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Anaplasma spp. infektion i små getbesättningar runt Gaborone, Botswana

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SUMMARY

Anaplasma is a genus of gram-negative, intracellular bacteria infecting different blood cells in animals. Three of the species infect the erythrocytes of ruminants, *A. marginale* and *A. centrale* most often infect cattle, while *A. ovis* primarily infects sheep and goats. Different species of wild ruminants can also become infected. The disease is called anaplasmosis and causes clinical signs like hemolytic anaemia, icterus and loss of production.

In this study samples were collected from smallholder goat flocks around Gaborone in Botswana. Blood samples were collected from 100 goats in 11 different flocks from three different villages; Modipane, Kopong and Gakuto. Body condition and FAMACHA© scores were estimated and the blood was used for PCV, blood smears, cELISA and PCR. Each farmer was interviewed about management, health and treatment of the goats.

Examination of blood smears in light microscopy showed inclusion bodies in 53% of the samples. A seroprevalence of 88% was found on cELISA and 76% of the goats were positive on PCR with a general primer for *Anaplasma* spp. The PCR positive samples were used for specific PCR for detecting *A. marginale* and *A. ovis*. All the PCR positive goats were infected with *A. ovis* and no goats were positive for *A. marginale*. Positive animals were found in all areas and in all flocks. The prevalence was highest in Modipane and lowest in Gakuto.

SAMMANFATTNING

Anaplasma spp. är ett genus av gramnegativa, intracellulära bakterier som kan infektera olika celler i blodet hos djur. Tre arter infekterar erythrocyterna hos idisslare, *A. marginale* och *A. centrale* infekterar främst nötkreatur, medan *A. ovis* infekterar får och getter. Även många vilda arter av idisslare kan infekteras av *Anaplasma* spp. Sjukdomen som ett infekterat djur kan utveckla kallas anaplasmos och kliniska fynd som sammankopplas med den är hemolytisk anemi, ikterus och nedsatt produktion.

I denna studie provtogs getter från små besättningar runt huvudstaden Gaborone i Botswana. Provtagningen utfördes i tre olika områden, Modipane, Kopong och Gakuto. Totalt 100 djur provtogs från 11 olika flockar. Från varje djur togs blod och en uppskattning av Body Condition och FAMACHA© score gjordes. Blodet användes till mikrohematokrit, blodutstryk, cELISA och PCR. En intervju med ägarna utfördes och frågorna gällde skötsel, hälsa och behandling av getterna.

Mikroskopering av blodutstryken gav en prevalens på 53% positiva med minst en inklusionskropp. En seroprevalens på 88% påvisades genom cELISA och hos 76% av getterna påvisades DNA från *Anaplasma* spp. genom PCR med en generell primer. Alla de getter som var positiva vid generell PCR visade sig bära på *Anaplasma ovis* vid PCR med en specifik primer för den arten. Ingen av getterna bar på *Anaplasma marginale* då alla getter var negativa vid PCR med en specifik primer för den arten. Positiva djur hittades i alla områden och i alla flockar. Prevalensen var högst i Modipane och lägst i Gakuto.

CONTENT

INTRODUCTION

Botswana is a landlocked country located in the southern part of Africa. It is now classified as an upper middle income country after being one of the poorest countries in Africa before its independence in 1966 (The World Bank, 2016). Because of a dry climate and limited access to farmable land, keeping cattle is the most important part of agriculture in Botswana. Not all farmers can afford cattle because it is a relatively expensive investment and therefore a lot of farmers keep goats instead that are cheaper to purchase (Panin, 2000). Goat herding is a significant part of the economy of the smallholders in Botswana since a part of their income is based on selling the goats (Panin & Mahabile, 1997) and an outbreak of disease can be devastating to the owner.

The health of the animals is important for all farmers. Tick-borne diseases like anaplasmosis can cause severe clinical signs like hemolytic anemia, or subclinical disease which can result in e.g. reduced production (Ndung'u *et al.*, 1995).

The aim of this study was to evaluate the prevalence of *Anaplasma* spp. in goats in small-holder flocks around Gaborone in Botswana. Information about the goats' health status can contribute to the owners' awareness of the disease which can impact the way they manage their goats, especially in regards to anti-tick treatment.

LITERATURE REVIEW

Anaplasma

Anaplasma is a genus of obligate intracellular, gram-negative bacteria that can be found in the blood cells of mammals. These bacteria can cause disease in vertebrates or just have them as reservoirs (Rymaszewska & Grenda, 2008). The *Anaplasma* species that can infect and cause disease in animals are *A. marginale*, *A. ovis*, *A. centrale*, *A. bovis*, *A. phagocytophilum* and *A. platys*, of which *A. marginale*, *A. centrale* and *A. ovis* are intraerythrocytic and infect ruminants (Liu *et al.*, 2011). In many tropical and subtropical areas of the world anaplasmosis with clinical signs as hemolytic anaemia is problematic (Fry & McGavin, 2012).

The spread of the bacteria is mostly through vectors, more specifically ticks. The most important species of ticks spreading *Anaplasma* spp. are *Ixodes*, *Dermacentor*, *Rhipicephalus* and *Amblyomma* (Rymaszewska & Grenda, 2008) of which *Rhipicephalus* and *Amblyomma* has been found on goats in Botswana (Mushi *et al.*, 1996). *Rhipicephalus evertsi* is the one considered to spread anaplasmosis to goats in the area of this study (Mushi *et al.*, 1996).

The age of the animal affects the severity of the disease, with older animals tending to have more severe clinical signs than younger animals (Fry & McGavin, 2012; Kocan *et al.*, 2003). The haemolytic anaemia is caused by immune-mediated extravascular hemolysis of the infected erythrocytes (Fry & McGavin, 2012). Persistent infection of *A. marginale* in cattle is necessary to spread the infection through ticks (Palmer *et al.*, 1998). It is assumed to be the same way for *A. ovis* in goats and persistent infection for up to 21 months has been shown in goats through PCR (Palmer *et al.*, 1998).

