Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Biomedical Sciences and Veterinary Public Health

Prevalence and Risk Factors for BVDV in Goats and Cattle in and around Gaborone, Botswana

Sara Lysholm

Uppsala
2016

Degree Project 30 credits within the Veterinary Medicine Programme

ISSN 1652-8697
Examensarbete 2017:56

Prevalence and Risk Factors for BVDV in Goats and Cattle in and around Gaborone, Botswana

Prevalens och riskfaktorer avseende BVDV infektion hos getter och nötkreatur i Gaborone, Botswana

Sara Lysholm

***Supervisor**: Mikael Berg, Department of Biomedical Sciences and Veterinary Public Health (BVF), Swedish University of Agricultural Sciences (SLU)*

***Assisting supervisors**:*
Jonas Johansson Wensman, Department of Clinical Siences (KV), Swedish University of Agricultural Sciences (SLU)

Solomon Stephen Ramabu, Department of Animal Science and Production, Botswana University of Agriculture and Natural Resources (BUAN).

***Examiner**: Maja Malmberg, Department of Biomedical Sciences and Veterinary Public Health (BVF), Swedish University of Agricultural Sciences (SLU)*

Degree project in Veterinary Medicine

***Credits**: 30 hp*
***Level**:Second cycle, A2E*
***Course code**: EX0751*

***Place of publication**: Uppsala*
***Year of publication**: 20xx*
***Number of part of series**: Examensarbete 2017:56*
***ISSN**: 1652-8697*
***Online publication**: http://stud.epsilon.slu.se*

***Keywords**: BVDV, Bovine Viral Diarrhoea Virus, seroprevalence, risk factors, Botswana, goats, cattle, livestock*
***Nyckelord**: BVDV, Bovint Virus Diarré Virus, seroprevalens, riskfaktorer, Botswana, getter, nötkreatur, boskap*

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
Department of Biomedical Sciences and Veterinary Public Health

SUMMARY

Bovine Viral Diarrhoea Virus (BVDV) is a cause of severe deterioration in animal health as well as grave economic losses globally. Infection is often inapparent but the virus can also cause respiratory signs, diarrhoea, pyrexia, decreased production, immunosuppression and reproductive problems such as increased calving intervals and abortions. Also, when naive dams in early pregnancy are infected, before the development of fetal immune competence, the fetus is at risk of developing persistent infection. These persistently infected individuals (PI) are of particular epidemiologic interest since they shed virus in large concentrations in all their bodily secretions throughout their life. Risk factors for disease transmission include, but are not limited to, herd size, animal trade and grazing on communal pastures. For goats, contact with cattle is a significant risk factor.

Several prevalence studies have been conducted on the African continent, but in the country of Botswana, the occurrence is largely unknown. Because of this, blood samples were obtained from 100 goats owned by 11 smallholder farmers, in three different villages just outside of Gaborone. Besides this, 361 blood samples from cattle collected as part of another study were analysed. The detected antibody prevalence was 0% in goats and 53.5% in cattle. In dairy cattle, the seroprevalence was 49.7% and in beef cattle 56.7%, but this difference was not statistically significant. The prevalence of virus in cattle was 0.27% on Ag-ELISA and PCR performed in Botswana, and 0.83% on PCR performed in Sweden. The viraemic animals all originated in the two herds with the highest prevalences (88.1% and 97.9% respectively). Finally, PCR analysis was performed and a short sequence of the genome of two of the detected viruses were sequenced, and found to belong to the BVDV-1a genotype.

The goat farmers were also subjected to a short interview regarding risk factors for BVDV transmission, as well as the general health status in their herd. All farmers allowed their animals to graze on communal pastures, and 64% reported to also keep cattle in close proximity to their goats. Also, 18% answered that they keep sheep and goats together in the same kraal (i.e. enclosure) during the night. Approximately 55% responded that they occasionally saw wildlife ruminants in the area where their goats were kept. However, only 18% purchased goats from other farmers as the majority relied solely on raising their own kidlings. The most common health problem described was abortion, which 91% occasionally struggled with. Besides this, 64% also reported problems with diarrhoea, 36% with coughing and 18% with ocular and nasal discharge. Last but not least, all farmers stated that they depend on their goats for food and cash income and that it would affect them greatly if their animals would fall sick or die in large numbers.

SAMMANFATTNING

Bovint Virus Diarré Virus (BVDV) är en global orsak till nedsatt djurhälsa samt ekonomiska förluster. Infektionen är ofta subklinisk men viruset kan också orsaka respiratoriska symptom, diarré, pyrexi, nedsatt produktion, nedsatt immunförsvar samt reproduktiva störningar såsom förlängda kalvningsintervall och aborter. När seronegativa hondjur infekteras under tidig dräktighet, innan fostret bildat immunokompetens, finns en risk att avkomman utvecklar persistent infektion (PI). Dessa djur är av särskilt epidemiologiskt intresse då de utsöndrar virus i höga koncentrationer i samtliga kroppsvätskor under hela deras liv. Riskfaktorer för virusspridning är exempelvis stor besättningsstorlek och hög djurdensitet. Även driftrutiner såsom djurhandel och utnyttjande av gemensamma betesmarker inverkar. För getter är kontakt med nötkreatur en riskfaktor.

Ett flertal prevalensstudier avseende BVDV har utförts på den afrikanska kontinenten, men i Botswana är förekomsten i stort sett okänd. På grund av detta utfördes blodprovstagning på totalt 100 getter, vilka ägdes av 11 småbrukande bönder hemmahörande i tre olika byar utanför Gaborone. Utöver dessa analyserades även 361 blodprover från nötkreatur, vilka tagits som en del av ett annat forskningsprojekt. Antikroppsprevalensen var 0% för get och 53,5 % för nötkreatur. För mjölkkor var seroprevalensen 49,7% och för köttkor 56,7%, men denna skillnad var ej statistiskt signifikant. Prevalensen av BVD-virus, baserat på resultat från Ag-ELISA och PCR, var 0,27%. När PCR:en senare upprepades i Sverige blev resultatet 0,83%. Samtliga viraemiska djur härstammade från de två besättningar med högst seroprevalens (88,1% resp 97,9%). Slutligen gjordes en PCR analys och en del av genomet sekvenserades för två av de detekterade virusen, och befanns tillhöra genotypen BVDV-1a.

Småbrukarna fick även i en kort intervju svara på frågor avseende riskfaktorer för spridning och smitta av BVDV samt den generella hälsostatusen i deras besättning. Intervjuerna påvisade flertalet riskfaktorer för virusspridning. Samtliga av bönderna lät sina djur beta fritt på allmänna betesmarker och 64% angav att de höll nötkreatur i nära anslutning till getterna. Ungefär 18% höll getter och får i samma inhägnad nattetid. Cirka 55% angav att de då och då såg vilda idisslare i sitt närområde. Endast 18% köpte getter från andra bönder, då majoriteten enbart använde sig av egen uppfödning av killingar. Det vanligaste hälsoproblemet var aborter, vilket 91% angav att de hade problem med till och från. Av bönderna hade även 64% bekymmer med diarré, 36% med hosta och 18% med ögon- och nosflöde. Samtliga angav att de är väldigt beroende av deras getter för föda och inkomst, och att ett större sjukdomsutbrott med dödsfall skulle ha en allvarlig påverkan på deras livssituation.

content

Introduction

The purpose of this study was to investigate the prevalence of Bovine Viral Diarrhoea Virus (BVDV) in cattle and in goats, and risk factors for BVDV infection in goats, in and around Gaborone, Botswana. Botswana is situated in southern Africa and is classified as an upper middle-income country. However, Botswana is also currently struggling with big income gaps and widespread poverty (The World Bank, 2016). The vast majority of the population is earning their living through agriculture (Svenska FN-förbundet, 2014). Due to harsh environmental conditions and poor soils, the rearing of livestock plays the most vital role (Bahta & Malope, 2014).

One obstacle for improving the productivity and welfare of livestock is infectious diseases. Some infections have devastating consequences with high morbidity and mortality. Others have more subtle clinical sings, but can lead to impaired production and hence decreased income for the farmer. One important disease that causes decreased production is Bovine Viral Diarrhoea (BVD). The causal agent of BVD is Bovine Viral Diarrhoea Virus (BVDV), a pestivirus that is endemic in a big part of the cattle producing world (Lindberg & Houe, 2005). The virus causes grave economic losses, first and foremost due to its' impact on reproduction (Houe *et a*l., 1993; Krametter-Froetscher *et a*l., 2007; Ståhl *et al*., 2012) as well as on the general health status in the herd (Krametter-Froetscher *et al*., 2007; Ståhl *et a*l., 2012). It also causes decreased production, for example due to reduced milk yield, decreased fertility, growth retardation and increased susceptibility to other diseases (Walz, 2015).

Cattle are the primary host of BVDV, but approximately 40 other species are also susceptible, including sheep and goats. Clinical signs vary somewhat between species, but in general the disease profile is similar (Nettleton, 1990). Primary infection is often inapparent and subclinical, but severe outbreaks with high mortalities have been reported. Some animals may display respiratory signs, pyrexia, diarrhoea, and reproductive signs such as abortions and stillbirths. Also, animals infected in early pregnancy, before the development of fetal immune competence, may give birth to persistently infected young. These individuals are immunotolerant, persistently viraemic and fail to seroconvert, and will therefore spread the virus in high concentrations in all their bodily secretions for the rest of their life. Therefore, persistently infected individuals play a key role in the epidemiology and transmission of the virus (Walz, 2015).

Very little research has been conducted on BVDV in Botswana. The prevalence studies that have been performed are, to the author's knowledge, few and old. Knowledge about prevalence and risk factors is cruical in establishing national control measures, which could lead to reduced disease incidence and risk nationwide. This would not only improve the health and welfare of Botswana livestock, but may also ultimately lead to increased income and wealth for farmers.

Literature review

Botswana

Botswana is situated in southern Africa and has a population of approximately 2 million people (Svenska FN-förbundet, 2014), the majority of which are inhabiting the eastern part of the country. In the capital Gaborone, the population is just over 200 000. The country is landlocked and borders South Africa, Namibia, Zambia and Zimbabwe (Central Intelligence Agency, 2016). Botswana is currently classified as an upper middle-income country, but the country is also struggling with big inequalities and income gaps, as well as widespread poverty (The World Bank, 2016).

Botswana has two different climate classifications depending on the investigated regions i.e., hot semi-arid and hot desert climate (Climate Data, 2017a). The South-East District where Gaborone is situated is classified as a hot semi-arid region, with an average temperature of 20.3 °C and average precipitation of 457 mm (Climate Data, 2017b). Most of the rainfall occurs during the rainperiod, which lasts from October to April (Landguiden, 2017). However, dryspells are common and sometimes leads to food shortages and hunger (FAO, 2014). More than 2/3 of the country is covered by the Kalahari Desert and approximately 1/5 of national parks and game reserves (Landguiden, 2017). The main income generating businesses are mining and tourism, but the vast majority of the people are making their living on subsistence agriculture (Svenska FN-förbundet, 2014).

Agriculture in Botswana

Approximately 39 % of people in Botswana live in rural areas (Svenska FN-förbundet, 2014), where economy is mainly based on subsistence smallholder farming (Panin, 2000). On a national scale, approximately 2% of the Botswana GDP per capita is contributed by agriculture (Central Intelligence Agency, 2016). Due to the harsh climate and poor soils, only about 0.65% of the land is suitable for arable agriculture (Central Intelligence Agency, 2016). Therefore, crop production is risky and often low in profit. Consequently, a lot of farmers choose to keep livestock instead or combine livestock and crop production (Bahta & Malope, 2014).

Livestock

Livestock is a significant contributor to smallholder farmers (Aganga *et al.*, 2005) and is extremely important from a socio-economic point of view (Central Intelligence Agency, 2016). Beef production remains the most important activity (Mrema & Rannobe, 1994; Aganga *et al*., 2005), and the country currently has a cattle population of just over 2 million, with an average flock size of approximately 52 (Statistics Botswana, 2016). However, the rearing of small stock is becoming increasingly more important. Ownership of goats is encouraged by the Botswana government through various projects and policies (FAO, 2014). The goat population in the country has been estimated to 1.26 million with an average flock size of 28 (Statistics Botswana, 2016), whereof approximately 99% are owned by smallholder farmers (Hellyer *et al*., 2015). However, the sheep population is only about 265 000 (Statistics Botswana, 2016). This difference is likely due to a greater profitability in keeping goats than sheep in the harsh local environmental conditions (Panin *et al*., 1994).

Almost every rural household owns at least one small stock, whereas only some farmers keep cattle. This is because cattle rearing demands more capital investment (Panin *et al*., 2000). In a study by Panin *et al*., (2000), the economic aspects of cattle vs small stock rearing in the Kgatleng agricultural district around Gaborone was compared. It was found that the return of invested capital was only slightly higher for cattle compared to goats (30% vs 28%), which suggests that both enterprises are almost equally cost-

effective (Panin *et al*., 2000). Another study by Panin *et al*., (1994) found that livestock contributed with about 49% of the household total net income, whereas crop growing only accounted for about 6%. In the livestock sector, cattle accounted for approximately 33%, and small stock for approximately 15% (Panin *et al*., 1994).

Livestock in Botswana are managed in two fundamentally different production systems i.e., the traditional and commercial system. The main difference between the two lies in tenure of land. In traditional systems, animals graze on communal pastures, without defined properties or rights to grazing resources. In the commercial systems, farms are generally fenced and rights to land resources exclusive. Approximately 81.5% of cattle and 95% of small stock utilise communal grazing (Statistics Botswana, 2016).

Constraints and risk factors in goat rearing

Several risk factors concerning the rearing of goats around Gaborone have been identified. Farmers that keep both cattle and small stock often manage them extensively together (Panin *et al*., 2000), which enables trans-species transmission of pathogens. Bucks are often rotated between herds, permitting inter-herd spread of infectious agents. In a study conducted around Gaborone by Panin *et al*., (1994), it was found that approximately ⅓ of the kids died before weaning. Diseases and parasites accounted for approximately 44.6% of the deaths, followed by predators at 26.8% and unknown causes such as poor mothering at 25%. A common predator of kids venturing outside of the kraals (i.e. enclosures) were jackals. Adult mortality was found to be 5.6%. Several of the farmers listed internal and external parasites as limiting factors for production, as well as unavailability of feed (Panin *et al*., 1994). In a study by Mmrema and Rannobe (1994), a major constraint to goat rearing in Botswana was identified as poor housing, with co-housing of different species in open kraals that are cleaned only occasionally. This is likely to contribute to to disease outbreaks. Also, keeping animals in large numbers in villages can lead to overgrazing, especially during winter, subsequently resulting in poorer feeding qualities each year. Additionally, a low ratio of veterinary assistants as well as high costs of veterinary services causes additional constraints (Mrema and Rannobe, 1994).

Pestiviruses

Pestivirus is a genus in the family *Flaviviridae*. Other members of the genus include Border Disease Virus (BDV) and Classic Swine Fever Virus (CSF) (International Committee on Taxonomy of Viruses, 2015). Traditionally, pestiviruses isolated from cattle were termed BVDV, from sheep BDV and pigs CSF. However, interspecies transmission is common, and classification today hence more focused on nucleotide sequencing and reactivity with monoclonal antibodies rather than host species (Nettleton *et a*l., 1998). Further, new members of the pestiviral family have recently been discovered, the so called HoBi-like Pestiviruses. So far, these viruses have not been detected in Africa. If they are introduced into naive regions they may have a significant impairing effect on animal production and welfare. Also, since they are genetically different, diagnostic test for BVDV may not be able to detect them (Bauermann *et al*., 2013).

