farmers for the questionnaire, some farmers were reluctant to answer some questions, and some may not have answered honestly. For example, some were afraid to say that their small ruminants had contact with wild ruminants since they did not want people to know that they had them in the area. Some were also afraid to confess that they had problems with certain diseases because they had heard of other farmers losing their animals after outbreaks of some diseases (stamping out). The veterinary assistant, and the district veterinary officer, have authority among the rural people and therefore there was a risk of them giving answers with the will to please, e.g. saying that they do not buy animals from other countries even though they do. Farmers may also have problems remembering, e.g. if the animal has been sick within the last twelve months, how old it is and where it came from. This constitutes recall bias, which was hard to avoid in this study since the questionnaire solemnly relied on the farmers' memory. Recall bias could have been decreased through e.g. looking at the animal's teeth to get more information about its age.

There were also some differences regarding what farmers considered to be another herd. Some considered their herd and the ones nearby as one herd and answered to the questions about other herds in that context. This could have been specified in the questionnaire to avoid this confusion.

Sample selection

One possible selection bias in this study is that snowball sampling was used for choosing the different households within a village. This could mean that farmers might send us to their friends and, assuming that friends might have similar management of animals, only households with similar management in a village could have been sampled. It could also mean that the farmers close by to each other were sampled, increasing the chance that their animals are in contact with the other households' animals being sampled. The general perception, though, was that it was hard to even find four households keeping small ruminants in one village in most cases and that the four households sampled were the majority of goat and/or sheep holders in the village. The answers regarding management of the animals in the questionnaire showed that the majority of the farmers had a similar management of their small ruminants during dry and rainy season, regardless of their location. Snowball sampling was probably the best method to reach this many households with small ruminants.

The aim was to sample herds of at least three different herd size categories within each village (as specified in the Material and Methods section). This was in most cases not possible because the majority of herds in a village were in general about the same size. Unfortunately, there was therefore no stratified selection of herds within the villages but the distribution of herd sizes in this study probably represents the general distribution of herd sizes in the sampled districts.

Another possible selection bias is how the individuals in each herd were picked out for the blood sampling. Most goats and sheep were roaming around freely during the time of the sampling and therefore they had to be caught to allow us to sample them. A large proportion of the herds were not used to be closed in during this period of the year and the animals were therefore hard to catch. It resulted in that it was impossible to randomly pick out specific animals for sampling, it was simply a matter of convenience sampling where we sampled the ones that were managed to be caught. It could have introduced selection bias as e.g. if more elderly or sick animals were easier to catch. Approximately a third ($n=163$) of the sampled individuals presented with signs of disease at the time of sampling, and only 3.3 percent were eight years or older at the time of sampling. This contradicts the hypothesis that a majority of elderly and sick animals were sampled.

Sample collection and lab work

Another possible observation bias in this study is that it was hard to keep the cold chain for the serum samples during transport from the field to the lab which could have affected the results. They were never warmer than 15 degrees Celsius but they were frozen and thawed several times during transport and during lab work. In the lab, many errors that can occur, e.g. wrong temperature in the incubation chamber, pipettes pipetting the wrong volume, incomplete mixing of sample or uneven incubation times between different sampling because of too slow transfer

of samples to wells, can have affected the results. It is not likely, though, since all the plates were validated and there was no skewed distribution of positive results between different batches of samples or different plates.

CONCLUSIONS

The study design was considered to have good external and internal validity. The low seroprevalences of both FMD and PPR, relatively to what were expected, are most likely representative of the true seroprevalences in the target population. This study found serological evidence of PPR in a goat in Zambia, but this result needs to be confirmed with other methods since there is a high risk of the result being falsely positive. No risk factors for FMD or PPR seropositivity could be identified in this study. It could be interesting to do further research on whether there are any protective factors keeping the small ruminants from encountering FMDV and PPRV in this area since the seroprevalence was lower than expected for both diseases.