Antibodies to *Anaplasma* spp. have also been found in blue wildebeest, eland, hartebeest, impala, Thomson's gazelle, Grant's gazelle, giraffe and plains zebra (Ngeranwa *et al.*, 2008). Infected wildlife can serve as a reservoir for the bacteria and may spread the disease to domestic animals through ticks (Ngeranwa *et al.*, 2008).

Anaplasmosis has a large impact on the economics of cattle production in many tropical and subtropical areas because of high morbidity and mortality. Parameters like reduction in milk production, low weight gain, abortion, mortality and the cost of treatment are important to consider when calculating losses due to anaplasmosis (Kocan *et al.*, 2003).

Anaplasma ovis

Anaplasma ovis infection in goats can cause acute anaplasmosis with intraerythrocytic inclusion bodies and severe anaemia (Ndung'u *et al.*, 1995). Goats can remain mildly to moderately anaemic after the acute disease stage, when no inclusion bodies in the erythrocytes are detectable, most likely influencing the milk and meat production (Ndung'u *et al.*, 1995). Often the microorganism only causes mild clinical signs but it has been reported to cause more severe disease in goats during stress factors as co-infection or hot and dry climate (Renneker *et al.*, 2013). The aspect of co-infection with other tick-borne diseases is an important factor to consider when evaluating the impact of *Anaplasma* spp. in animals (Renneker *et al.*, 2013). The more severe clinical signs could be due to *Anaplasma* spp. having an impact on the immune system or that an affected immune system has a harder time fighting *Anaplasma* spp. Co-infection with other pathogens than those tick-borne probably have the same impact on severity of disease but no studies of this has been found. Some species may be more susceptible to disease like Rocky Mountain Bighorn Sheep in the Unites States that developed severe clinical disease with icterus and anaemia after experimental infection with *A. ovis* (Tibbitts *et al.*, 1992).

Anaplasma marginale

Anaplasma marginale is the species that causes bovine anaplasmosis. Number of bacteria infecting blood cells affects the incubation period which can vary between 7-60 days, and in average 28 days (Kocan *et al.*, 2003). Infected erythrocytes become phagocytized by bovine reticuloendothelial cells causing different degrees of anaemia and icterus. Cattle can become persistently infected and will gain a lifelong immunity to clinical disease but they remain reservoirs for vectors (Kocan *et al.*, 2003). This is also the case for calves even though they rarely get infected at all. Transmission can be mechanical through blood contamination of objects like needles, dehorning saws and castration instruments or through biological transmission by ticks (Kocan *et al.*, 2003). *A. marginale* can infect goats but does not usually cause clinical disease (Shompole *et al.*, 1989).

Anaplasma centrale

A. centrale is a less pathogenic bacteria that infect ruminants but rarely causes clinical disease. It has been used as live vaccine for *A. marginale* due to cross-reactivity (Kocan *et al.*, 2003). The mild strain that is used for live vaccination can be picked up by ticks and transmitted to other individuals, and it is believed to have been naturally spread in areas where vaccination is

common (Potgieter & Van Rensburg, 1987). This is believed to improve the enzootic stability of anaplasmosis in some areas due to a natural vaccination (Potgieter & Van Rensburg, 1987).

Diagnostics

Blood smears

The most practiced method of detecting *Anaplasma* spp. in blood is through light microscopy examination of blood smears stained with Giemsa (figure 1) (Ndung'u *et al.*, 1995; Shompole *et al.*, 1989; Renneker *et al.*, 2013). Infected erythrocytes can be detected up to 52 days after infection (Ndung'u *et al.*, 1995). For blood smears to be an effective way of detecting the infection, preferably more than 0.2% of the erythrocytes should be infected (Shompole *et al.*, 1989). The bacteria cannot be differentiated from Howell-Jolly bodies (nuclear remnants), especially if the level of bacteraemia is low (Shompole *et al.*, 1989). Blood smears are considered an insensitive method that requires expertise (Renneker *et al.*, 2013).

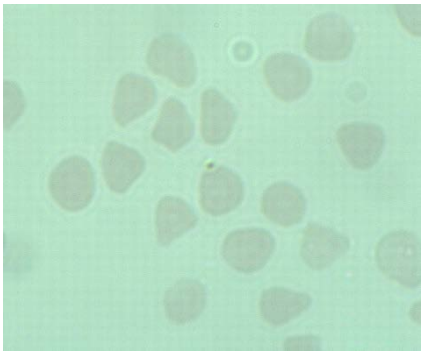

Figure 1. *Inclusion body in an erythrocyte from a goat.*

Serology

Anaplasma spp. infection can also be detected through antibody ELISA (Shompole *et al.*, 1989). Goats can remain seropositive for at least 6 months after seroconversion (Ndung'u *et al.*, 1995). One study (Ndung'u *et al.*, 1995) showed that the amount of antibodies continue to increase following the acute phase of the infection, which suggests that goats can get persistently infected with *A. ovis* similar to persistent *A. marginale* infection in cattle. The serological test can be positive for infection with several different types of *Anaplasma* because of cross-reactivity (Renneker *et al.*, 2013).

PCR

PCR is a way to detect the bacteria in the blood after a DNA extraction has been performed. It is a sensitive way of detecting *Anaplasma* spp. in the blood, but it requires more resources and is therefore mostly used for research (Jalali *et al.*, 2013). Special primers must be used to amplify the DNA of the specific bacteria of interest. There have been primers designed both for general detection and amplification of *Anaplasma* spp. and specific primers for *A. ovis* and *A. marginale*. The primers target major surface proteins (MSP) like MSP4 for the general primer (de la Fuente *et al.*, 2007). The primers used to amplify *A. ovis* also target MSP4 but it is not

the same base pairs as the general one and the one used to amplify *A. marginale* targets MSP1b (Michelet *et al.*, 2014).