Bovine viral diarrhoea virus (BVDV)

BVDV is an enveloped, positive-sense single-stranded (ss+) RNA-virus of approximately 12.5 kb in length (Baule *et al*., 1997; Nettleton *et al*., 1998; Kabongo *et al*., 2003). The virus is flanked by NCR (non-coding regions) on both the 5’ and 3’ end (Vilcek *et al*., 2001). The 5’ NCR is highly conserved

among pestiviruses and is commonly utilized for phylogenetic analysis (Van Vuuren *et al*., 2005; Bauermann *et al*., 2013). There are two different genotypes, BVDV-1 and -2, and within these several subgenotypes exists (Vilcek *et al*., 2001; Fulton *et al*., 2003). According to Peterhans *et al*., (2010) BVDV-1 can be separated into at least 16 subgenotypes (1a-1p), e.g. BVDV-1a with a reference strain termed NADL, and BVDV-2 into three subgenotypes (2a-b) (Peterhans *et al*., 2010). However, these subgenotypes are not acknowledged by the ICTV (International Committee on Taxonomy of Viruses) (Otonel *et al*., 2014).

There are also two biotypes, designated as cytopathogenic (CP) and non-cytopathogenic (NCP). The designation depends on their effect on tissue culture cells, where the cytopathogenic strains will cause vacuolisation and cell death (Walz, 2015). NCP is the most common biotype and it is the one that causes persistent infection in animals (Meiring *et al*., 2011). The CP biotype causes a disease known as mucosal disease, which only affects persistently infected individuals. It usually arises due to genetic alterations in the NCP but the source can also be external, such as vaccination with a modified live vaccine. These animals typically have severe necrotizing lesions in the gastrointestinal tract with hemorrhagic diarrhoea (Walz, 2015).

Mucosal disease only affects persistently infected animals, in cattle often between the ages of 6-18 months (Houe, 1992). If the superinfecting CP strain is genetically similar to the NCP strain, usually caused by genetic alteration of the NCP, disease is typically more severe and leads to death more quickly. These animals display severe depression, pyrexia, anorexia, profuse diarrhoea as well as erosions in the mouth. This syndrome is inevitably fatal. However, if the superinfecting strain is more heterologous to the NCP strain that the animal is immunotolerant to, for example due to vaccination with a CP modified live vaccine, clinical signs are similar but often less serious and fast-acting. In some cases, the animal may respond to the strain immunologically and then clear the superinfection, but this is a rare occurrence (Walz, 2015).

Susceptible species

Pestivirus infects even-toed ungulates in the order Artiodactyla (Nettleton, 1990). Cattle is the primary host but serological evidence of pestivirus infection has been found in over forty different species, for example sheep, goats, giraffe, eland, kudu, nyala, oryx, waterbuck, lechwe, wildebeest, impala, duiker and african buffalo (Nettleton, 1990). Sheep and goats are of particular interest in the epidemiology of BVDV infection in bovids, since they are more often in contact with cattle than other species, which therefore will facilitate transmission (Bachofen *et al*., 2013).

Clinical signs

BVDV causes a diverse set of clinical signs. These include, but are not limited to, respiratory signs, diarrhoea, pyrexia, trombocytopaenia, depression, inappetence, nasal discharge, salivation, erosion/ulceration of oral mucosa, reduced production, anemia and sudden death. The virus is also known to cause leukopenia and immunosuppression, which in turn renders the animal more susceptible to secondary infections (Van Vuuren *et al*., 2006; Martin *et al*., 2011; Walz, 2015). The frequency of retained placenta and mastitis has been shown to increase with BVDV infection in dairy herds (Niskanen *et al*., 1995). BVDV has also been implicated in the Bovine Respiratory Disease complex (BRD), which causes severe economic losses in cattle feedlots and among intensively reared calves (Meiring *et al*., 2011; Walz, 2015). The virus can cause respiratory disease on its own but its biggest role is likely in impairing immune defence and rendering the animal more susceptible to secondary pathogens (Walz, 2015). In Kenya, an association has been found between BVDV, IBR (Infectious bovine rhinotracheitis) and PIV-3 (Parainfluenza virus 3), since any calf infected with one of the viruses was shown to be significantly more likely to also be infected with one or both of the others as well (Callaby *et al*., 2016).

Several studies indicate that respiratory signs are the most common symptom when an animal is infected with BVDV, followed by diarrhoea (Fulton *et al*., 2000; Fulton *et al*., 2003; Kabongo & Van Vuuren, 2004) and abortion (Fulton *et al*., 2000; Fulton *et al*., 2003). However, in most cases the infection is subclinical (Walz, 2015), but serious outbreaks have been described with death in several age groups as well as severe economic losses (Carman *et al*., 1998; Ridpath *et al*., 2006). Signs in domestic smallstock are in general similar to the ones observed in cattle (Taylor *et al*., 1977) but infection with BVDV can also give rise to symptoms typically associated with Border Disease Virus infection (BDV) (see below) (Passler & Walz, 2009).

Reproductive disease

BVDV can affect an animal's reproductive system in many ways. In males, both persistently and acutely infected animals will shed virus in their semen (Walz, 2015). In females, infection prior to insemination or mating can cause lowered fertility rates due to ovarian dysfunction (Walz, 2015). Calving intervals are usually increased as well as the need of oestrus stimulating treatment (Niskanen *et al*., 1995).

When a pregnant seronegative dam is infected she will inevitably transmit the virus to her fetus. Outcome of the fetal infection depends most notably on the age of the fetus and strain of the virus (Houe & Meyling, 1991; Walz, 2015). Early embryonic death and abortion can occur throughout gestation but are more common during the first trimester. Infection at a later stage can result in congenital malformation, including but not limited to cerebellar hypoplasia, microencepahly, hydrocephalus, brachygnathism, cataract and growth retardation. Infection during the last trimester, after immuno competence has developed and organogenesis is completed, will often result in the birth of clinically normal offspring with antibodies to BVDV (Walz, 2015).

Persistent infection

The most serious implication of fetal infection is the birth of persistently infected animals. If the fetus is infected prior to the development of fetal immune competence, it will become immunotolerant to the virus and persistently viraemic, and fail to mount an antibody response (Houe & Meyling, 1991; Walz, 2015). This happens if infection occurs during the first trimester, for cattle approximately between day 45-125 (Walz, 2015). Persistently infected individuals shed virus in high concentrations in all their bodily secretions throughout their life, and are therefore the most important viral transmittors (Houe & Meyling, 1991). They are usually small, unthrifty and will often die prematurely. However, they can also appear clinically normal. Some may survive to adulthood and breeding age, and then pregnant persistently infected dams will inevitably give rise to persistently infected young (Walz, 2015).

BVDV in goats

Clinical signs in domestic smallstock are in general similar to the ones observed in cattle (Taylor *et al*., 1977). However, when seronegative pregnant goats are infected, abortion is the most common result, and can occur at any stage in gestation. Studies indicate that the development of persistently infected kidlings is rare and a much less likely outcome compared to in cattle and sheep (Depner *et al*., 1991a; Broaddus *et al*., 2009; Bachofen *et al*., 2013; Passler *et al*., 2014). In a study conducted by Broaddus *et al*., (2009), only one out of 25 kidlings delivered by dams infected with BVDV at various stages of pregnancy, developed persistent infection. Persistently infected goats are sometimes clinically normal but more often ill-thrifting and occasionally displaying neurologic signs (Bachofen *et al*., 2013). Congenital malformations are sometimes seen in aborted kids and includes for example facial deformities such as prognathia and brachygnathia inferior. Brain lesions such as encephalitis and choroid plexitis are also occasional findings (Broaddus *et al*., 2009). The low prevalence of persistently infected goats as well as their low survival rate implies that goats likely do not play an important role in the epidemiology of BVDV in cattle. However, BVDV can be transmitted between goats, and

persistently infected kids subsequently generated. Therefore, the virus is able to persist in goat populations, independent of contact with persistently infected cattle (Bachofen *et al*., 2013).

Border Disease Virus

Border Disease Virus (BDV) is a pestivirus that is closely related to BVDV, so close that diagnostic tests that detect antibodies directed at the p80 protein, for example the antibody ELISA used in this study, will also detect antibodies for the same protein on BDV. Also, the two viruses have similar disease profiles and BVDV has been known to cause signs in sheep and goats that previously were thought to be unique for BDV (Passler & Walz, 2009). The virus is usually transmitted through direct contact with persistently infected animals, and outbreaks of BDV will most commonly occur with introduction of these individuals during the breeding season. The virus may also spread through contaminated modified live vaccines. BDV is usually non-cythopatogenic, but cytopathogenic strains exist as well, and has been isolated from sheep suffering from illnesses resembling mucosal disease. However, it is the non-cytopathogenic biotype that causes persistent infection and congenital disease (Nettleton, 1990).

Susceptible species

BDV mainly affects sheep but other species such as cattle, pigs, red deer, roe deer and fallow deer are susceptible and hence potential reservoirs (Nettleton, 1990). Infection in goats is occasionally seen but is a rare occurence (Nettleton *et al*., 1998).

Clinical signs

There are several signs that may indicate the presence of Border Disease in a herd of sheep. Disease in adult non-pregnant sheep is usually subclinical, but sometimes a mild pyrexia and transient viremia will occur. However, some isolates of BDV have been associated with severe diarrhoea, pyrexia and high mortalities, especially amongst younger lambs (Nettleton, 1990). Outcome of infection of pregnant sheep varies most notably with the age of the fetus, but also with the viral strain and breed of sheep. During the first 60 days of gestation, fetal death is likely to occur. Lambs that survive until birth are often persistently infected and will hence shed virus in large amounts throughout their life. Some persistently infected lambs have abnormal body conformations, pelt changes, and neurologic signs such as tremors. These lambs are known as “hairy shaker lambs” and will often die shortly after birth. Survivors are generally stunted in growth and extra sensitive to other infections. As the lamb matures, nervous signs will often gradually diminish, but in some cases these signs may reappear later in life. However, some persistently infected lambs will appear normal. These animals often survive to breeding age and will subsequently give rise to new persistently infected animals (Nettleton, 1990).

Lambs that are infected after 80 days of gestation i.e., after the development of foetal immune competence, are often clinically normal and seropositive. Outcome for lambs that are infected between these periods is unpredictable, as they can be both viraemic/non-viraemic as well as both antibody positive/negative. They can also develop severe lesions in the central nervous system (CNS) with cavitation and dysplasia (Nettleton, 1990).

Transmission of BVDV

Nose-to-nose contact between a susceptible animal and a persistently infected individual is regarded as the most efficient route of transmission. However, the virus can utilise indirect routes as well. Usage of improperly disinfected materials such as vaccines and medicine bottles can transmit the virus (Niskanen & Lindberg, 2003). Also, insufficient cleaning of pens that previously held persistently infected animals constitutes another risk. In a study by Niskanen & Lindberg (2003), two persistently infected calves were kept in two separate pens for one week each. After removal of the animals, five naive calves were placed in the pens, 2 hours and 4 days after the removal of the persistently infected calves. The majority of the calves that were introduced after 2 hours seroconverted in 2-3 weeks, but none of the calves that were introduced on day 4 seroconverted (Niskanen & Lindberg, 2003).

BVDV can also be transmitted with ambient air over short distances. Niskanen & Lindberg (2003) found that four calves kept in the same building but in separate pens at a distance of 1.5m and 10m from a persistently infected calf would seroconvert after 3-4 weeks. The calves that were kept 1.5m away both seroconverted after 3 weeks, whereas the calves that were kept 10m away seroconverted after 4 weeks. Also, in a study conducted by Mars *et al*. (1999), two persistently infected calves and 5 naive calves were housed separately, but the housings had a joint ventilation system allowing passive airflow from the persistently infected calves to the naive calves. All naive calves had seroconverted within three weeks (Mars *et al*., 1999).

Epidemiology

BVDV is endemic in the majority of the world (Lindberg & Houe, 2005). Seroprevalence varies between regions but is in endemic regions often 60% or more (Houe & Meyling, 1991; Houe, 1999). Differences in seroprevalence is dependent on various factors, for example on trade routines, animal grouping, as well as the usage of vaccines and communal pastures for grazing (Houe, 1999). The prevalence of persistently infected individuals is also highly variable but is generally around 1-2% in endemic settings (Houe & Meyling, 1991; Houe, 1999).

The biggest threat to infection in a herd is posed by persistently infected animals (Lindberg & Alenius, 1999; Lindberg & Houe, 2005; Walz, 2015). Seropositivity is significantly higher in herds with persistently infected animals compared to herds with only transient infection (Houe & Meyling, 1991). In herds with persistently infected cattle, where the animals are kept under close confinement, antibody prevalence is usually 90% or more by the time the persistently infected calf has reached 3-4 months of age (Houe *et al.*, 1993). In practice, a herd should not be considered infected with BVDV until persistent infections have been established (Lindberg & Alenius, 1999). For acutely or transiently infected (AI or TI) animals, the infection is of short duration and virus only intermittently shed, which means that the probability of horizontal transmission is lower compared to that for persistently infected individuals (Lindberg & Houe, 2005). In some studies, acutely infected animals have even been unable to transmit the virus to susceptible peers (Niskanen *et al*., 2000; Niskanen *et al*., 2002). These results may indicate that transiently infected individuals only rarely will propagate the virus horizontally. Contradictory to this, there are also studies that indicate continued circulation of the virus in herds despite an apparent lack of persistent infection (Moen *et al*., 2005). However, this may be caused by these individuals escaping detection, for example if they are aborted, die early or are sold before reaching a testable age. It may also be due to periodical re-introductions of the virus from the outside. The usual sequence of

events when all the persistently infected animals in a herd has been removed is that active infection eventually fades out (Lindberg & Alenius, 1999).

Despite the inefficiency of horisontal transmission, acutely infected animals are very capable of vertical transmission, i.e. from a mother to her young. The majority of new persistent infections in a herd is likely a consequence of transient infections in pregnant dams (Lindberg & Houe, 2005). These dams are often refered to as Trojan cows and they are of particular epidemiologic interest. This is because the persistently infected offspring that they carry hidden in their uterus will pose a great infectious threat when they are born. Unfortunately, there are no reliable and economically feasible test to detect Trojan cows. The antibody levels of these dams will often rise considerably towards the end of gestation but not in every case, which makes this trait unreliable for diagnostics (Lindberg *et al*., 2001).

There are mainly three factors that influence the sequence of events following BVDV introduction into a herd. The first is the route of viral introduction, which can occur via persistently or transiently infected animals as well as be direct, indirect or through ambient air. The second factor is the features of the recipient group, for example their immune status and number of pregnant and non-pregnant animals. The third is management factors that affect within-herd spread, for example building formation, animal grouping and usage of quarantines (Lindberg & Alenius, 1999). If no introduction of persistently infected animals occur, BVDV can probably only persist in a herd if a dam in early pregnancy is infected and later gives birth to a persistently infected calf. No or only minor transmission of BVDV will occur until this happens. This period is referred to as the first period of latency. After the birth of the persistently infected calf, a period of increased viral spread will commence (Lindberg & Alenius, 1999). The extent of transmission will mainly depend on the number of persistently infected animals born, how long they survive and which animals they come into contact with. If these calves subsequently are removed, viral persistence is dependent on wether new persistent infections have successfully developed. If this is the case, a second period of latency will take place, followed by another period of increased spread. However, if no persistent infection develops, self clearance is likely to occur (Lindberg & Alenius, 1999).

Self clearance

Self clearance is a process where a group of animals manage to eliminate an infection, without the need for active human intervention. In the case of BVDV, self clearance is likely to happen if no persistently infected animals are present and no periodic reintroductions of the virus occurs (Lindberg & Alenius, 1999). This is a phenomenon that works in favour of control in infected herds (Ståhl *et al*., 2008). The probability of self-clearance is mainly related to the number of susceptible naive dams in early pregnancy and the amount of contact between the persistently infected individuals and these animals. The high transmission rate of BVDV within a herd will lead to a steadily decreasing number of susceptible pregnant females. This coupled with the high mortality of persistently infected animals will work in favour of self clearance (Lindberg & Alenius, 1999; Lindberg & Houe, 2005; Ståhl *et al*., 2008). The process is genereally believed to occur more readily in smaller herds but it has been known to take place in flocks with as many as 300 animals (Lindberg & Houe, 2005). Research conducted in Peru by Ståhl *et al*., (2008) could not detect a statistically significant difference between small and large herds, and the results even showed a small increase in the probability of self-clearence in bigger herds. The authors believed that this was due to the in general more extensive biosecurity measures taken in bigger herds, as well as more intensive management procedures which may promote early death in the animals suffering from persistent infection (Ståhl *et al*., 2008).