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POPULAR SCIENCE SUMMARY

Viral diseases in sheep and goats in northeastern Zambia

Foot and mouth disease and sheep and goat plague are two very contagious diseases affecting sheep and goats. They have a large impact on economy and both human and animal welfare in many developing countries. Sheep and goats are important to the rural poor, providing them with food and income. More than half of Zambia's population is living in poverty, most of them in rural areas, and many of them keep sheep and goats. Zambia shares borders with many countries and due to illegal movement of animals across borders, there is a constant threat of incursion of new diseases into Zambia. This study searched for the diseases sheep and goat plague and foot and mouth disease in sheep and goats in northeastern Zambia on the border to Tanzania.

Foot and mouth disease (FMD) is also a highly contagious disease that affects sheep and goats but also cattle, pigs and buffalo. The disease is mild in sheep and goats and can often go undetected. The virus causes the formation of blisters on the feet and in the mouth, which can result in the animal having a mild lameness and being unwilling to eat. Affected animals usually recover on their own except for young ones that can die suddenly due to heart affection.

Sheep and goat plague (peste des petits ruminants, PPR) is a very contagious disease affecting sheep and goats. It is caused by a virus that spreads between animals through airborne droplets or secretions from diseased animals. When the virus infects an animal, it suppresses its immune system and the animal develops fever, nasal discharge and loses its appetite. It can sometimes struggle to breathe because of a very profound and thick nasal discharge that can block the airways. Most diseased animals also develop blisters in the mouth and severe diarrhea and the disease often leads to death.

Poverty is increasing the fastest in sub-Saharan Africa and in 2015, more than half of Zambia's population was living in poverty, the majority in rural areas. Sheep and goats are important livestock species for the rural poor in many developing countries, including Zambia. They provide their owners with food, wool, skin and manure and can serve as an insurance if the household is struck by drought or low crop yields. Women are often the ones taking care of the small ruminants which empowers them since it allows them to decide when to sell, how to use the income and thereby provides a sense of security. The income from small ruminants are important to see to that children get adequate nutrition and can attend school. Sheep and goats are also easy to keep, require very little management and do not compete with food resources for humans.

FMD and PPR exist in many parts of the world including large parts of Africa. FMD is endemic in parts of Zambia which means that animals affected by the disease can constantly be found in those areas. The last outbreak of FMD in Zambia was in March 2018 in cattle. PPR is not yet present in Zambia but exists in many of its neighboring countries in the north: the Democratic Republic of Congo and Tanzania.

The International Livestock Research Institute, located in Nairobi in Kenya, carried out a study with the purpose to identify research opportunities within the field of animal health that could have significant impact on poverty reduction. Within this study, sheep and goats were identified to be important species to do further research on, as one step in reducing poverty. The same study also identified FMD and PPR as important diseases for research in the future. Control or even eradication of FMD and PPR have also been identified to be key steps in alleviating poverty by other organizations such as the World Bank, the Food and Agricultural Organization and the World Animal Health Organization.

This study's purpose was to investigate the proportion of goats and sheep that had encountered the viruses causing FMD and PPR in the two districts Mbala and Nakonde in northeastern Zambia, on the border to Tanzania. It also aimed to find if there were any factors making certain animals more prone to encounter these diseases. The study area was chosen due to its proximity to Tanzania, where FMD and PPR are highly present, and because it is characterized as a high-risk area for both FMD and PPR.

The study was carried out in September-December 2018. Blood samples were collected from goats and sheep from 160 herds in 40 villages that had been randomly picked within the study area. Three goats and/or sheep were sampled from four different households in each village, 480 animals were sampled in total. The age, sex, breed and disease history were noted for each sampled individual. One person in every household was also interviewed regarding management of the herd and trade. The interview was carried out by local veterinary staff in the local language.

The majority of the sampled animals were goats, and most were female. Most farmers let their goats and sheep graze freely on their own in the dry season and most tethered them during the rainy season. The majority of the herds met other herds of sheep, goats and/or cattle at least once per week but never wild ruminants. Only eight households claimed to have bought sheep and/or goats from outside of Zambia. None of the farmers had had their animals vaccinated.