Prevalence in other areas

There are many studies on the prevalence of *Anaplasma* spp. performed on cattle but fewer on goats. Most studies do not specify which species of *Anaplasma* the goats are infected with.

One study on *Anaplasma* spp. in goats in Botswana has found a prevalence of 8% (Mushi *et al.*, 2002).

In a study from Kenya, 85% of goats were positive either on blood smears or with a DNA hybridization with a known DNA-probe for *A. ovis* (Ndung'u *et al.*, 1995). Of the positive goats, 94% were antibody-positive on ELISA (Ndung'u *et al.*, 1995). Another study from Kenya showed a prevalence of *A. ovis* between 22 to 87% in different areas using DNA-hybridization (Shompole *et al.*, 1989). In a more recent study from Kenya, 85% of the goats sampled were antibody-positive for *Anaplasma* spp (Ngeranwa *et al.*, 2008).

In Angola, 100% of sampled goats had *Anaplasma ovis* according to PCR with the msp4 gene (Kubelová *et al.*, 2012). The number of sampled goats were only 13 and the result do not reflect the actual prevalence in the study area, as pointed out by the authors. A study in Ghana on goats showed a prevalence of *Anaplasma* spp. on blood smears of 46% (Bell-Sakyi *et al.*, 2004).

Vaccination

The way of controlling anaplasmosis outbreaks is through tick control (with acaricides), antibiotic treatment and vaccination. Tick control is not always practically possible and does not protect against mechanical transmission. Antibiotics should not be used carelessly since it can cause selection of resistant strains (Kocan *et al.*, 2003).

Vaccination can therefore be the most efficient and economical way of protecting against bovine anaplasmosis. There are both live and killed vaccines and both types use *A. marginale* from infected bovine erythrocytes. Vaccines prevent or reduces clinical disease but do not protect cattle from becoming persistently infected (Kocan *et al.*, 2003).

Infection with the less pathogenic *A.centrale* can induce cross-reactive immunity to *A. marginale*, and *A. centrale* has therefore been used as a live vaccine. This type of vaccination has been used in cattle in Africa, Australia, Israel and Latin America (Kocan *et al.*, 2003).

Killed vaccines have also been used against anaplasmosis. It has advantages compared to live vaccines, such as cheaper storage, lower risk of contamination and a low risk of post-inoculation reactions. Disadvantages of killed vaccines, like higher cost of purification, lack of cross protection and need of a yearly booster, are probably the reason why live vaccines are more frequently used (Kocan *et al.*, 2003).

A vaccine that would be ideal for anaplasmosis would need to prevent infection and also induce immunity, and the vaccines currently being used do not live up to that (Kocan *et al.*, 2003). No

records of vaccination of goats or sheep has been found in the literature review preceding this study.

Ticks in Botswana

Anaplasma spp. are mainly spread through vectors as ticks (Rymaszewska & Grenda, 2008). Ticks collected from the Kgatleng district in Botswana, close to this study's sample area, were most abundant during January-March because of heavy rainfalls (Mushi *et al.*, 1997). The same study showed that relatively few ticks were found during the dry and cold winter months of May-August. A correlation between mean numbers of ticks and the monthly total rainfall has also been found (Mushi *et al.*, 1996). Moreover, the monthly average maximum and minimum temperature have been correlated to tick infestation in goats (Mushi *et al.*, 1996). Mushi *et al.* (1996) considered the infestation rate of the goats (1-3.5 ticks per goat per month) to be low which might be explained by a natural resistance among an indigenous goat breed. The most abundant tick found was *Rhipicephalus evertsi evertsi* (Mushi *et al.*, 1997), which can act as a vector for *Anaplasma* (Rymaszewska & Grenda, 2008).

Body condition score

Body condition score (BCS) is a way of measuring the nutritional status of an animal and a score from 1-5 is often used, where 1 is emancipated and 5 is fat. In goats in Botswana, BCS has been proved to be a more useful indicator of the nutritional status than body weight and heart girth. The season affects the mean body condition score; it is lower during the dry season (Nsoso *et al.*, 2003). Optimal BCS for goats is 2-3. Fat goats are at risk of pregnancy toxaemia, while emaciated goats are too weak to be productive (Luginbuhl *et al.*, 2002 cited in Nsoso *et al.*, 2003).

FAMACHA©

A FAMACHA© chart is a tool to help determine if an animal has anaemia without having to draw blood from the animal. The five-step colour scale on the chart is compared to the ocular mucus membrane of the goat to determine if it is normally red (1), anaemic white (5) or somewhere in between. It was initially developed to help farmers determine which animals to treat for internal parasites, but can determine anaemia due to other reasons as well (Kaplan *et al.*, 2004). Initially the FAMACHA© chart was intended to use on sheep but it has been validated for goats as well (Vatta *et al.*, 2001). To maximise the sensitivity and specificity of the test goats having a score of 1-2 is considered normal while 3-5 is classified as anaemic (Vatta *et al.*, 2001)

Packed Cell Volume

Packed cell volume (PCV) is a parameter used to determine the percentage of erythrocytes in the total blood volume, mainly used to evaluate anaemia. It can be determined directly in modern cell counting machines or through microhematocrit centrifugation (Hillström *et al.*, 2013) A variation in PCV between different breeds has been seen in goats (Daramola *et al.*, 2005). In the Tswana goat, an indigenous breed in Botswana, the mean PCV is 24.5% when availability of water is unlimited, whereas the mean PCV is 25.1% when they are allowed to drink only once a day (Adogla-Bessa & Aganga, 2000).