Risk factors

Antibody prevalence is generally higher in adults compared to young animals (Houe & Meyling, 1991; Nigussie *et a*l., 2009). This has also been shown to apply in African settings (Hyera 1991) as well as for sheep and goats (Mischra *et al*., 2009). However, the risk of viraemia is significantly higher for younger animals than for adults (Houe & Meyling, 1991). Residing in an area where cattle density is high is likely to lead to an increased antibody prevalence (Saa *et al*., 2014). For small ruminants, regular contact with cattle has been shown to constitute a risk factor (Mischra *et al*., 2009). According to a study conducted in Cameroon by Handel *et al*. (2011), a herd is more likely to have persistently infected cattle if they are simultaneously farming goats and/or if the animals are in contact with antelopes (Handel *et al*., 2011). Many studies also indicate that seroprevalence is higher in large than in small herds (Mockeliuniene *et al*., 2004; Almedida *et a*l., 2013; Graham *et al*., 2013; Sarrazin *et al*., 2013). With the use of a stochastic model developed for beef herds, Damman *et al*., (2015) showed that eradication of BVDV in bigger herds is likely to take about 1-2 years longer than in small herds. Also, the virus is more likely to persist over time in large than in small herds (Damman *et al*., 2015).

Contact between animals on pasture or over fences between neighbouring farms is a risk factor (Lindberg & Alenius, 1999; Valle *et al*., 1999; Walter *et al*., 2005; Handel *et al*., 2011). This first and foremost poses a risk for seronegative dams to get infected in early pregnancy, but also for other animals attaining transient infection and subsequently spreading it further in their destination herd. Persistently infected animals on pasture constitutes a big risk. Generally, sharing pastures is considered to be a bigger risk than over-fence contact (Lindberg & Alenius, 1999). In a study conducted in the Austrian Alps by Krametter-Froetscher *et al*. (2007), communal pasturing was found to be a significant risk factor in transmission of Border Disease Virus in sheep. Also, in the eradication programme in Austria, only allowing BVDV-free herds on to common grasslands had a considerable reducing effect on seroprevalence and prevalence of persistently infected animals (Krametter-Froetscher *et al*., 2007).

Livestock trade

Livestock trade is a significant risk factor for transmission of BVDV and plays a central role in the epidemiology of the virus (Lindberg & Alenius, 1999; Valle *et al*., 1999; Graham *et al*., 2013). Herds that are closed to trade have been shown to have significantly reduced seroprevalence in both beef and dairy herds (Gates *et al*., 2013). It has been shown in an epidemiological study based on data from Scottish beef herds that cattle movement, for example due to trade, had about three times greater explanatory power than the combined factors contributing to local spread (i.e. sharing pasture, regular contact with wildlife etc.). In dairy herds the two had almost the same effect, which the authors hypothesised was due to Scottish dairy cattle in general being kept in higher density areas as well as under more intensive settings (Gates *et al*., 2013).

Two major risk factors associated with trade is purchase of persistently infected animals or dams pregnant with persistently infected young (i.e. Trojan cows). It is likely that because of the high mortality of persistently infected individuals, the prevalence of Trojan cows in trade is higher (Lindberg & Alenius, 1999). Besides this, trade of seronegative animals in early pregnancy is a risk since they can be infected during the process of sale. In general, purchasing breeding cattle significantly contributes to increased seropositivity compared to purchasing store cattle (Gates *et al*., 2013). However, trade of transiently infected animals may also constitute a risk since they can transmit the virus to susceptible naive pregnant dams in the destination herd (Lindberg & Alenius, 1999). Increasing the number of cattle purchased as well as the number of source farms will significantly increase antibody prevalence (Gates *et al*., 2013).

Modified Live Vaccines, Embryos, Artificial Insemination

Modified live vaccines (MLV) manufactured using fetal calf serum (FCS) are often contaminated with BVDV-1 or -2 (Bolin *et al*., 1991; Ridpath *et al*., 1994). There are control measures that aim to inactivate these contaminants, but they are not efficient in every case (Ridpath, 2013). The risks related to this are introducing new viral strains into naive populations and regions, as well as enabling recombination between natural strains and vaccine strains, giving rise to new variants with new genetic properties (Lindberg & Alenius, 1999).

Embryos and semen are other potential vectors of BVDV that can introduce the virus to new naive populations (Houe, 1999; Lindberg & Alenius, 1999). These risks can be avoided by appropriate washing procedures (embryos) and testing of donor animals (embryos and semen) (Houe, 1999). Persistently infected animals shed BVDV in large quantitites in their semen throughout their life, while transiently infected individuals shed smaller amounts and during a short isolated time frame (Walz, 2015). Also, bulls that persistently shed BVDV in their semen, despite being seropositive and non-viraemic, have been detected. These animals may be difficult to detect and to prevent from entering artificial insemination stations (Niskanen *et al*., 2002). However, this is likely not a common occurrence (Lindberg & Alenius, 1999).

Economy

Even though infection with BVDV in general is mild to subclinical, the infection can cause serious economic losses. Overall, costs in individual dairy herds vary from a few thousands up to $100 000, and are on a population level approximately $10-40 million per million calvings (Houe, 2003). These losses are first and foremost due to the virus' effect on reproduction (Houe *et al*., 1993; Krametter-Froetscher *et al*., 2007; Ståhl *et al*., 2011), as well as its impact on the general health status of the herd (Krametter-Froetscher *et al*., 2007; Ståhl *et al*., 2012). Production losses due to infection with BVDV include, but are not limited to, decreased fertility, reduced milk production, abortions, congenital defects, growth retardation and increased susceptibility to other diseases. Infection of the foetus in early gestation may result in birth of a persistently infected individual, that will often be unthrifty and die prematurely (Walz, 2015). BVDV infection is also associated with mastitis, retained placenta, longer calving intervals and an increased need of treatments to stimulate oestrus (Niskanen *et al*., 1995).

With the use of a stochastic model, Damman *et al*., (2015) have calculated the economic implications of BVDV infection in a beef cattle herd. It showed that the losses were highest during the first three years after introduction into a naive herd, peaking at the second year. The losses during this period could be as much as six times higher than in subsequent years. The economic losses then subsided as the herd entered the endemic phase, due to the development of herd immunity. However, cumulative losses over the next 10 years in the endemic phase were shown to vastly surpass the costs during the acute phase. The impact and level of spread within a naive beef herd is, according to the model, most greatly affected by herd size and the type of initial BVDV introduction. The model also showed that regular reintroductions of BVDV every 2-3 years in an endemic herd almost doubled the losses associated with the virus, as compared to no introductions or introductions only every 6-7 years. Abortions and mortality due to persistent infection, caused the greatest losses in the first two years and the costs per bred female tended to be higher in small than in large herds (Damman *et al*., 2015).

BVDV in Botswana

Mucosal disease was suspected for the first time in Botswana in 1970 when an outbreak of diarrhoea, salivation due to mouth lesions and lameness due to foot lesions, took place (Hunter & Carmichael, 1975). In a study by Hunter and Carmichael (1975), seroprevalence in two herds displaying clinical signs of infection with BVDV was estimated to 42% and 70%. Samples were also taken in a nearby village without clinical signs, with a seropositivity of 19% (Hunter & Carmichael, 1975). Also, prevalence was in 1973 estimated to 88% in 100 tested Botswana cattle (Theodoris *et al.*, 1973, refered by Depner *et al*., 1991b).

Prevalence of BVDV on the African continent

Several prevalence studies in cattle have been conducted on the African continent. In neighbouring South Africa, seroprevalence in dairy cattle with clinical signs suggestive of BVDV was 79.9-100% in a study by Ferreira *et al*. (2000). In feedlots, seroprevalence of 37.3% have been found among animals displaying signs of BVDV (Ularamu *et al*., 2012). In the Gauteng province in northeast South Africa, a seroprevalence of 49.4% among cattle owned by non-commercialized farmers was detected (Njiro *et al*., 2011).

In Namibia, 49% of cattle sampled randomly across the country had neutralizing antibodies for BVDV. For cattle that displayed signs indicative of BVDV, seroprevalence was 77.5% (Depner *et al*., 1991b). In northern Tanzania, a 34% seroprevalence has been found (Hyera, 1991). In Kenya, prevalence has been estimated to 19.8% in indigenous East African Shorthorn Zebu calves (Callaby *et al*., 2016). In Uganda, Jönsson (2013) found an animal level prevalence of 23.4% and a herd-level prevalence of 39.3% (Jönsson, 2013). In Ethiopia, a 11.5% seroprevalence was found (Nigussie *et al*., 2010). In Cameroon, a 34.6% seroprevalence was detected on an individual level, as well as 92% on herd level (Handel *et al*., 2011).

Also, a number of prevalence studies on sheep and goats have been conducted on the continent. In Namibia, 9% and 5% seroprevalence have been found in sheep and goats, respectively. In sheep displaying signs of BVDV, the same study detected a 41% seroprevalence (Depner *et al*., 1991b). Hyera (1991) found a seroprevalence of 32.1% in sheep and 24.9% in goats in northern Tanzania (Hyera, 1991). Päärni (2016) detected a seroprevalence of 2.93% in goats on an individual level, and 10.3% on herd-level in Tanzania. In Nigeria, 12.7% of sheep and 4.5% of goats had neutralizing antibodies (Taylor *et al*., 1977). In Sudan, 39.1% of sheep and 14.8% of goats were found to have antibodies (Ali *et al*., 2013).

Genotypes circulating in Africa

A number of studies that investigate the BVDV genotypes circulating on the African continent have been conducted. In Zimbabwe, pestiviruses were isolated from three elands, whereof at least one is likely to have been persistently infected. These three isolates were most similar to BVDV-1a (Vilcek *et al*., 2000). In Fayom, Egypt the genotypes 1a, 1b and 1f have been detected (Emran *et al*., 2014). In Ethiopia, BVDV-2a and BVDV-1b have been identified on two different farms (Thabti *et al*., 2005).

In southern Africa, significant genetic diversity exists (Baule *et al*., 1997; Van Vuuren, 2005). In South Africa, Kabongo *et al*., (2003) found BVDV-1a, BVDV-1b and a separate cluster termed BVDV-1*. No

relationship was found between subtype, clinical signs and geographic origin. Ularamu *et al*., (2012) sequenced samples from feedlots from all over South Africa and Namibia, and found that 82.5% belonged to BVDV-1 and 17.5% to BVDV-2. Baule *et al*., (1997) sequenced BVDV-1 on isolates from South Africa and Mozambique. These isolates were divided into two genetically distinct groups (A and B), which were further subdivided into different clusters. Group A was subdivided into three clusters, out of which two were similar to strains isolated in Europe and North America (NADL-like and Osloss-like respectively) (Baule *et al*., 1997). NADL-like is the reference strain for BVDV-1a, and Osloss for BVDV-1b (Peterhans *et al*., 2010; Vilcek et al., 2001). The third was concluded to represent strains that are rare or absent in Europe and North America. The viruses assigned to group B all originated from Mozambique, and appeared to predominately induce respiratory signs. This group consisted of strains that are rare or absent in Europe and North America (Baule *et al*., 1997).

Role of wildlife in the epidemiology of BVDV

BVDV infection in wildlife is not only a possible cause of clinical disease, but may also play a role in the epidemiology in domestic livestock. In order for wildlife to constitute a potential reservoir for domestic ruminants, the virus needs to be able to persist in these populations without regular reintroductions from cattle. Also, infected wildlife species need to come into sufficient contact with livestock to enable viral transmission (Lindberg & Houe, 2005). Whether or not infection in wildlife can inhibit control in domestic animals is currently unclear. On the African continent, buffalo and eland have been highlighted as potential reservoirs due to the occurence of persistent infections in these species (Vilcek *et al*., 2000). Besides these, pestivirus has also been isolated from giraffes (Harasawa *et al*., 2000).

In Zimbabwe, BVDV-1 very closely related to cattle isolates have been detected in eland antelopes, indicating that the infection originally is derived from cattle. This could in turn indicate that contact with cattle poses a greater threat to eland antelopes than the other way around (Vilcek *et al*., 2000). Studies conducted in the European alps indicate that it is more likely that wildlife in these settings constitutes an incidental spillover host and that infection in the studied populations is of domestic origin (Becher *et al*., 1997; Martin *et al*., 2011; Causabon *et al*., 2012). Also, in many if not all countries where BVDV has been or are well on its way of eradication, control has been successfully achieved without first managing the disease in wildlife (Handel *et al*., 2011).

In Zimbabwe, antibodies have been detected in nyala, eland, bushbuck, buffalo, waterbuck, reedbuck, sable, giraffe, tsessebe, kudu and wildebeest, the highest prevalences in nyala (75%) and eland (46%) and bushbuck (41%) (Anderson & Rowe., 1998). Also, one out of 167 tested black rhinos was positive for antibodies in an indirect ELISA. Warthog and white rhino was also tested but no antibodies detected. Also, virus was isolated from a seronegative eland on two occasions, and it was hence classified as persistently infected (Anderson & Rowe., 1998).

Three similar studies have been conducted in Namibia. Depner *et al*. (1991b) discovered neutralising antibodies in giraffe, gemsbock, roan, eland, kudu and sable. The seroprevalence was 46% for giraffe, 15% for gemsbok, 7% for roan, 18% for blue wildebeest, 57% for eland, 6% for kudu and 9% for sable. Red hartebeest, black wildebeest and springbock were also tested but without detection of antibodies (Depner *et al*., 1991b). Soiné *et al*., (1992) detected a 65% seroprevalence in giraffe, 16% in oryx and 5% in Roan antelope. The same study found a seropositivity of 67% in Kudu as well as 9% in Sable antelope and Blue wildebeest respectively. However, for these species the sample sizes were small,

ranging from 6-14. No antibodies were detected in Springbooks, despite a large sample size. Scott *et al*., (2013) found antibodies in 71% of 38 tested Namibian Kudu, 71% out of 7 tested South African Kudu as well as 40% out of 5 tested Namibian Eland.

Aim of study

The aim of this study was to determine seroprevalence of BVDV-antibodies and -antigen in goats and cattle in and around Gaborone, Botswana. The aim was also to determine risk factors for infection in the sampled goats.

Materials and Methods

Sample collection

Three villages situated in or just outside of Gaborone, namely Modipane, Gakuto and Kopong, were visited (Fig 1). Five smallholder farmers in Modipane and three in Gakuto and Kopong respectively were selected for sampling. The choice of farmers was non-random and with the inclusion criteria of owning a herd consisting of approximately 20-30 adult animals, i.e. the average herd size in Botswana. However, this criterion was not always met since on three occasions the farmer was found to possess less or more goats than this number at the time of sampling. The GPS-coordinates for each location was recorded, and verbal consent acquired from the farmers before sampling was initiated.

From every herd 10 goats were sampled, except in two neighbouring flocks where five animals each were utilized instead. This was due to the small number of goats kept by these farmers and their close proximity to one another, which enabled the goats from the two herds to closely interact with each other. Therefore, they were considered one epidemiological unit, and hence sampling was divided between the two. The selection of goats was randomized by only sampling every second or every third animal that was caught. Whether every second or every third animal was sampled depended on the total number of goats in the herd i.e., in flocks with 20 animals every second goat was sampled, in flocks with 30 animals every third was sampled etc.