The collected blood samples were analyzed for antibodies for the viruses that cause FMD and PPR. Antibodies are part of the animal's immune system and are created when an animal gets infected by a specific virus. They stay in the blood stream even after the animal has recovered from the illness and can thereby be used as indicators of previous disease. The results indicated that six goats had encountered FMD and one goat had encountered PPR. There is however always a risk that some of the positive results might be falsely positive due to that the diagnostic tests used in this study are not entirely perfect. This imperfection is pronounced when a very small proportion, as in this case, are positive. It would therefore be necessary to confirm these positive results with an additional diagnostic test.

The details of the animals and the answers from the interviews were analyzed statistically together with the results from the blood analysis to see if any factors that predisposed the animal for disease could be found. Hypotheses tested were for example if an animal's species, age or gender had an impact on the risk of encountering FMD or PPR, or if herds that met other herds with sheep, goats and/or cattle on a regular basis were predisposed to these diseases. No

predisposing factors could be identified in this study and one reason for this could be the low number of positive animals. Previous studies in other countries have identified increasing age and contact with other herds to be risk factors for FMD and mixed herds, contact with other sheep and/or goat herds, large herd size, grazing freely and a poor veterinary organization to be risk factors for PPR. Many of these risk factors were present in the sampled herds and the seroprevalences found in this study were therefore lower than expected.

In summary, this study showed evidence of FMD in goats in Zambia. There was one goat that tested positive for PPR but this result needs to be confirmed since there is a high risk of it being falsely positive. If PPR was to spread within Zambia's sheep and goat population it could have a devastating outcome, because it spreads fast and is highly lethal. The number of goats that had antibodies for FMD was lower than expected but control or even eradication of FMD would still be economically beneficial, especially because of its impact on cattle production.

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APPENDICES

Appendix 1 – Questionnaire

1) What grazing system are you utilizing?

The comment column is for example if the respondent utilizes several grazing systems, then ask him/her to clarify what influences his/her decision on which system to use and indicate this in the comment column.

Grazing system	1= YES 0 = NO	Comment F e only during rainy season, only pregnant mothers
Communal grazing		
Commercial grazing/Fenced grazing		
Tethering		
Herding		
Zero-grazing		
Other, please specify _____		

2 a) How often are your sheep and/or goats in contact with sheep and goats from other herds?

FREQUENCY	Check the one that applies
Daily	
At least once a week	
At least once a month	
At least once every 6 months	
At least once a year	
More rarely	
Never	

2 b) ASK only if respondent did not answer NEVER on 1.2a):

Does this vary over the year? If yes, how?

(For example; more contact during dry season etc)

3 a) How often are your sheep and goats in contact with cattle from other herds?

FREQUENCY	Check the one that applies
Daily	
At least once a week	
At least once a month	
At least once every 6 months	
At least once a year	
More rarely	
Never	

3 b) ASK only if respondent did not answer never on 1.3a):

Does this vary over the year? If yes, how?

(For example; more contact during dry season etc)

4 a) How often are your sheep and/or goats in contact with wild ruminants?

FREQUENCY	Check the one that applies
Daily	
At least once a week	
At least once a month	
At least once every 6 months	
At least once a year	
More rarely	
Never	

4 b) ASK only if respondent did not answer never on 1.4a)

Does this vary over the year? If yes, how?

(For example; more contact during dry season etc)

5) Do you vaccinate your sheep and/or goats?

YES	NO
<p>If YES; for what diseases?</p> <p>If YES; when was the last time they were vaccinated?</p> <p>If YES; were all animals vaccinated?</p>	

6) Have you ever bought sheep and/or goats from other countries?

YES	NO
<div data-bbox="327 414 502 504"><input type="text"/></div> <p data-bbox="204 683 630 728">Please specify country/countries:</p> <div data-bbox="204 806 606 817"><hr/></div> <p data-bbox="204 929 742 1019">Approximately how many times have you bought goats/sheep from other countries?</p> <div data-bbox="204 1097 606 1108"><hr/></div> <p data-bbox="204 1344 678 1433">When was the last time you bought a goat/sheep from another country?</p> <div data-bbox="204 1478 606 1489"><hr/></div>	<div data-bbox="925 414 1101 504"><input type="text"/></div>