Some studies have found no difference in PCV in goats infected with *Anaplasma* spp. compared to those uninfected (Bell-Sakyi *et al.*, 2004), while others have seen mild anaemia (Obi & Anosa, 1980).

Small ruminants' importance for the economy of smallholders

Small ruminants are an important part of the economy for small-scale farmers in Botswana (Panin & Mahabile, 1997; Aganga *et al.*, 2005; Panin, 2000). The income from livestock was 49% of the household's total income, of which small ruminants contribute with 15%. The net margin profitability per animal is far higher for cattle, but the return on the capital invested is almost as high for goats, which means that it is almost as efficient to keep goats (Panin, 2000). For many smallholder farmers in Botswana it is more practical to own small ruminants, because the capital needed to invest is significantly bigger for cattle (Panin, 2000). Most farmers that keep small ruminants have only goats in their flocks, while some have a mix of goats and sheep, and the flock-sizes are usually small with an average of 20 goats (Panin & Mahabile, 1997). Households keep goats rather than sheep, because most people prefer goat meat to mutton and goats survive and adapt better in the environment (Panin & Mahabile, 1997), even though the limited amount of quality feed during the dry season is not optimal for the productivity (Aganga *et al.*, 2005). During the dry season the grasses are destroyed by the heat, but goats can eat from drought-resistant acacia bushes (Mushi *et al.*, 1997). Goats are also considered to be less prone to disease (Panin & Mahabile, 1997).

Goats are often sold when farmers are in need of cash to purchase food, invest in the farm, pay school fees or medical expenses (Kocho *et al.*, 2011; Kosgey *et al.*, 2008; Dossa *et al.*, 2007). Major problems for goat owners are outbreaks of disease that could result in high mortality and decreased productivity (Dossa *et al.*, 2007). If farmers had the knowledge and economy to adopt proper disease control and prophylactic measures the risk of outbreaks could be reduced.

MATERIAL AND METHODS

Study design, sample collection and clinical signs

Blood samples from 100 goats were collected in three different areas, Modipane, Kopong and Gakuto (figure 2 & 3), around Gaborone, Botswana, during September 2016, which is at the end of the dry season. The goat owners were smallholders and chosen depending on the area they lived in, how many goats they kept and if they were willing to participate. Ten goats were sampled from each flock, except for two of the flocks, where five goats were sampled in each. These two flocks were close to each other and the goats grazed together so they were regarded as one flock. All goat owners gave their permission to sample their goats.

Figure 2 & 3. *Map of Botswana and the location of the villages Modipane (1), Kopong (2) and Gakuto (3).*

Only goats more than one year old were sampled, both males and females. Because most farmers had more females than males no effort were made to get an even number of animals of both sexes. Systematic random sampling was used in the following way; goats available for sampling were counted and then divided by ten to get the frequency of goats to sample. If the flock consisted of 30 adult animals, every third animal was sampled but all the animals were caught. In that way both easily caught and animals that were harder to catch were sampled because that may have been affected by their condition.

All animals sampled were subjected to a FAMACHA© reading of the mucous membrane and a Body Condition Score (BCS) estimation. This was carried out by the same person for all animals to minimize the risk for subjective differences between the groups. Blood samples were collected sterile with a vacutainer system (BD Biosciences, New Jersey, USA) from the jugular vein. Two tubes of blood were filled for each animal, one with added EDTA and one without additive for later separation of serum. The coordinates of the kraal were noted.

Interviews

After the sampling a short semi-structured interview was conducted with the goat owner and other people, often family members, taking care of the goats. The questions were asked in English but in most cases needed to be translated into Setswana, the national language of Botswana. The translation was done by employees from the Botswana University of Agriculture and Natural Resources who also assisted with the sampling. Answers from the owners were written down and the interview took between 10-20 minutes. The questions were as follows:

1. How many goats do you have? How many are adults and how many are kids?
2. Do you keep any other animals? Which species? Do they get in contact with each other?
3. Do the goats graze in a pen/holding or in the bush?
4. Do they get in contact with goats or other ruminants from other herds/villages?

5. What species of wildlife do you observe around you? Does wildlife ever come in contact with your herd?
6. How do you consider the health of your animals?
7. Have you ever observed any of the following clinical signs; abortion, stillbirths, diarrhoea or respiratory symptoms?
8. Do you vaccinate your animals?
9. Do you use anti-tick treatment?
10. How do you acquire new animals?
11. How would it affect you if a lot of your animals got sick and died?

Treatment of the blood samples

EDTA blood was used for preparing thin blood smears that later were stained with Giemsa and examined for inclusion bodies. The blood was also used to examine microhematocrit with microhematocrit tubes that were spun for 5 min at 12000 rpm and read with a microhematocrit reader. EDTA blood was then frozen at -20°C until further used for DNA extraction. Blood tubes without additive were left to coagulate and serum was separated from the coagulate and frozen at -20°C until further used for ELISA.

Diagnostic tests

Blood smears

Blood smears were stained with Giemsa and examined under a microscope with x100 magnification (Leica DM500, Leica Microsystems, Switzerland). Each slide was examined for five minutes and samples with one or more inclusion bodies were classified as positive.

cELISA

Sera were used in a competitive, enzyme-linked, immunosorbent assay (cELISA) for detection of antibodies to *Anaplasma* spp. The cELISA was carried out according to the instructions from the manufacturer (Veterinary Medical Research and Development, Pullman, USA). In brief, 50 µl of controls and samples were transferred to the antigen-coated plate and left to incubate at room temperature for one hour. After this incubation, the plate was washed two times before 50 µl of diluted antibody-peroxidase conjugate was added. After an incubation at room temperature for 20 minutes, the plate was washed four times before adding the substrate solution and incubated in darkness at room temperature for 20 minutes. Stop solution was added and the plate was read in a microplate absorbance spectrophotometer (Multiskan FC, Thermo Scientific, Waltham, USA) with a wavelength of 620 nm. The % of inhibition was calculated the following way:

% I = 100 x (1- (Sample OD / Negative control OD))

Samples with an inhibition of ≥ 30% were considered positive, while samples with an inhibition of <30% were considered negative.