Jugular blood samples were obtained with a closed vacutainer system and serum tubes. Every tube was marked with two specific numbers to enable identification of the individual herd and animal. A short description of each animal was recorded, as well as a basic assessment of Body Condition Score (BCS). BCS was estimated by inspecting the goats visually as well as palpating the lumbar and sternal area, and then assigning them a number from 1-5, 1 being extremely thin, 5 being obese, and 3 ideal. Also, the color of the animal's conjunctivae was evaluated using a Famacha chart. This system is developed to aid in estimating wether a sheep or a goat is anemic and in need of treatment for the bloodsucking intestinal parasite *Haemonchus contortus*. The chart is scaled 1-5, 1 being ideal and 5 severly pale and aenimic (Van Wyk et al., 2002). All animals were marked with spray to enable individual identification. After sampling, the tubes were left standing for some time in room temperature, and then serum was separated and placed in cryotubes. The serum was always separated within 24 hours from sampling. It was then stored at -20°C until being used.

Afterwards, the farmers were subjected to a short interview regarding management procedures, contact with other herds and wildlife, health status etc. (Appendix 1). In the majority of cases this was done with the help of an interpretor, but for the farmers that were comfortable with speaking English the interview was performed in this language.

Fig 1. *Approximate locations of the villages where goats were sampled. Since the coordinates for Kopong 2 and Gakuto 1 are incorrect, the individual locations are not displayed here. Modipane and Gakuto are situated approximately 25 km from Gaborone, Kopong about 20 km.*

In addition, 361 blood samples from cattle previously obtained in 2014 as a part of a project in Anaplasma, were tested (unpublished result, personal contact via email, Dr. Solomon Ramabu, Botswana University of Agriculture and Natural Resources, 2016-12-13). The samples were taken within an approximate 150 km radius of Gaborone (Fig 2), from both beef and dairy cattle. The selection of farmers was non-random. Approximately 75-100% of the animals at each of the nine farms were sampled. After sampling the test tubes were left standing in order to allow serum to separate. The serum was then placed in cryotubes and stored at -20°C until being used.

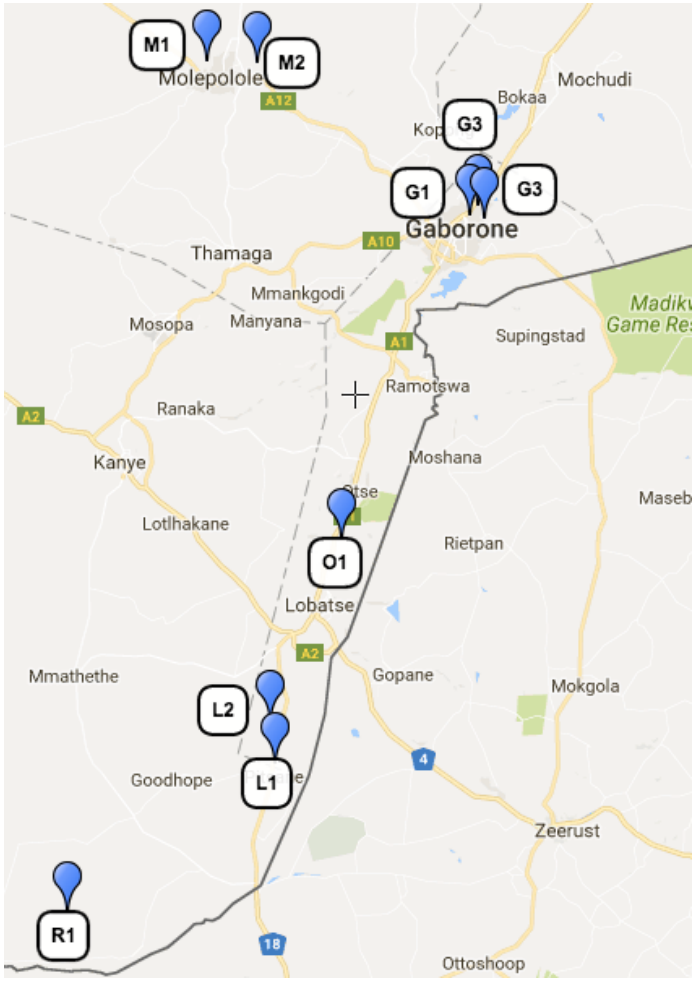

Fig 2. *Approximate locations of the cattle sample sites. R1= Ramatlabama 1. L1= Lobatse 1. L2 = Lobatse 2. O1 = Otse 1. G1= Gaborone 1. G2= Gaborone 2. G3= Gaborone 3. M1= Molepolole 1. M2= Molepolole 2. M1 and R1 are situated approximately 60 km and 150 km from Gaborone respectively.*

Antibody detection

For detection of antibodies, IDEXX BVDV/MD/BDV p80 Protein Antibody Test Kit (IDEXX, Westbrook, Maine, USA) was used. This is an enzyme linked immunoassay that detects antibodies directed to the p80 protein. The test was used according to the instructions provided by the manufacturer. First, 50 µL of dilution buffer was added to each well, followed by adding 50 µL of negative control to two wells and 50 µL of positive control to one well. After this 50 µL of serum sample was added into the remaining wells, the contents homogenized by lightly tapping the plate, and then the plate covered and left to incubate for 60 minutes at room temperature. This was then followed by washing with a previously prepared washbuffer, 300 µL in each well repeated 5 times. In between each washing step the plate was tapped onto paper to remove remaining fluid. After this, 100 µL of conjugate was added into each well, and the plate covered and left to incubate for 30 minutes at room temperature. This was followed by three repetitions of washing performed in the same manner as above. Thereafter 100 µL of TMB substrate was added and the plate covered and left to incubate in the dark at room temperature for 20 minutes. This was then followed by adding 100 µL of Stop Solution to each well and then the absorbance values were measured immediately at 450 nm, with a Thermo Scientific Multiskan FC ELISA reader (Thermo Scientific, Vantaa, Finland). The validity of the positive and negative controls was calculated. The mean of the negative control needed to be higher than 0.8 to be valid, and the

positive control divided with the negative control less than 0.2. For invalid assays, the assay was not repeated due to shortage of material. The percent of inhibition was then calculated by dividing the sample absorbance value with the mean of the negative control, and then multiplied with 100. Samples with a higher value than 50% was considered negative and lower than 40% positive. Samples with a value between 40-50% were denoted as suspect.

For the goat samples, both the above protocols designed for cattle and a protocol for sheep was used. In the sheep protocol, 50 µL of dilution buffer was added in three previously chosen wells, and 75 µL in the remaining ones. After this, 50 µL of negative control was added into two wells and 50 µL of positive control into one well. Subsequently, 25 µL of sample was added to the remaining wells. The contents were homogenized by lightly tapping the plates and then covered and incubated for 20 hours at 3°C. From the first washing step the protocol was the same as above.

Antigen detection

Subsequently, the antibody negative serum samples were subjected to antigen ELISA. In order to detect BVDV antigen, an enzyme immunoassay was performed, namely IDEXX Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus (IDEXX, Westbrook, Maine, USA). First, 50 µL of detection antibodies were dispensed into each well. Then, 50 µL of negative control and positive control was dispensed into two wells each. Then 50 µL of sample were placed into the remaining wells. The contents were homogenized by lightly tapping the plate, and thereafter covered and left to incubate for two hours at room temperature. Thereafter the wells were washed with 300 µL of wash solution, which was repeated five times. After each wash the plates were tapped onto paper in order to remove remaining fluid. After this 100 µL of conjugate was added to each well, and the plates then covered and left to incubate for 30 minutes at room temperature. The washing was then repeated in the same manner as above. After this 100 µL of TMB substrate was added into each well, followed by a 10 minutes incubation in the dark and at room temperature. After this 100 µL of stop solution was added to each well and and the absorbance measured immediately at 450 nm with a Thermo Scientific Multiskan FC ELISA reader (Thermo Scientific, Vantaa, Finland). The validity of the negative and positive control was calculated. For the negative control the mean value should be equal to or less than 0.25. For the positive control, the number when subtracting the negative control from the positive control should be more than or equal to 0.416. The percent of inhibition was calculated by subtracting the mean of the negative control from the absorbance value of the samples. The result was interpreted as negative if the difference was less than 0.3, but positive if it was more than 0.3.

RNA Isolation

After this, RNA isolation was performed on the sample that was positive for antigen as well as the serum samples that were negative for antibodies and antigen in herds with seroprevalences of 50% or more. Macherey-Nagel Viral RNA isolation Nucleospin RNA Virus kit (Macherey-Nagel, Düren, Germany) was used according to the instructions provided by the manufacturer. Then, 600 µL of the lysis buffer RAV1 was added to 150 µL of samples, mixed by vortexing, and then incubated for 5 minutes in a 70 °C water bath. After this, 600 µL of ethanol was added and then vortexed. Thereafter, 700 µL of the solution was added to the NucleoSpin RNA Virus Columns, and then centrifuged for 1 minutes at 8000 x g. The flow-through was subsequently discarded and the column placed in another collection tube, and the the remaining solution was added, and centrifuged for 1 minutes at 8000 x g. After this, 500 µL of buffer RAW was added to the column and then centrifuged for 1 min at 8000 x g. The flowthrough was then discarded. Then, 600 µL of buffer RAV3 was added to the column and then centrifuged for 1 min at 8000 x g. The collection tube was then discarded. The column was then placed in a new collection

tube and 200 µL of buffer RAV3 was added, and then centrifuged for 4 min at 11000 x g. Then the column was placed in a new microcentrifuge tube, 50 µL of RNase-free water was added and left to incubate for 2 min. Then the mixture was centrifuged for 1 min at 11000 x g and the virus column discarded. The microcentrifuge tubes was then stored in -20°C for a maximum of 7 days before being used.

PCR

PCR was performed within 7 days of RNA isolation, using applied biosystems AgPath-ID One-Step RT-PCR Reagents kit (Applied Biosystems/Thermo Scientific, Foster City, California, USA). The primers OPES13A and OPES14A were used to amplify a 296 bp PCR-product of the 5' NCR (non-coding region) (Elvander *et al*., 1998), which is highly conserved among pestiviruses (Bauermann *et al*., 2013). This was performed in a 25 µL reaction volume, containing 1 µL OPES13A and OPES14A respectively, both diluted to 100 µM, 12.5 µL 2X RT-PCR-buffer, 1 µL 25X RT-PCR Enzyme mix, and 9.5 µL of extracted RNA. One negative control consisting of nuclease free-water instead of extracted RNA was also used. The mastermix and the samples were stored on ice throughout the procedure. The PCR machine Applied Biosystem 2720 Thermal Cycler (Applied Biosystems/Thermo Scientific, Foster City, California, USA) was used. The thermoprofile was: Reverse transcription: 45 °C 10 minutes, followed by an inactivation/initial denaturation step at 95 °C for 10 minutes. After this, an amplification step at 95 °C for 15 seconds, annealing step at 50 °C for 1 minutes and extension step at 72 °C for 1 minutes was used. These steps (amplification-extension) were repeated 40 times. After this, there was a final extension step at 72 °C for 7 minutes. The samples were then kept at 4 °C until being used.

After this, gel electrophoresis was performed. One gram of agarose gel and 100 ml of TBE buffert was mixed and boiled. After this 5 µl of ethidium bromide was added and the gel left to set. Then 1 µL of loading dye and 5 µL of sample/negative control was mixed and transfered to a well. The electrophoresis was then set to 100 amperes and 150 volts for 90 minutes. After this the gel was photographed in UV-light.

FTA-cards

All the samples that were subjected to RNA-isolation and PCR were subsequently applied to SIGMA-ALDRICH Whatman FTA-cards (Sigma-Aldrich, Saint-Louis, Missouri, USA). From each sample, 125 µL of serum, 30 µl RNA-isolate and 10 µL PCR-material were applied. The cards were then transported to the Swedish University of Agricultural Sciences in Uppsala, Sweden.

PCR performed on material from FTA-cards

In Sweden, the cards were placed on a punch pad and two 2mm punches of the applied PCR-material were taken using a Harris micro punch. These were thereafter placed in individual microcentrifuge tubes. One of the punches from each sample was then subjected to eluation by adding a RNA-processing buffer, i.e. 50 µL buffer (10 mM Tris-Hcl, pH 8, 0,1 mM EDTA) and 1 µL DTT. This was then left to incubate for 15 min at room temperature, after which the plate was removed from the solution. PCR was then performed using both the eluated RNA and an unprocessed punch from each sample as template. The procedure was repeated in almost exactly the same manner as above, with a few exceptions. The PCR machine ProFlex PCR System (Applied Biosystems, Foster City, California, USA) was used. Only 5 µL sample was used and instead 4.5 µL nuclease-free water was added to reach the desirable reaction

volume of 25 µL. The electrophoresis was set to 120 volts for 20 minutes, instead of 150 volts for 90 minutes.

This procedure was initially attempted using the applied serum, but when this failed PCR-material was used instead.

DNA extraction from gels

DNA-extraction was performed on the gels with positive samples that were detected on gel electrophoresis, using a Thermo Scientific GeneJET Gel Extraction Kit (Thermo Scientific, Foster City, California, USA). Gel slices containing the amplified fragments were excised with a scalpel, placed in a microcentrifuge tube and weighed. Binding buffer was then added, the volume equal to the weight of the gel slice. The mixture was then incubated at 55 °C for approximately 10 minutes and then vortexed until the gel had been completely dissolved. The color of the solution at this point was yellow, which indicates optimal pH for binding of DNA. Approximately half of the mixture was then transferred to the GeneJET purification column and subsequently centrifuged for 1 minute, after which the flow-through discarded. This was then repeated with the remaining mixture.

After this 700 µL of Wash Buffer was added to the column and then centrifuged for 1 minute, and the flow-through discarded. The column was then centrifuged an additional time to ensure that all the wash buffer had been removed. The column was then transferred to a microcentrifuge tube, 25 µL Elution Buffer was added to the center of the purification column membrane and centrifuged for 1 minute. The tubes were then placed in a refrigerator and stored for approximately three weeks.

DNA-sequencing

For DNA sequencing 10 µL of PCR product from gels was added to two separate microcentrifuge tubes containing 5 µL of the primers OPES13A (5 µM) and 5 µL of the OPES14A (5 µM) respectively. These samples were subsequently sent to Macrogen Lab in the Netherlands for Sanger sequencing. The sequencing data was then entered into the NCBI Blast database for analysis.

Results

Sampling

Goats

The average herd size was 32.2 adults and 19.8 kidlings. In Modipane the average number of adults was 26.6, in Kopong 41, and in Gakuto 32.7 (Fig 3). The average Body Condition Score on a five-point scale (1-5) was 3.4. In the village of Modipane the average BCS was 3.1, for Kopong 3.4 and Gakuto 3.4 (Fig 4). On Famacha the average was 2.4 on a five-point scale. The average in Modipane was 2.2, in Kopong 2.7, in Gakuto 2.3 (Fig 5).

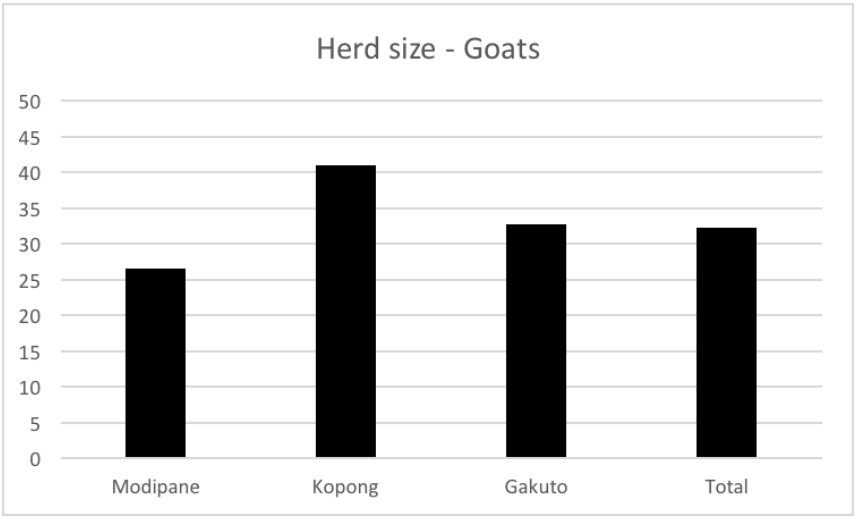

Fig 3. *Average herd size of goats on the sampled farms. The averages in the villages as well as the total average are displayed here.*

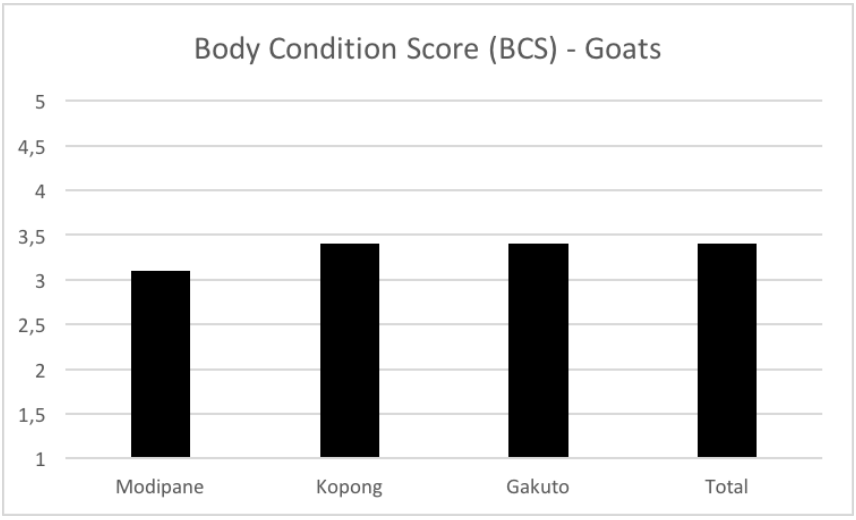

Fig 4. *Average Body Condition Score (BCS) of the sampled goats on a five- point scale. The averages in the different villages and in total are displayed here. 1 is extremely thin, 3 ideal, 5 obese.*

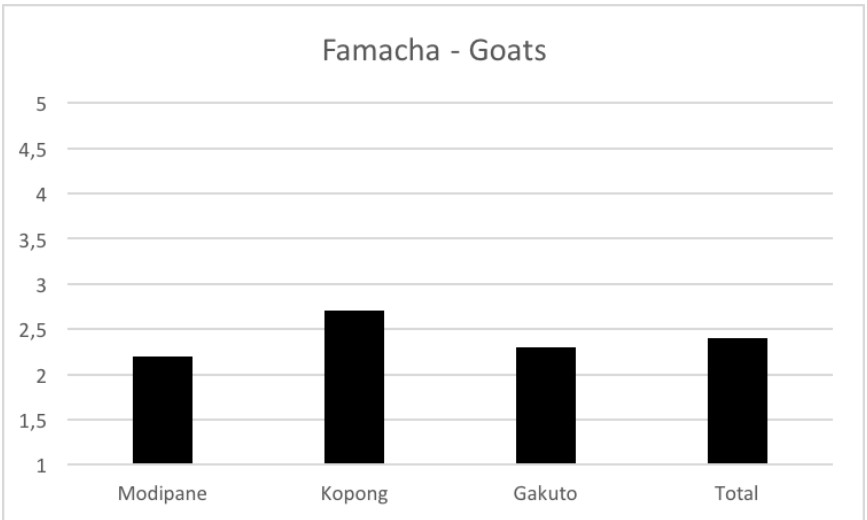

Fig 5. *Average Famacha scoring of the sampled goats on a five-point scale, 1 being ideal and 5 extremely pale. The averages in the different villages as well as the total average are displayed here.*

All of the sampled goats were negative for antibodies to BVDV on Ab-ELISA (Fig 6). Therefore, Ag-ELISA, RNA extraction and PCR were not performed on these samples.

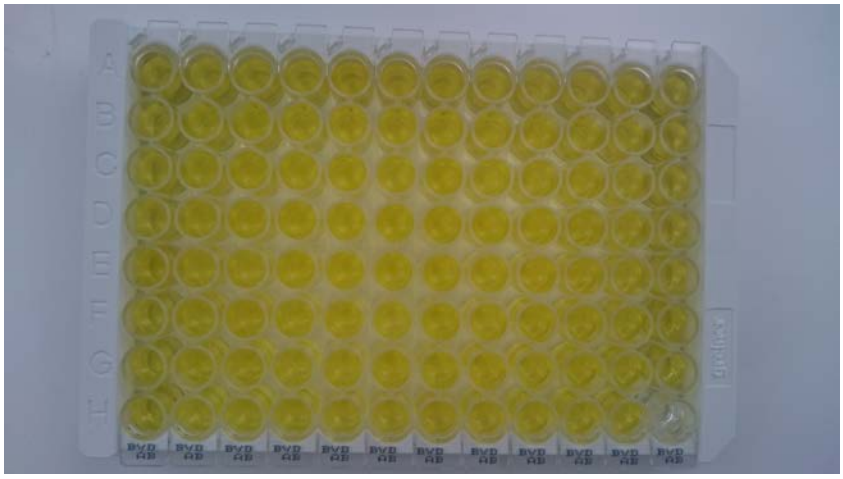

Fig 6. *Ab-ELISA results on goat samples. All samples are negative except for the positive control (H12). Negative controls are found in wells A1-2.*

Cattle

Antibodies

In total 361 samples from cattle were tested. Out of these, 53.5% (193/361) were seropositive for BDV/BVDV on Ab-ELISA on an individual level. The seroprevalence within the tested herds ranged from 16.7-97.9% (Fig 7 and Table 1). All of the herds (100%) had at least one seropositive animal. Seroprevalence in the sampled dairy farms was 49.7%, and 56.7 % in beef farms (Fig 8 and Table 2). However, the difference between dairy and beef was not statistically significant using Minitab17 Two-proportion Test (p=0.087).

A total of 20 samples (5.5%) got results denoted as ”suspected” according to the cutoff values provided by the manufacturer.

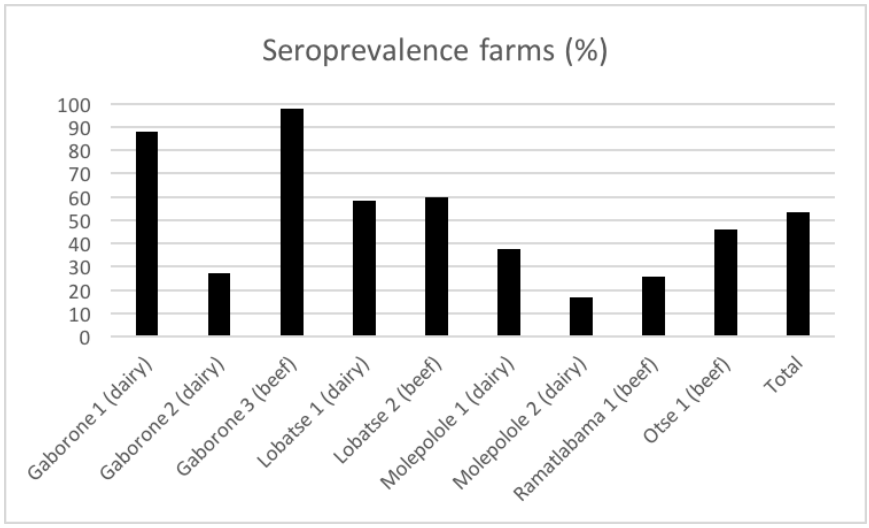

Fig 7. *Differences in antibody-prevalence between the sampled farms. The Y-axis shows seroprevalence in percent.*

Table 1: *The sampled farms, their seroprevalence and the associated confidence interval (CI)*

Farm	Seroprevalence (%)	CI (95%)
Gaborone 1 (dairy)	88.1 (37/42)	+/- 9.79
Gaborone 2 (dairy)	27.1 (16/59)	+/- 11.34
Gaborone 3 (beef)	97.9 (47/48)	+/- 4.04
Lobatse 1 (dairy)	58.3 (21/36)	+/- 16.1
Lobatse 2 (beef)	60.0 (27/45)	+/- 14.3
Molepolole 1 (dairy)	37.5 (9/24)	+/- 19.4
Molepolole 2 (dairy)	16.7 (1/6)	+/- 29.8
Ramatlabama 1 (beef)	25.5 (13/51)	+/- 12.0
Otse 1 (beef)	46.0 (23/50)	+/- 13.8
Total	53.5 (193/361)	+/- 5.15

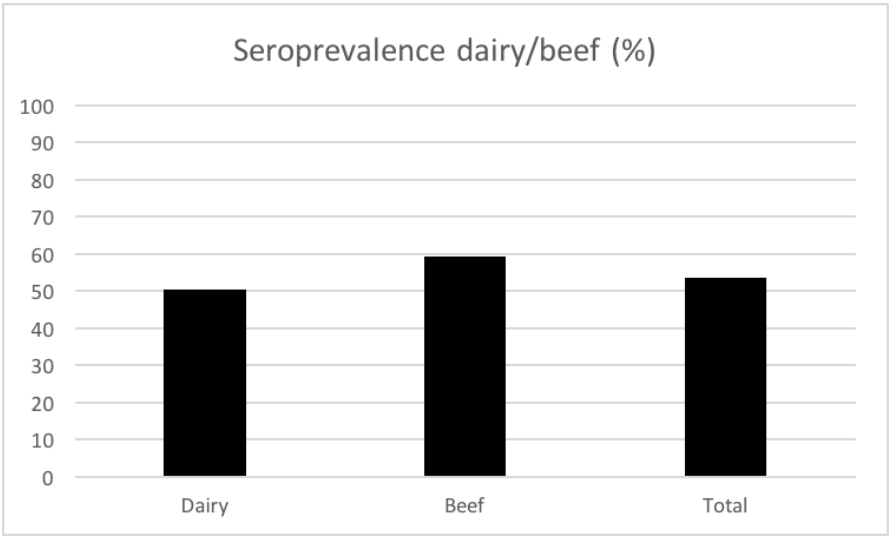

Fig 8. *Seroprevalences in beef and dairy cattle. The Y-axis shows seroprevalence in percent*

Table 2. *Seroprevalences in dairy and beef cattle and their associated confidence interval*

Dairy/Beef	Seroprevalence (%)	CI (95%)
Dairy	50.3 (84/167)	+/- 7.58
Beef	59.3 (115/194)	+/- 6.91

Total	53.5 (193/361)	+/- 5.15

Antigen

Almost all of the antibody negative samples were thereafter tested with Ag-ELISA. However, the residual volume of serum of five cattle in Lobatse 2 (beef) and one in Ramatlabama (beef) were too small, and testing could therefore not be resumed with these. One sample from Gaborone 3 (beef) was found to be positive for antigen (Fig 9). This is equivalent to a prevalence of 0.27%.

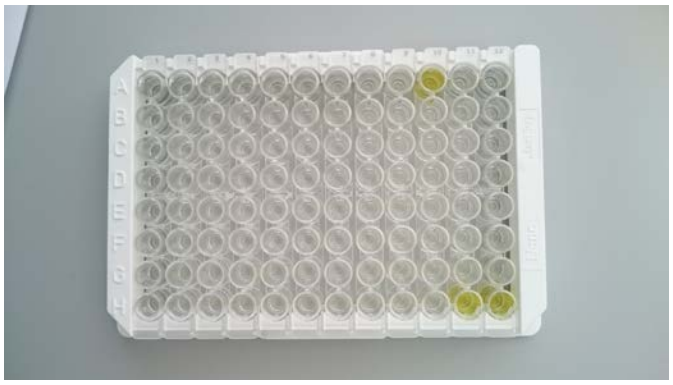

Fig 9. *The result of the Ag-ELISA. Negative controls are found in wells A1-2, positive controls in well H11-12. The sample in well A10 is positive.*

PCR performed in Botswana

The sample that was positive on Ag-ELISA was also positive with PCR (Fig 10). However, only the first PCR run was successful since multiple bands appeared on subsequent runs (Fig 11). On these, approximately nine other samples had bands in locations indicating a possible positive result. However, these results need to be interpreted with caution.

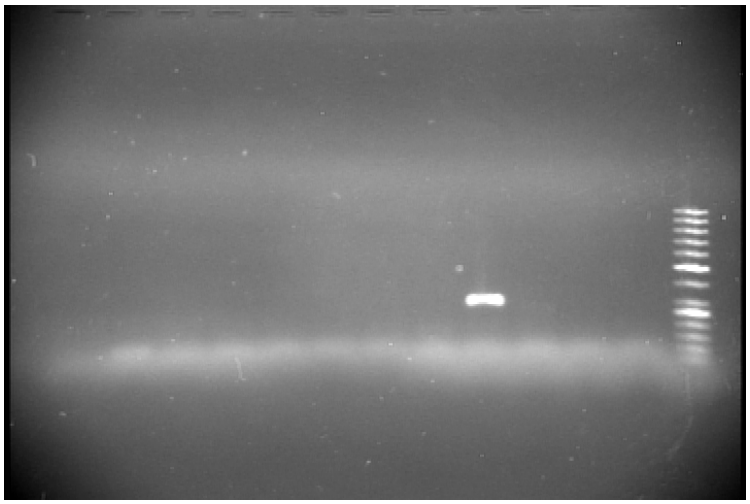

Fig 10: *PCR-results, performed in Botswana. One positive result (well number 5). Negative control situated immediately left to the ladder but, no positive control was used.*

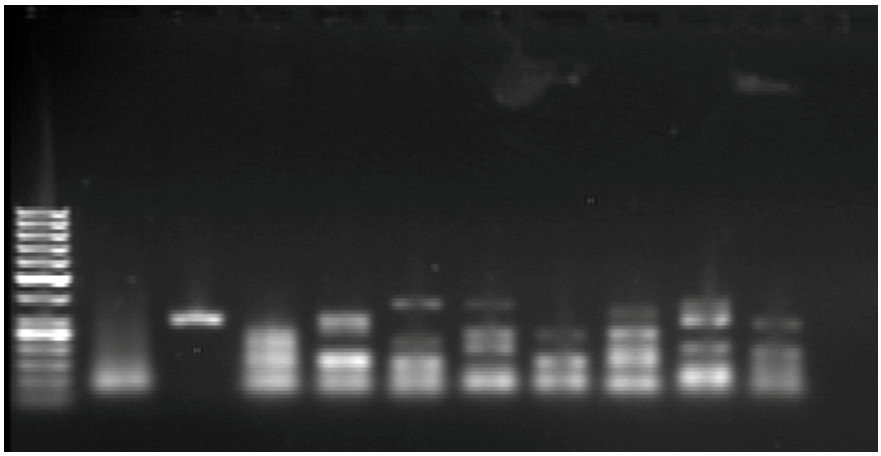

Fig 11: *Multiple bands. The negative control is situated immediately right to the ladder, followed by the positive sample which was used as a positive control in subsequent runs.*

PCR performed in Sweden

The PCR was subsequently repeated in Sweden because of the problems with multiple bands experienced in Botswana, and to acquire sufficient material for genetetic sequencing. The sample that was positive on Ag-ELISA and PCR only showed weak bands, even though the procedure was repeated several times in order to get a more distinct result. In addition, two other samples that were negative on both Ag-ELISA and PCR in Botswana were positive (Fig 12). These two samples originated from the same herd (Gaborone 1) with an antibody seroprevalence of 88.1%. One of these samples had a band in a location indicative of a possible positive result also on one of the PCR runs in Botswana with multilple bands. Three positive samples (3/361) is equivalent to a prevalence of 0.83%.

The sample that was positive on Ag-ELISA and PCR performed both in Botswana and Sweden originated in the herd Gaborone 3 which had the highest seroprevalence (97.9%). The two samples that were positive on PCR in Sweden originated from the herd Gaborone 1 which had the second highest seroprevalence (88.1%). The seroprevalence in Gaborone 3 was significantly higher than in all of the other herds, except Gaborone 1 ($p < 0.001$) with Minitabs 2-proportion test. In Gaborone 1, seroprevalence was significantly higher in all of the others except for Gaborone 3 and Lobatse 2 using the same test (p-value for Lobatse 2 was 0.051, for the rest <0.05).