DNA extraction

DNA was extracted from 50 µl of anti-coagulated blood using DNeasy Blood and Tissue kit according to the manufacturer's instructions (Qiagen, Poland). The extracted DNA product was stored in -20°C until further use.

PCR

Extracted DNA from all samples was subsequently used in a PCR assay for detection of *Anaplasma* spp. Five µl of DNA were mixed with 12.5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, Life technologies, California USA), 0.4µM of forward and reverse primer respectively in a total volume of 25 µl. Primers for general *Anaplasma* spp. detection were used; MSP45: 5'-GGGAGCTCCTATGAATTACAGAGAATTGTTTAC-3' and MSP43: 5'-CCGGATCCTTAGCTGAACAGGAATCTTGC-3' (de la Fuente *et al.*, 2007). The following conditions were used for the thermocycler (2720 Thermal Cycler, Applied Biosystems, Waltham, USA): initial denaturation at 95°C for 10 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 53°C for 30 sec and extension at 72°C for 60 sec, followed by a final extension at 72°C for 7 min. Generated PCR products were stored at -20°C.

PCR products were analysed by gel electrophoresis in a 1% agarose gel with a 50-base pair ladder and visualised under UV-light. Expected length of the PCR product was around 850 base pairs.

Samples positive for *Anaplasma* spp. in the general MSP4-PCR were further analysed in specific PCR assays for *A. marginale* and *A. ovis*. For *A. marginale,* msp1b primer An_ma_msp1_F: 5'-CAGGCTTCAAGCGTACAGTG-3' and An_ma_msp1_R: 5'-GATATCTGTGCCTGGCCTTC-3' were used (Michelet *et al.,* 2014). For *A. ovis*, MSP4 primer An_ov_msp4_F: 5'- TCATTCGACATGCGTGAGTCA-3' and An_ov_msp4_R: 5'-TTTGCTGGCGCACTCACATC-3' were used (Michelet *et al.*, 2014). Following thermoprofile was used: initial denaturation at 95°C for 10 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 53°C for 30 sec and extension at 72°C for 60 sec, followed by a final extension at 72°C for 7 min. Generated PCR products were stored at -20°C.

PCR products were analysed by gel electrophoresis in a 2% agarose gel with a 50-base pair ladder and visualised under UV-light. No positive control was used for this step due to lack of a certain positive sample. The *A. marginale* and *A. ovis* products were expected to be 85 and 92 base pairs respectively.

Statistical analysis

Data gathered from sampling and diagnostic tests were compiled in an excel document. All statistical analysis was performed in Minitab 17 Statistical Software. For the proportions a confidence interval of 95% were calculated. Difference between the villages result were calculated through ANOVA (analysis of variance) and Spearman's rank correlation coefficient was used to calculate correlation.

RESULTS

Interviews

In total 11 interviews among goat farmers were carried out, one for each flock. Goat owners kept between 9-70 adult goats, mean flock size was around 30 adults. The exact number could not be calculated since all owners did not know how many animals they kept. The goat owners had between 0-30 kids. Majority of the owners kept other ruminants like cattle or sheep and most of them grazed together with, or in the same area, as the goats. All owners reported that their goats stayed in the kraal (pen) at night and grazed freely on communal grazing ground during the day. The owners stated that their goats were mixed with goats from other herds during grazing.

All owners from Modipane reported seeing wild ruminants in the same areas where their goats grazed. In Kopong and Gakuto, one out of three owners reported seeing wild ruminants, but did not mention as many species as the owners in Modipane. Species that had been observed were among others; kudu, impala, duiker and springbok.

The owners had different opinions about the health of their animals. Some thought they were in good condition while some thought the animals had some health problems. When asked about specific clinical signs most owners had experienced abortion and diarrhoea.

Some owners never treated their animals with medications, while others did deworming and occasionally treated sick animals with antibiotics. Most owners did not vaccinate their goats. All owners had treated their animals at least once with prophylactic drugs against ticks through dipping. Most of them stated that they treated the goats when they consider the ticks to be a problem instead of doing it regularly.

Mostly, goat owners acquired new animals by raising the kids born in their own flock, but two of them said they also occasionally bought from other farmers in the village or from nearby villages.

All goat owners stated that it would greatly affect them in a negative way if the majority of their animals got sick and died. Most of the owners make their living from selling live goats so they would primarily be affected economically. Some also stated that it would be difficult emotionally since the goats are important to them, and some owners even used the word devastating.

Body Condition Score, FAMACHA© Score and Packed Cell Volume

The mean BCS, FAMACHA and PCV for all groups were 3.3, 2.4 and 33.0% respectively. Mean values for the different villages are shown in Table 1. Significant difference between the villages BSC (p-value = 0.05), FAMACHA© (p-value = 0.02) and PCV (p-value < 0.0005) were found and the comparison is presented in figure 4, 5 and 6. None of the goats were anaemic according to Packed cell volume but 9/100 goats had a FAMACHA© score of 4, undoubtedly classified as anaemic.