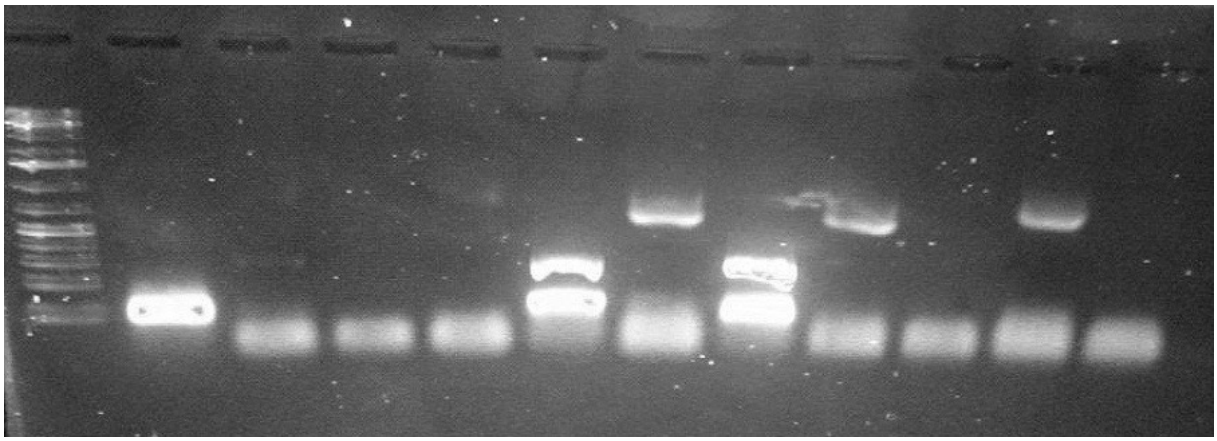

Fig 12. *Negative control situated just right to the ladder. Well number two contains the sample that was positive on Ag-ELISA and PCR in Botswana. Well number 6 and 8 (including the ladder) had bands in the correct location.*

Genetic Sequencing

The PCR products from two animals from the same kraal (Gaborone 1) were sent for sequencing. The two products were 338 and 337 base pairs long. The sequences were identical to each other and belonged to the BVDV-1 genotype. The segments showed high sequence resemblance with several BVDV-1 strains in the NCBI database. The top hit, when sorted after maximum score, was USMARC-53875 (Workman *et al*., 2015) (Table 3). This was followed by two NADL-strains (Vassilev & Donis, 2000). The top hits originating from the African continent was five strains from Mozambique isolated in 1991 and 1992, and three strains isolated in South Africa (Baule *et al*., 1997). These African strains were also the top hits when sorted after identity.

Table 3. *Information from the NCBI Blast consolidated into a table. The table is sorted in accordance to the maximum score appointed by the NCBI Database. The three strains with the highest maximum scores are included in the table, followed by the African isolates with the highest maximum scores. The African isolates shared the highest identity similarities with the strains that were sequenced in this study. Also, a BVDV-2 strain is included for reference*

Strain	Genotype	Max score	Identity	E-value	Origin
USMARC-53875	BVDV-1	473	95%	9.00E-130	USA
Ncp NADL	BVDV-1	473	96%	9.00E-130	USA
NADL	BVDV-1	473	96%	9.00E-130	USA
M278A/91	BVDV-1	420	98%	1.00E-113	Mozambique
M589A/92	BVDV-1	414	98%	6.00E-112	Mozambique
M139B/91	BVDV-1	414	98%	6.00E-112	Mozambique
S-ALT7/K	BVDV-1	412	97%	2.00E-111	South Africa
USMARC-60765	BVDV-2	231	87%	7.00E-62	USA

Interview

All farmers (100% or 11/11) reported to utilize communal pastures where their goats could get into contact with animals from other herds. The majority (9/11 or 82%) of the sampled herds had other domestic ruminant species in the household. All of the nine reported to keep cattle in addition to goats, and two of them (18%) to also keep sheep. Seven farmers (78%, 7/9) kept cattle in close proximity to the goats, allowing them to share grazing grounds. The farmers who also owned sheep admitted to housing them in the same kraal during the night. A total of 56% (5/9), kept goats and cattle in kraals next to each other during the night. However, none of the farmers kept cattle and goats in the same kraal at night time (Fig 13).

For the acquisition of new stock, the vast majority solely depended on rearing kidlings from their current stock. Only 18% (2/11) of the farmers reported that they sometimes bought goats from other farmers, whereof one occasionally purchased from a local farmer and one from a farmer in another village (Fig 13). The majority of the farmers used medicines when their animals were ill. For example, 72.7% (8/11) dewormed their animals occasionally or on a more regular basis. Antibiotics, such as sulfonamide and oxytetracycline, were used by 45% (5/11). Vaccination was practiced by 36% (4/11), three of them against Pulpy Kidney Disease and one against Pulpy Kidney Disease and Pasteurella. However, there was confusion about the terms deworming/anthelmintics, antibiotics and vaccination. Some farmers answered that they vaccinated their animals, then mentioned the name of an antibiotic when asked to specify. Therefore, these numbers may be incorrect.

Several (55% or 6/11) of the farmers reported to observe wildlife species in their home area (Fig 13). In Modipane all (100%) of the sampled kraals reported sightings of wildlife ruminant species in the area. Impala, duiker and kudu were a common sighting. One also stated to occasionally see springboks. Jackals were commonly sighted in the area as well, and one farmer reported to occasionally spot hyenahs and cheetahs. Rabbits were also commonly seen. Wildlife sightings in Kopong were less diverse than in Modipane, and only 33% (1/3) stated to occasionally spot wildlife ruminants (duiker) in the area. One of the farmers reported not having seen wildlife in the area in the last 5 years, whereas one reported to occasional see jackals. Also in Gakuto, sightings of wildlife were less diverse. Two farmers reported spotting jackals and one hyenahs. Kudu and duikers were occasionally seen by 67% (2/3). None of the interviewed farmers was sure of wether their animals ever got in direct contact with wildlife, but stated that they shared grazing grounds.

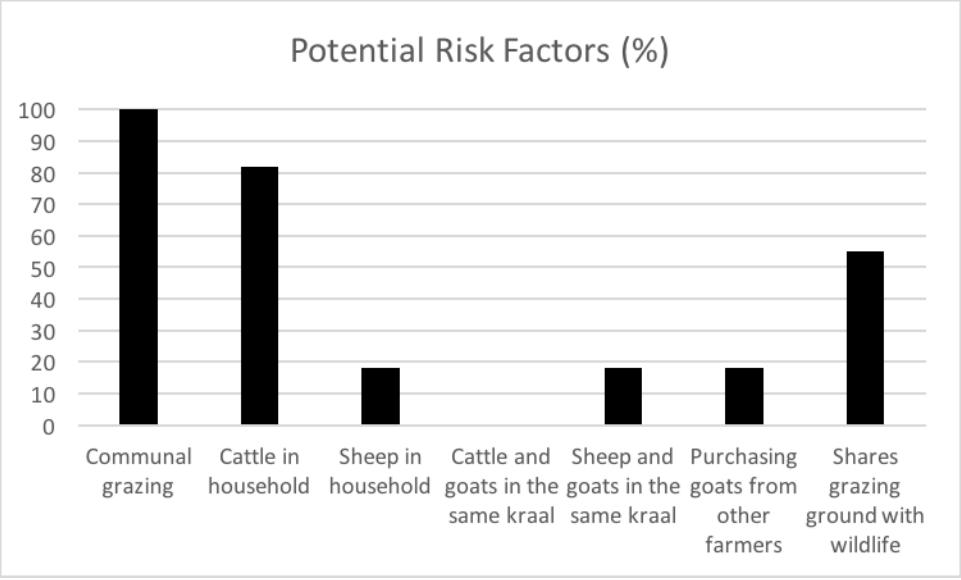

Fig 13. *Potential risk factors for transmission of BVDV. The Y-axis shows the percentage of the farmers whose goats are subjected to the risks listed on the X-axis.*

Abortion was a commonly listed health problem (10/11 or 91%). The extent of the abortions varied since some only had occasional problems whereas others considered it a major issue. Diarrhoea was reported by 64% (7/11). Coughing was reported as an occasional problem by 36% (4/11), whereas two farmers admitted to not pay attention to coughing. Only two farmers (18%) experienced problems with runny eyes and noses (Fig 14).

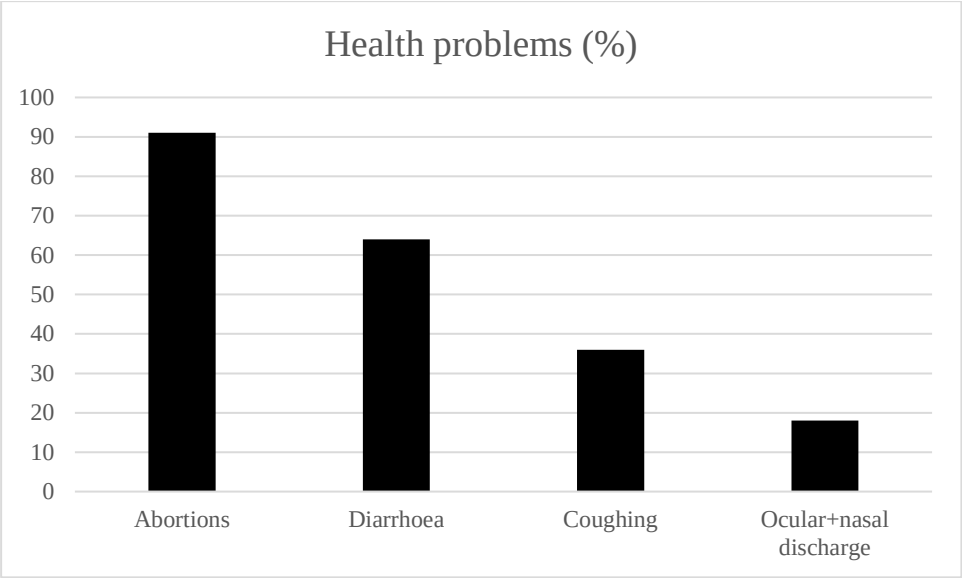

Fig 14. *The Y-axis shows the percent of the farmers who experienced problems with the syndromes listed on the X-axis.*

All farmers stated that they are very dependent in their goats for food and/or cash income and that it would have devastating consequences if their animals were to fall ill or die in large numbers. Two of the smallholders also admitted an emotional connection to their stock and that it is painful to witness illness and death among them. For some smallholders, goats were their only livelihood while others also had other occupations.

Discussion

Botswana is an upper middle-income country situated in southern Africa. The vast majority of the country's inhabitants are dependent on agriculture and livestock to survive (Svenska FN-förbundet, 2014). BVDV is an endemic pathogen in most of the cattle producing world, and is a cause of decreased production and impaired animal health globally (Lindberg & Alenius, 1999). Because of this, many countries have implemented control measures that aim to reduce disease incidence in their nation. Some countries, e.g. Sweden, have even successfully eradicated the virus (SVA, 2017).

In principal, there are two different methods to control BVDV. There are vaccination regimes and control programs of which the purpose is to improve biosecurity as well as to identify and remove persistently infected animals. In some instances, the two methods are combined (Lindberg & Alenius, 1999). Which one of the two that is the most economically viable vary between herds, since it is dependent on the specific conditions in different flocks. For example, in herds where the risk of infection is high, e.g. due to extensive animal trade, vaccination may be more profitable (Houe, 2003). There are both modified live vaccines (MLV) as well as killed vaccines available today with acceptable efficacy (Ridpath, 2013). The primary purpose of vaccination is to prevent prenatal infection, i.e. persistent infection, and thereby also the establishment of BVDV in a herd if it is introduced. Vaccination can also prevent or limit the clinical effects and economic implications of acute infection.

However, vaccination regimes also have clear disadvantages. First of all, BVDV is a highly genetically variable virus, which makes it difficult to produce vaccines that are able to prevent infection of all genotypes/subgenotypes (Lindberg & Houe, 2005; Ridpath, 2013). In addition to this, MLVs may be contaminated with BVDV and can hence contribute to its spread (Ridpath, 2013). Besides this, there are currently no available vaccines that evoke an antibody response that is easily differentiated from natural BVDV-infection, i.e. no DIVA vaccine (Raue *et al*., 2011). Also, in order to achieve long term control, vaccination alone is usually insufficient. This is because of the extremely high infectious pressure posed by persistently infeced animals, which means that the efficacy of the vaccination regime must be close to 100%. Achieving 100% protection is very difficult or even impossible, especially if the vaccination strategy is not implemented in a controlled manner. Therefore, in order to achieve long-term control, vaccination should ideally be combined with appropriate biosecurity measures. Vaccination implemented on its own may even convey a false sense of security and hence lead to detrimental breaches in biosecurity, thereby allowing introduction and establishment of the infection (Lindberg & Houe, 2005; Ridpath, 2013).

Because of these limitations, control programs aiming to systematically identify and remove persistently infected animals coupled with biosecurity measures, are generally viewed as the superior alternative (Lindberg & Houe, 2005). However, the establishment of such programs is currently unlikely in the vast majority of African countries. This is due to, for example, a lack of veterinary infrastructure, widespread animal movement and lack of trade control (Handel *et al*., 2011). Also, control measures that are only instituted on an individual farm have limitations, mainly due to the lack of control of the infectious pressure that neighboring farms/smallholders put on the environment. They are therefore generally more effective when implemented at a regional or national level (Houe, 2003). Due to the absence of a national control program that is supervised and financed by the Botswana government, the most economic alternative for farmers in Botswana may be vaccination. Ideally, this should be combined with biosecurity measures and monitoring, as mentioned above (Ridpath, 2013).

Prevalence of a disease as well as the infectious status of herds in a region is necessary knowledge in order to implement a control program (Lindberg & Houe, 2005). The seroprevalence in cattle found in this study was 53.5%. According to Houe *et al*. (1999) seroprevalence is usually 60% or more in endemic regions. However, this number varies greatly with differences in management such as animal trade and vaccination. Previous studies conducted on the African continent have found seroprevalences ranging from approximately 10-50% in cattle without signs of BVDV-infection (Depner *et al*., 1991b; Hyera, 1991; Nigussie *et al*., 2010; Handel *et al*., 2011; Njiro *et al*., 2011; Callaby *et al*., 2016). This comparatively low prevalence is maybe more likely due to differences in management strategies, for example less intensive production systems, rather than the virus not being endemic in the region.

The cattle samples that were used in this study were obtained as part of another research project that was not focusing on BVDV. Because of this, it was not recorded whether the farmers vaccinated their animals for BVDV or not. It can therefore not be excluded that the detected seroprevalence was due to vaccination rather than natural infection. However, in Gaborone 1 and 3 antigen-positive animals were found, which indicates active infection. In Gaborone 1 and 3, seropositivity was 88.1% and 97.9%. Rigourious vaccination schemes can lead to seroprevalences that are this high, but it is more likely that natural infection due to contact with viraemic animals has caused at least the majority of this high seropositivity.

The Ab-ELISA that was used detects antibodies for both BVDV and Border Disease Virus (BDV). Because of this it can not be said for certain that the antibodies found were directed to BVDV or BDV. Both infections can affect both cattle and goats (Nettleton, 1990). However, the virus from two of the

viraemic animals was designated as BVDV-1 with sequencing. The third was positive on Ag-ELISA as well as PCR, and the Ag-ELISA is specific for BVDV. The high seroprevalence in the two herds, where viraemic animals were found, is likely to be due to either the presence of persistently infected animals or active infection with BVDV. Also, to the author's knowledge, Border Disease Virus has never been detected in Southern Africa. However, this may be due to a lack of surveys and/or failure of detection, rather than the virus truly being absent in the region.

When using the antibody-ELISA, the negative control was continously invalid. Reportedly, this was a common problem with the ELISA reader that was used. However, for the antigen-ELISA the same ELISA reader was utilised without similar problems. Both procedures were performed by the same person and using the same basic techniques. The problem with the negative control in the antibody-ELISA may have resulted in antibody prevalence values lower than the actual value. To calculate the percentage of inhibition, the sample absorbance value was divided with the mean of the two negative controls, and then multiplied with 100. Therefore, with low negative control values, the percentage of inhibition value may get misleadingly high and therefore falsely classify samples as negative or suspect when they were in fact positive.

The prevalence of animals positive for antigen was 0.27% or 0.83% in this study. The difference between the two may be due to the usage of more sensitive equipment in Sweden. Ideally, when an Ag-positive animal is detected, testing should be repeated after 3-4 weeks to differentiate between acute and persistent infections. Due to the time lapse between sampling and analysis, this was not possible in this study. According to Houe *et al*., (1999) the prevalence of persistently infected animals in endemic populations is usually around 1-2%. In this study, Ag-ELISA was only performed on the animals that were seronegative in herds with at least one seropositive animal. However, an animal can be immunotolerant, and hence seronegative to one strain, but seropositive to a heterologous strain due to vaccination or natural infection. Because of this, it is possible that some viraemic animals were missed. However, according to Lindberg and Alenius (1999) this risk is likely negligible. Also, six of the antibody negative samples were not subjected to antigen-ELISA and PCR because the sample volume was too low. Five of these samples originated from Lobatse 2 where seroprevalence was 60%, and one from Ramatlabama 1 where prevalence was 25.5%. According to Houe *et al*., (1993) seroprevalence in herds with persistent infection is usually 90% or more. It is therefore unlikely that any of these samples were Ag-positive. Nevertheless, the fact that these animals could in fact have been viraemic can not be ignored.

There was not enough available material to perform RNA-isolation and PCR on all the seronegative samples. Therefore, a cut off value of 50% was chosen, which means that RNA isolation and PCR was performed on antibody negative animals in herds with an antibody prevalence of 50% or more. According to Houe *et al*., (1993), seroprevalence is usually 90% or more in herds with persistently infected animals. However, this number is influenced by a number of factors, for example management strategies, such as contact between different groups of animals, etc. Since this information was not available for the tested cattle herds, a lower cut off value was chosen, in order to minimize the risk of a viraemic animal escaping identification.

The number of cattle that was positive for antigen may be falsely low due to repeated freeze-thawing cycles of the samples as well as storage for about two years at -20 °C. It has been shown for the RNA-virus Hepatitis C virus that RNA titers dropped significantly after five cycles of freeze-thawing (16%), as well as after 6 months storage at -20 °C (23%), compared to storage at -80 °C (10%) (Halfon *et al*., 1996). The goat samples were also subjected to freeze-thawing cycles and storage at -20 °C, but not to

the same extent nor for the same length of time. To the author's knowledge, no similar study on the effect of storage on BVDV concentrations have been conducted.

The two viral segments that were sequenced in this study belonged to the BVDV-1 genotype, which according to previous studies is more common in Africa than BVDV-2 (Baule *et al*., 1997; Vilcek *et al*., 2000; Kabongo *et al*., 2003; Thabti *et al*., 2005; Ularamu *et al*., 2005; Van Vuuren, 2005; Emran *et al*., 2014). The two isolates were identical to each other, which is not surprising given the fact that they originated from animals in the same herd. The two segments were similar to a high number of sequences in the NCBI nuclotide database besides the ones discussed here. This is not surprising either since the sequenced part of the genome, i.e. the 5'NCR, is a highly conserved region (Bauermann *et al*., 2013; Van Vuuren *et al*., 2005).

The sequence that shares the most similarities, according to the NCBI nuclotide database, when sorted after maximum score, is USMARC-53875, which was isolated in the USA (Workman *et al*., 2015). This is followed by two NADL-strains (Vassilev & Donis, 2000) (Table 3). NADL is a reference strain for BVDV-1a that was isolated for the first time in North America (Baule et al., 1997; Mendez *et al*., 1998). The most similar sequences from the African continent originate in Mozambique and South Africa. These strains were also similar to the BVDV-1a reference strain NADL (Baule *et al*., 1997).

In the goats sampled in this study, no evidence of antibodies to BVDV/BDV could be found. Previous studies conducted in other African countries have found seroprevalences of 5% in neighbouring Namibia (Depner *et al*., 1991b), and 2.93% (Päärni, 2016) as well as 24.9% (Hyera, 1991) in Tanzania. All goats sampled in this study originated from herds around Gaborone, which is the same region as the viraemic cattle were found. Because of this, it is unlikely that the 0% seroprevalence is due to BVDV not existing in the area. It is more likely that the goats were insufficiently exposed to the virus to develop antibodies. Also, seroprevalence in goats is in general considerably lower compared to cattle. This is probably because persistent infection is an unusual occurrence in this species (Depner *et al*., 1991a; Broaddus *et al*., 2009; Bachofen *et al*., 2013; Passler *et al*., 2014).

Important risk factors for infection of BVDV include, among others, animal trade (Lindberg & Alenius, 1999; Valle *et al*., 1999; Graham *et al*., 2013;) and sending animals to communal pastures (Lindberg & Alenius, 1999; Valle *et al*., 1999; Walter *et al*., 2005; Krametter-Froetscher *et al*., 2007). In goats, regular contact with cattle has been shown to increase the risk of infection (Mischra *et al*., 2009). In this study, only 18% of the farmers admitted to purchase goats from other smallholders. Also, none of the farmers reported to house goats and cattle in the same kraal. However, 82% kept other ruminant species in the household, the majority cattle, and more than 3/4 of these kept them in close proximity to the goats. Also, all of the farmers utilized communal grazing grounds for their animals.

All of the farmers admitted to being very dependent on their goats for income. Fortunately, the average Body Condition Score and Famacha score (3.4 and 2.4) were within normal ranges, indicating that the goats were in good health, at least in these aspects. However, abortions were a commonly listed health problem, since 91% of the herds struggled with this on a varying level. Diarrhoea was also a common problem since 64% occasionally experienced this. Respiratory problems were less common. All of these clinical signs are seen when goats are infected with BVDV. However, these signs can have numerous other causes besides pestiviral infection. This study can not predict the underlying causes, but it does however demonstrate that they are not caused by infection with BVDV or BDV.

There is a great need of further research on BVDV in Botswana and on the African continent. More knowledge is required about the implications of BVDV in these countries and its economic significance to smallholder farmers. This should not only focus on the direct effects of BVDV but also on its role as

a co-pathogen in e.g. respiratory and enteric diseases. Also, more extensive knowledge is needed on viral prevalence as well as local risk factors for viral transmission, since this knowledge is imperative when deciding on the most efficient and cost-effective way to control the infection. Last but not least, there is a great need for extended knowledge regarding control methods applicable to the local settings. This coupled together could lead to a reduced incidence of BVDV, and thereby not only improve animal health and welfare, but also help in reducing hunger and poverty in Botswana.

Conclusion

Seroprevalence of BVDV in and around Gaborone was 0% in goats and 53.5% in cattle. The seroprevalence in dairy cattle was 49.7% and 56.7% in beef cattle, however, this difference was not statistically significant. Antigen prevalence in cattle was 0.27% on Ag-ELISA and PCR in Botswana, and 0.83% on PCR in Sweden. These animals originated from the two herds with the highest antibody prevalences (97.9% and 88.1%), and these seroprevalences were, with one exception, significantly higher compared to the other herds. Also, the viruses of two viraemic animals were sequenced and subsequently assigned to the BVDV-1 genotype. They also resembled NADL-strains, i.e. the reference strain for BVDV-1a, which has a North American origin.

Several risk factors for BVDV transmission were present. All the farmers allowed their goats to graze on communal pastures, and 64% reported to keep cattle in close proximity to the goats. Only 18% admitted to keep sheep and goats toegether in the same kraal during the night, while 55% reported to occasionally spot wildlife ruminants in the area. Only 18% purchased goats from other farmers, the majority relied solely on raising their own kidlings to adulthood. The most common health problem encountered was abortion, which 91% occasionally struggled with. Diarrhoea was a problem in 64% of the herds, while 36% also had problems with coughing and 18% with ocular and nasal discharge. All the farmers reported that they are very dependent on their goats for food and/or cash income and that it would have devastating consequences if their animals were to fall sick or die in large numbers.

Acknowledgment

I would like to gratefully acknowledge my primary supervisor professor Mikael Berg for his guidance throughout the project. I am also thankful to my assisting supervisor Dr Jonas Johansson Wensman as well as local supervisor Dr Solomon Ramabu for all their help and thoughtful comments. I am also grateful to my examiner Dr Maja Malmberg for her helpful critique. I would also like to express my gratitude to Emeli Torsson, Sumbikane Gasebonwe, Nico Mosweu, Moemedi Legodimo, Mathio Solomon Mathaio, Noliwe Nontokozo Lebani and Kutlwano Eunice Samakabadi for all their assistance. Last but not least, I would like to thank my fellow MFS-students Jessica Berthelsson and Tove Johansson, for sharing this experience with me.

This project was sponsored by the Swedish governmental agency SIDA through a Minor Field Study (MFS) scholarship, as well as a scholarship from the Faculty of Veterinary Medicine Scholarship Fund

(SLU.ua 2016.3.1.5-914). The project was also financed by the Swedish Research Council (Grant no. 348-2014-4293). I would like to express my sincere gratitude for these fundings.

References

Ali, Y., Intisar, K., Ishag, O., Baraa, A., Haj, M., Taha, K., Tamador, M., Hussein, M., Elfahal, A. 2013. Seroprevalence of pestiviruses in small ruminants in Sudan. *African Journal of Microbiology Research* (7): 3988-3991

Almeida, L., Miranda, I., Hein, H., Santiago Neto, H., Costa, E., Marks, F., Rodenbush, C., Canal, C., Corbellini, L. 2013. Herd-level risk factors for bovine viral diarrhea virus infection in dairy herds from Southern Brazil. *Research in Veterinary Science* (95): 901-907

Anderson, E., Rowe, L. 1998. The prevalence of antibody to the viruses of bovine virus diarrhoea, bovine herpes virus 1, rift valley fever, ephemeral fever and bluetongue and to Leptospira sp in free-ranging wildlife in Zimbabwe. *Epidemiology & Infection* (121): 441-449

Aranga, A., Omphile, U., Chabo, R., Kgosimore, M., Mochankana, M. 2005. Goat Production under Traditional Management in Gaborone Agricultural Region in Botswana. *Journal of Animal and Veterinary Advances* (5): 515-519

Bachofen, C., Vogt, H-R., Stalder, H., Mathys, T., Zanoni, R., Hilbe, M., Schweizer, M., Peterhans, E. 2013. *Veterinary Research* (44): 10 pages

Bahta, S., Malope, P. 2014. Measurement of competitiveness in smallholder livestock systems emerging policy advocacy: An application to Botswana. *Food Policy* (49): 408-417

Bauermann, F., Ridpath, J., Weiblen, R., Flores, E. 2013. HoBi-like viruses: an emerging group of pestiviruses. *Journal of Veterinary Diagnostic Investigation* (25): 6-15

Baule, C., van Vuuren, M., Lowings, J., Bélak, S. 1997. Genetic heterogeneity of bovine viral diarrhoea viruses in Southern Africa. *Virus Research* (52): 205-220

Becher, P., Orlich, M., Shannon, A., Horner, G., König, M., Thiel, H-J. 1997. Phylogenetic analysis of pestiviruses from domestic and wild ruminants. *Journal of General Virology* (78): 1357-1366.

Bolin, S., Matthews, P., Ridpath, J. 1991. Methods for detection and frequency of contamination of fetal calf serum with bovine viral diarrhea virus and antibodies against bovine viral diarrhea virus. *Journal of Veterinary Diagnostic Investigation* (3): 199-203

Broaddus, C., Holyoak, R., Dawson, L., Step, D., Funk, R., Kapil, S. 2007. Transmission of bovine viral diarrhea virus to adult goats from persistently infected cattle. *Journal of Veterinary Diagnostic Investigation* (19): 545-548

Callaby, R., Toye, P., Jennings, A., Thumbi, S., Coetzer, J., Conradie Van Wyk., J., Hanotte, O., Mbole-Kariuki, M., deC Bronsvoort, B., Kruuk, L., Woolhouse, M., Kiara, H. 2016. Seroprevalence of respiratory viral pathogens of indigienous calves in Western Kenya. *Research in Veterinary Science* (108): 120-124

Carman, S., van Dreumel, T., Ridpath, J., Hazlett, M., Alves, D., Dubovi, E., Tremblay, R., Bolin, S., Godkin, A., Anderson, N. 1998. Severe acute bovine viral diarrhea in Ontario, 1993-1995. *Journal of Veterinary Diagnostic Investigation* (10): 27-35

Causabon, J., Vogt, H-R., Stalder, H., Hug, C., Ryser-Degiorgis, M-P. 2012. Bovine viral diarrhea virus in free-ranging wild ruminants in Switzerland: low prevalence of infection despite regular interactions with domestic livestock. *BMC Veterinary Research* (8): 14 pages

Central Intelligence Agency. 2016. *Botswana*. https://www.cia.gov/library/publications/the-world-factbook/geos/bc.html (2016-12-05)

Climate Data, 2017a. *Climate: Botswana*. https://en.climate-data.org/country/115/ (2017-01-23).

Climate Data, 2017b. *Climate: Gaborone*. https://en.climate-data.org/location/653/ (2017-01-23).

Damman, A., Viet, A-F., Arnoux, S., Guerrier-Chatellet, M-C., Petit, E., Ezanni, P. 2015. Modelling the spread of bovine viral diarrhea virus (BVDV) in a beef cattle herd and its impact on herd productivity. *Veterinary Research* (46): 14 pages

Depner, K., Hubschle, O.J.B., Liess, B. 1991a. BVD-virus infection in goats – experimental studies on transplacental transmissibility of the virus and its effect on reproduction. *Archives of Virology* (3): 253-256

Depner, K., Hubschle, O.J.B., Liess, B. 1991b. Prevalence of ruminant Pestivirus Infections in Namibia. *Onderstepoorts Journal of Veterinary Research* (58): 107-109

Elvander, M., Baule, C., Persson, M., Egyed, L., Ballagi-Pordány, A., Belák, S., Alenius, S. 1998. An Experimental Study of a Concurrent Primary infection with Bovine Respiratory Syncytial Virus (BRSV) and Bovine Viral Diarrhea Virus (BVDV) in calves. *Acta Vet Scand* (39): 251-264

Emran, R., Arafa, A., Aly, N., Amin, D., Selim, A., El Kalamawey, N. 2014. Heterogenicity of Bovine Viral Diarrhea Virus in Egypt. *Global Veterinaria* (13): 966-971

FAO. 2014. *Country Programming Framework for Botswana*. Food and Agriculture Organization of the United Nations.