Table 1. *Mean values of BCS, FAMACHA© and PCV for the different villages*

	Body Condition Score (optimal 2-3)	FAMACHA© (normal 1-2)	PCV[1] in % (95% CI)
All villages	3.3	2.4	33.0 (32.0; 34.1)
Modipane	3.1	2.2	34.2 (32.8; 35.6)
Kopong	3.4	2.7	29.6 (28.1; 31.2)
Gakuto	3.4	2.3	34.9 (32.9; 36.9)

1. Normal PCV range in goats 22-38% (Fielder, 2016).

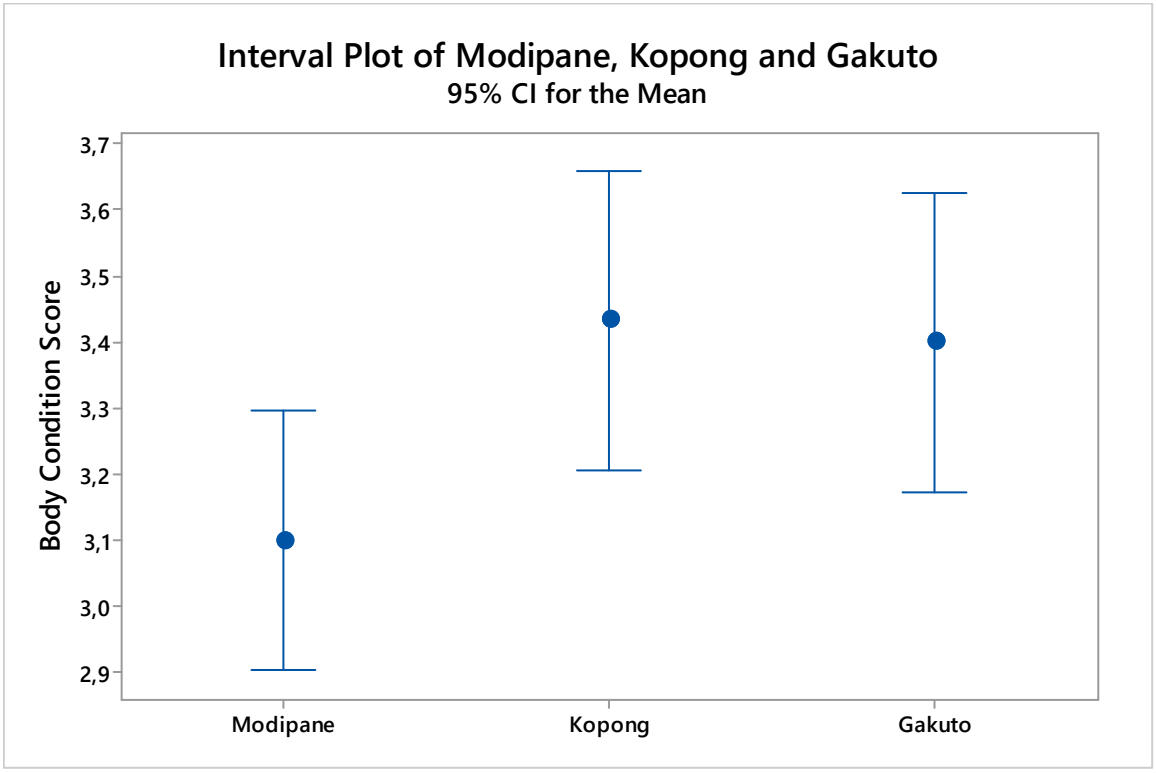

Figure 4. *Mean BCS with 95% confidence interval*

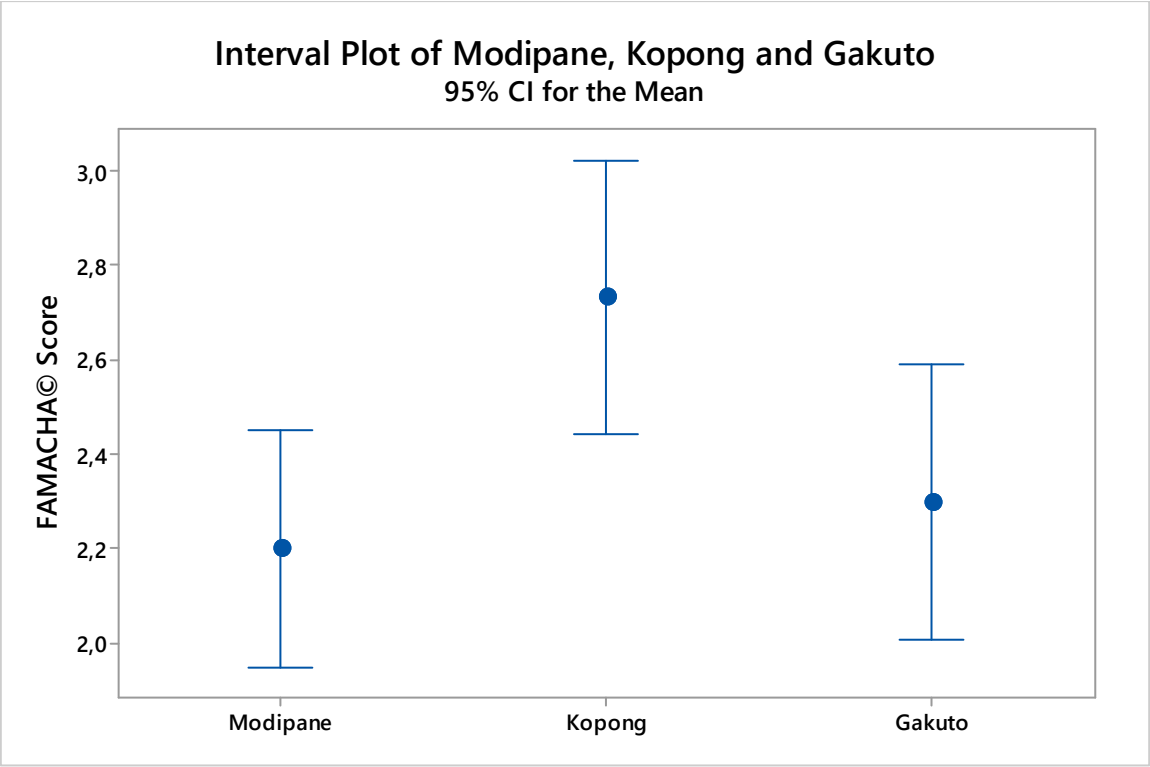

Figure 5. *Mean FAMACHA© with 95% confidence interval*

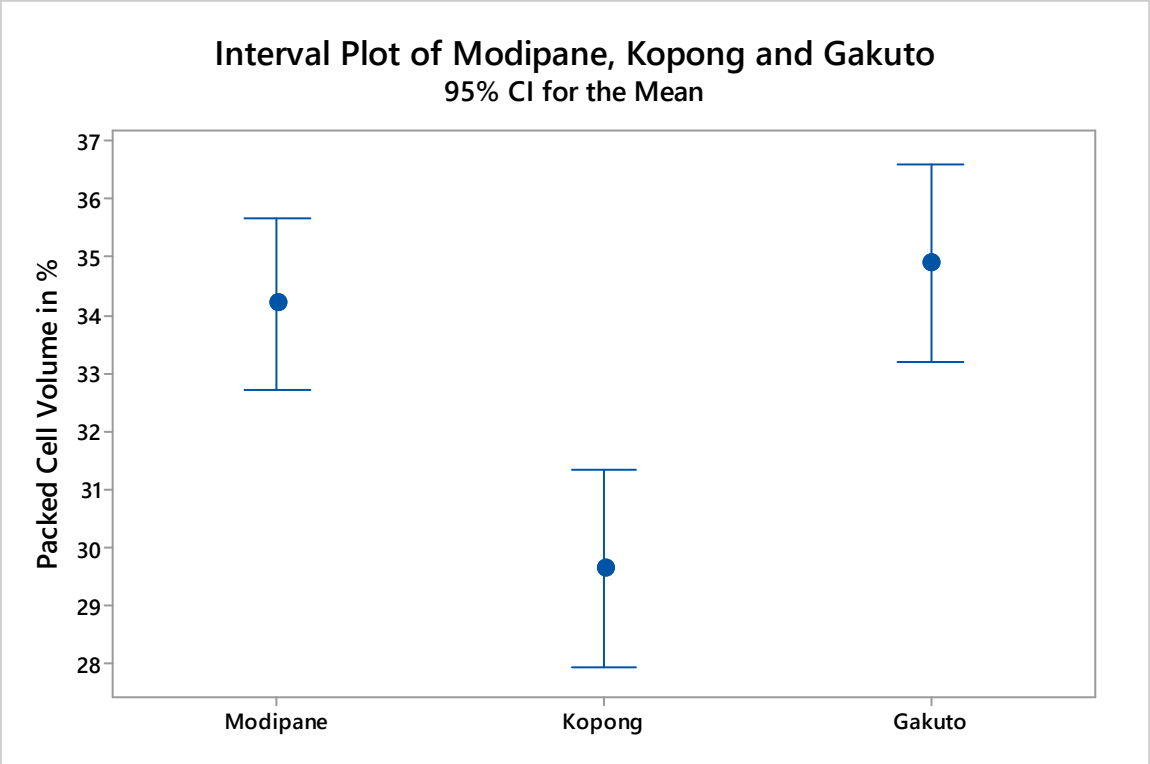

Figure 6. *Mean PCV with 95% confidence interval*

Diagnostic tests

Blood smears

When examining thin blood smears with light microscopy, 53% of the goats were positive with one or more inclusion bodies detected in the erythrocytes. See Table 2 for difference between flocks and areas.

Table 2. *Number and proportion of positives for each village and diagnostic method*

	Blood smears		ELISA		PCR	
	n	Prevalence (95% CI)	n	Prevalence (95% CI)	n	Prevalence (95% CI)
Modipane (n=40)	31	78% (61.5; 89.2)	40	100% (91.2; 100)	35	88% (73.2; 95.8)