Ferreira, G., Lourens, D., Van Vuuren, M. 2000. The prevalence of bovine viral diarrhoea antibodies in selected South African dairy herds, and control of the disease. *Journal of the South African Veterinary Association* (71): 10-13

Fulton, R., Saliki, J., Confer, A., Burge, L., d'Offay, J., Helman, R., Bolin, S., Ridpath, J., Payton, M. 2000. Bovine viral diarrhea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle. *Journal of Veterinary Diagnostic Investigation* (28): 33-38

Fulton, R., Ridpath, J., Confer, A., Saliki, J., Burge, L., Payton, M. 2003. Bovine viral diarrhoea virus antigenic diversity: impact on disease and vaccination programmes. *Biologicals* (31): 89-95

Gates, M., Woolhouse, M., Gunn, G., Humphry, R. 2013. Relative associations of cattle movements, local spread, and biosecurity with bovine viral diarrhoea virus (BVDV) and seropositivity in beef and in dairy herds. *Preventive Veterinary Medicine* (112): 285-295

Graham, D., Clegg, T., Lynch, M., More, S. 2013. Herd-level factors associated with the presence of bovine viral diarrhoea virus in herds participating in the voluntary phase of the Irish national eradication programme. *Preventive Veterinary Medicine* (112): 99-108

Halfons, P., Khiri, H., Gerolami, V., Bourliere, M., Feryn, J., Reynier, P., Gauthier, A., Cartouzou, G. 1996. *Journal of Hepatology* (25): 307-311

Handel, I., Willoughby, K., Land, F., Koterwas, B., Morgan, K., Tanya, V., deC. Bronsvoort, B. 2011. Seroepidemiology of Bovine Viral Diarrhoea Virus (BVDV) of Cameroon and Use of the SPOT Test to Identify Herds with PI Calves. *PLoS ONE* (6): 11 pages

Harasawa, R., Giangaspero, M., Ibata, G., Paton, D. 2000. Giraffe Strain of Pestivirus: Its Taxonomic Status Based on the 5'-Untranslated Region. *Microbiology and Immunology* (44): 915-921

Hellyer, M., Solomon, M., Manda, E., Gideon, P. (2015). *Value chain analysis in the goat sector under the framework of the Private Sector Development Programme (PSDP), Botswana*. BWP/1304/01/GO.

Houe, H. 1992. Age Distribution of Animals Persistently Infected with Bovine Virus Diarrhea Virus in Twenty-two Danish Dairy Herds. *Canadian Journal of Veterinary Research* (56): 194-198

Houe, H. 1999. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Veterinary Microbiology* (64): 89-107

Houe, H. 2003. Economic impact of BVDV infection in dairies. *Biologicals* (31): 137-143

Houe, H., Meyling, A. 1991. Prevalence of bovine virus diarrhoea (BVD) in 19 Danish dairy herds and estimation of incidence of infection in early pregnancy. *Preventive Veterinary Medicine* (11): 9-16

Houe, H., Pedersen, K., Meyling, A. 1993. The effect of bovine virus diarrhea virus infection on conception rate. *Preventive Veterinary Medicine* (15):117-123

Hunter, A., Carmichael, I. 1975. Mucosal Disease in Botswana. *Tropical Animal Health and Production* (7): 41-44

Hyera, J., Liess, B., Frey, H-R. 1991. Bovine Viral Diarrhoea Virus Infection in Cattle, Sheep and Goats in Northern Tanzania. *Tropical Animal Health and Production* (23): 83-94

International Committee on Taxonomy of Viruses. 2016. *Virus Taxonomy: 2015 Release*. http://www.ictvonline.org/virustaxonomy.asp (2016-12-05).

Jönsson, E. 2013. *Seroprevalence and risk factors for brucellosis, salmonellosis and bovine viral diarrhea in urban and peri-urban areas of Kampala, Uganda*. Master thesis, Swedish University of Agricultural Sciences.

Kabongo, N., Baule, C., Van Vuuren, M. 2003. Molecular analysis of bovine viral diarrhoea virus isolates from South Africa. *Onderstepoort Journal of Veterinary Research* (70): 273-279

Kabongo, N., Van Vuuren, M. 2004. Detection of bovine viral diarrhea virus in specimens from cattle in South Africa and possible association with clinical disease. *Journal of the South African Veterinary Association* (75): 90-93

Krametter-Froetscher, R., Kohler, H., Benetka, V., Moestl, K., Golja, F., Vilcek, S., Baumgartner, W. 2007. Influence of Communal Alpine Pasturing on the Spread of Pestiviruses among Sheep and Goats in Austria: First Identification of Border Disease Virus in Austria. *Zoonoses and Public Health* (54): 209-213

Landguiden. 2015. *Botswana*. http://www.landguiden.se/Lander/Afrika/Botswana (2017-01-23).

Lindberg, A., Alenius, S. 1999. Principles for eradication of bovine viral diarrhoea virus (BVDV) infection in cattle populations. *Veterinary Microbiology* (64): 197-222

Lindberg, A., Groenendaal, H., Alenius, S., Emanuelsson, U. 2001. Validation of a test for dams carrying fetuses persistently infected with bovine viral-diarrhoea virus based on determination of antibody levels in late pregnancy. *Preventive Veterinary Medicine* (51): 199-214

Lindberg, A., Houe, H. 2005. Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. *Preventive Veterinary Medicine* (72): 55-73

Mars, M., Bruschke, C., van Oirschot, J. 1999. Airborne transmission of BHV1, BRSV, and BVDV among cattle is possible under experimental conditions. *Veterinary Microbiology* (66): 197-207

Martin, C., Letellier, C., Caij, B., Gauthier, D., Jean, N., Shaffii, A., Saegerman, C. 2011. Epidemiology of Pestivirus infection in wild ungulates of the French South Alps. *Veterinary Microbiology* (147): 320-328

Mendez, E., Ruggli, N., Collett, M., Rice, C. 1998. Infectious Bovine Viral Diarrhea Virus (Strain NADL) RNA from Stable cDNA Clones: a Cellular Insert Determines NS3 Production and Viral Cytopathogenicity. *Journal of Virology* (72): 4737-4745

Meiring, T., Prozesky, L., Preez, E., Verwoerd, D. 2011. The diagnosis and prevalence of persistent infection with bovine viral diarrhea virus in South African feedlot cattle. *Onderstepoort Journal of Veterinary Research* (78): 8 pages

Mishra, N., Rajukumar, K., Tiwari, A., Nema, R., Behera, S., Satav, J., Dubey, S. 2009. Prevalence of Bovine viral diarrhoea virus (BVDV) antibodies among sheep and goats in India. *Tropical Animal Health and Production* (41): 1231-1239

Mockeliuniene, V., Šalomskas, A., Mockeliunas, R., Petkevicius, S. 2004. Prevalence and epidemiological features of bovine viral diarrhoea virus infection in Lithuania. *Veterinary microbiology* (99): 51-57

Moen, A., Sol, J., Sampimon, O. 2005. Indication of transmission of BVDV in the absence of persistently infected (PI) animals. *Preventive Veterinary Medicine* (72): 93-98

Mrema, M., Rannobe, S. 1994. Goat Production in Botswana: Factors affecting production and marketing among small-scale farmers. (Lebbie, S.H.B., Kagwini, E.) *Small Ruminant Research and Development in Africa, Proceedings of the Third Bienniel Conference of the African Small Ruminant Research Network UICC, Kampala, Uganda, 5-9 December 1994.* ILRI (International Livestock Research Institute) p 105-108

Nettleton, P.F. 1990. Pestivirus infections in ruminants other than cattle. Scientific and Technical Review of the Office International des Epizooties (Paris) (9):131-150

Nettleton, P.F., Gilray, J., Russo, P., Dlissi, E. 1998. Border disease of sheep and goats. *Veterinary Research* (29): 327-340

Nigussie, Z., Mesfin, T., Sertse, T., Tolosa Fulasa, T., Regassa, F. 2010. Seroepidemiological study of bovine viral diarrhea (BVD) in three agroecological zones in Ethiopia. *Tropical Animal Health and Production* (42): 319-321

Njiro, S., Kidanemariam, A., Tsotetsi, A., Katsande, T., Mnisi, M., Lubisi, B., Potts, A., Baloyi, F., Moyo, G., Mpofu, J., Kalake, A., Williams, R. 2011. A study of some infectious causes of reproductive disorders in cattle owned by resource-poor farmers in Gauteng Province, South Africa. *Journal of the South African Veterianry Association* (82): 213-218

Niskanen, R., Alenius, S., Belák, K., Baule, C., Voges, H., Gustafsson, H. 2002. Insemination of Susceptible Heifers with Semen from a Non-Viraemic Bull with Persistent Bovine Virus Diarrhoea Virus Infection Localized in the Testes. *Reproduction in Domestic Animals* (37): 171-175

Niskanen, R., Emanuelson, U., Sundberg, J., Larsson, B., Alenius, S. 1995. Effects of infection with bovine viral diarrhoea virus on health and reproductive performance in 213 dairy herds in one county in Sweden. *Preventive Veterinary Medicine* (23): 229-237

Niskanen, R., Lindberg, A. 2003. Transmission of Bovine Viral Diarrhoea Virus by Unhygienic Vaccination Procedures, Ambient Air, and from Contaminated Pens. *The Veterinary Journal* (165): 125-130

Niskanen, R., Lindberg, A., Alenius, S. 2000. Lack of Virus Transmission from Bovine Viral Diarrhoea Virus Infected Calves to Susceptible Peers. *Acta Veterinaria Scandinavia* (41): 93-99

Panin, A. 1994. Profitability and income contribution of small ruminant production to rural African households: A case study of Kgatleng and Kweneng districts (Lebbie, S.H.B., *Bienniel Conference of the African Small Ruminant Research Network UICC, Kampala, Uganda, 5-9 December 1994.* ILRI (International Livestock Research Institute) p 105-108

Panin, A. 2000. A Comparative Economic Analysis of Smallholde Cattle and Small Ruminant Production Systems in Botswana. *Tropical Animal Health and Production* (32): 186-196

Passler, T., Riddell, K., Edmondson, M., Chamorro, M., Neill, J. 2014. Experimental infection of pregnant goats with bovine viral diarrhea virus (BVDV) 1 or 2. *Veterinary Research*: 45 10 pages

Passler, T., Walz, P. 2009. Bovine biral diarrhea virus infections in heterologous species. *Animal Health Research Reviews* (11): 191-205

Päärni, M. 2015. *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*. Master thesis., Swedish University of Agricultural Sciences.

Raue, R., Harmeyer, S., Nanijani, S. 2011. Antibody responses to inactivated vaccines and natural infection in cattle using bovine viral diarrhea virus ELISA kits: assessment of potential to differentiate infected and vaccinated animals. *The Veterinary Journal* (187): 330-334

Ridpath, J. 2013. Immunology of BVDV vaccines. *Biologicals* (41): 14-19

Ridpath, J., Bolin, S., Dubovi, E. 1994. Segregation of Bovine Viral Diarrhea Virus into Genotypes. *Virology* (205): 66-74

Ridpath, J., Neill, J., Vilcek, S., Dubovi, E., Carman, S. 2006. Multiple outbreaks of severe acute BVDV in North America occurring between 1993 and 1995 linked to the same BVDV2 strain. *Veterinary Microbiology* (114): 196-204

Saa, L., Perea, A., Garcia-Bocanegra, I., Arenas, A., Jara, D., Ramos, P., Carbonero, A. 2011. Seroprevalence and risk factors associated with bovine viral diarrhea virus (BVDV) infection in non-vaccinated dairy and dual purpose cattle herds in Ecuador. *Tropical Animal Health and Production* (44): 645-649

Sarrazin, S., Veldhuis, A., Méroc, E., Vangeel, I., Laureyns, J., Dewulf, J., Caij, A.B., Piepers, S., Hooyberghs, J., Ribbens, S. 2013. Serological and virological BVDV prevalence and risk factors analysis for herds to be BVDV seropositive in Belgian cattle herds. *Preventive Veterinary Medicine* (108): 28-37

Scott, T., Stylianides, E., Markotter, W., Nel, L. 2013. Serological survey of bovine viral diarrhoea viruses in Namibian and South African kudu (*Tragelaphus strepsiceros*) and eland (*Taurotragus oryx*). *Journal of the South African Veterinary Association* (84): 3 pages

Soine, C., Uatanaua, G., Depner, K. 1992. Prevalence of antibodies to bovine viral diarrhoea virus in Namibian Wildlife. *Tropical Animal Health Production* (24): 125-126

Statistics Botswana. 2016. *Agricultural Census Stats Brief 2015.* Gaborone: Statistics Botswana

Ståhl, K., Alenius, S. 2012. BVDV control and eradication in Europe – and update. *Japanese Journal of Veterinary Research* (60): 31-39

Ståhl, K., Lindberg, A., Rivera, H., Ortiz, C., Moreno-López, J. 2008. Self-clearance from BVDV infections – A frequent finding in dairy herds in an endemically infected region in Peru. *Preventive Veterinary Medicine* (83): 285-296

SVA. 2017-01-09. *BVD – Bovin Virus Diarré.* http://www.sva.se/djurhalsa/notkreatur/endemiska-sjukdomar-notkreatur/bvd-bovin-virusdiarre-notkreatur (2017-01-13)

Svenska FN-förbundet. 2014. *Botswana*. http://www.globalis.se/Laender/Botswana (2016-12-05).

Taylor, W., Okeke, A., Shidali, N. 1977. Prevalence of Bovine Virus Diarrhoea and Infectious Bovine Rhinotracheitits Antibodies in Nigerian Sheep and Goats. *Tropical Animal Health and Production* (9): 171-175

Thabti, F., Bakkali Kassimi, L., M'Zah, A., Ben Romdane, S., Russo, P, Ben Said, M.S., Hammami, S., Pepin, M. 2005. First detection and genetic characterization of bovine viral diarrhoea viruses (BVDV) types 1and 2 in Tunisia. *Revue de Médecine Vétérinaire* (156): 419-422

The World Bank. 2016. *Botswana*. http://www.worldbank.org/en/country/botswana/overview (2017-01-16)

Theodoris, A., Boshoff, S., Botha, M., 1973. Mucosal disease in southern Africa. *Journal of the South African Veterinary Association* (44): 61-63.

Ularamu, H., Sibeko, K., Bosman, A., Venter, E., Van Vuuren, M. 2013. Genetic characterization of bovine viral diarrhoea (BVD) viruses: confirmation of the presence of BVD genotype 2 in Africa. *Archives of Virology* (158): 155-163

Valle, P., Martin, S., Tremblay, R., Bateman, K. 1999. Factors associated with being a bovine-virus diarrhoea (BVD) seropositive dairy herd in the More and Romsdal County of Norway. *Preventive Veterinary Medicine* (40): 165-177

Van Vuuren, M. 2006. Bovine viral diarrhea virus infection in livestock in southern Africa. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* (1): 1-5

Van Wyk, J., Bath, G. 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research* (33): 509-522

Vassilev, V., Donis. R. 2000. Bovine viral diarrhea virus induced apoptosis correlates with increased intracellular viral RNA accumulation. *Virus Research* (2): 95-107

Vilcek, S., Paton, D., Durkovic, B., Strojny, L., Ibata, G., Moussa, A., Loitsch, A., Rossmanith, W., Vega, S., Scicluna, M., Palfi, V. 2001. Bovine viral diarrhea virus genotype 1 can be separated into at least eleven genetic groups. *Archives of Virology* (146): 99-115

Vilcek, S., Paton, D., Rowe, L., Anderson, E. 2000. Typing of Pestiviruses from Eland in Zimbabwe. *Journal of Wildlife Diseases* (36): 165-168

Walter, O., Klemens, F., Josef, K. 2005. BVDV infection risk in the course of the voluntary BVDV eradication program in Styria/Austria. *Preventive Veterinary Medicine* (72): 127-132

Walz, P. 2015. Diseases of the Alimentary Tract. I Bradford Smith (red.). *Large Animal Internal Medicine*. St. Louis : Elsevier, 750-759

Workman, A., Harhay, G., Heaton, M., Grotelueschen, D., Sjeklocha, D., Smith, T. 2015. Full-Length Coding Sequences for 12 Bovine Viral Diarrhea Virus Isolates from Persistently Infected Cattle in a Feedyard in Kansas. *Genome Announcements* (3): 1-3

Appendix 1

Interview questions

How many goats do you own? How many adults, how many kidlings?

Do you have any other animal species in your households?

Does your goats graze in a pen/holding or out in the bush?

Do they get in contact with goats or other ruminants from other herds or other villages?

What species of wildlife do you observe in this area? Do wildlife ever come in contact with your herd?

How do you consider the health of your animals? Do you have any specific health problems?

Have you ever observed any of the following symptoms; abortions, diarrhea, respiratory symptoms (cough, runny nose etc)

Do you use medicines such as antibiotics and antihelmintics?

Do you vaccinate your animals?

Do you use anti-tick treatment?

How do you acquire new goats?

How important are your goats to you? How would it affect you if they got ill or died?