M 1 (n=10)	10	100%	10	100%	9	90%
M 2 (n=10)	8	80%	10	100%	10	100%
M 3 (n=10)	8	80%	10	100%	9	90%
M 4 (n=10)	5	50%	10	100%	7	70%
Kopong (n=30)	15	50% (31.3; 68.7)	28	93% (77.9; 99.2)	24	80% (61.4; 92.3)
K 1 (n=10)	5	50%	10	100%	6	60%
K 2 (n=10)	7	70%	9	90%	10	100%
K 3 (n=10)	3	30%	9	90%	8	80%
Gakuto (n=30)	7	23% (9.9; 42.3)	20	67% (47.2; 82.7)	17	57% (37.4; 74.5)
G 1 (n=10)	2	20%	4	40%	5	50%
G 2 (n=10)	1	10%	9	90%	9	90%
G 3 (n=10)	4	40%	7	70%	3	30%

CI = Confidence interval

cELISA

Results from competitive inhibition ELISA detecting antibodies is shown in table 2. Overall 88% of the goats were positive and those wells are clear in colour (figure 7).

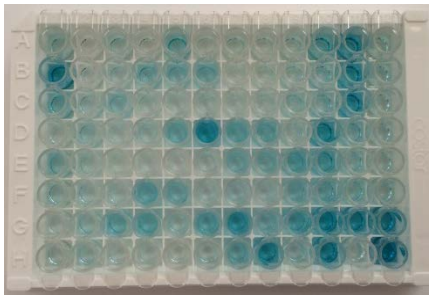

Figure 7. *ELISA plate.*

PCR

Anaplasma spp.

The PCR product was run through an agarose gel (figure 8). Results from the conventional PCR detecting *Anaplasma* spp. is shown in table 2. Number of goats positive after PCR with the MSP4 primer was 76/100 (76%). A p-value of 0,22 indicates that no statistically significant difference in prevalence could be found when comparing the PCR results from the different villages through ANOVA.

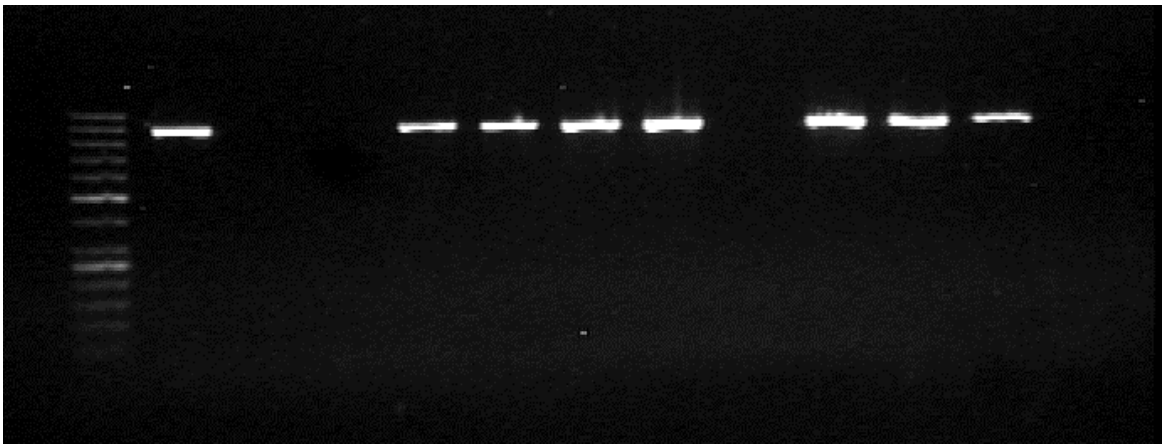

Figure 8. *Agarose gel with Anaplasma* spp. *From the left; ruler, positive control, negative control, samples 5:1-5:10.*

A. ovis

Samples positive for *Anaplasma* spp. (n=76) were analysed in a specific PCR for *A. ovis*. Samples were run twice due to contamination in the first run that made the negative control invalid. During the second run the negative control was once again slightly positive (figure 9). Due to lack of resources the PCR could not be run a third time. All samples tested were positive with the specific primer for *A. ovis* which gives a prevalence of 76%.

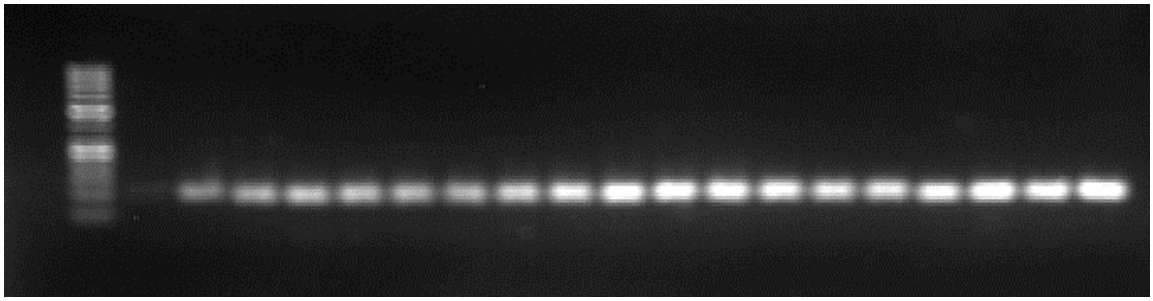

Figure 9. *Anaplasma ovis PCR. Negative control to the far left, next to the ruler.*

A. marginale

All samples positive for *Anaplasma* spp. were tested with the specific primer for *A. marginale*. All samples were tested negative. No positive control was used.

Correlation

A weak negative correlation between PCV and FAMACHA© was found (Spearman's -0,52 p-value <0.0005), i.e. a higher FAMACHA© score was correlated with a lower PCV. Even though a correlation was found none of the goats classified as anaemic on the FAMACHA© score were anaemic according to PCV.

Lower FAMACHA© score are not associated with a positive PCR result (Spearman's rank correlation coefficient = 0.35, p-value <0.0005).

BCS are not correlated with a positive PCR result (Spearman's rank correlation coefficient = -0.31 p-value = 0.002)

DISCUSSION

Prevalence and management

The prevalence of *Anaplasma* spp. was 76% according to PCR which is consistent with other studies conducted on goats in Africa (Ndung'u *et al.*, 1995; Shompole *et al.*, 1989; Kubelová *et al.*, 2012). From microscopic examination, only 53% of the animals had inclusion bodies and this difference in prevalence is in accordance with other studies that have shown PCR to have a higher sensitivity than blood smear examination (Jalali *et al.*, 2013).

There was a difference in prevalence between the different sampling areas but it was not statistically significant. Gakuto was the area with least number of infected animals (17/30) in all three diagnostic tests. Modipane was the area with most infected animals (35/40). One environmental difference between the different groups are the reported incidence of wild animals in the areas. The owners in Gakuto and Kopong had in general not seen many wild species in the areas where their goats foraged and since wild ruminants can be reservoirs for the bacteria this could have affected the prevalence of infection. This cannot be determined just from the owners' information but would have to be examined more closely.

None of the goats were perceived to be sick during the sampling according to the owners. The goats were mostly kept for selling and there were no production records. However, none of the owners reported a loss in production. Nevertheless, there could have been a loss in production but this would have to be examined closer.

Some of the owners gave their goats water once a day while others had unlimited access to water. Either way the mean PCV of the sampled goats (33.0%) in this study were higher than previously reported figures (24.5% and 25.1%) for healthy goats in Botswana (Adogla-Bessa & Aganga, 2000). This could be because the sampling occurred during the dry season or that the goats might not be able to drink enough even if they have been offered water. The normal PCV range of goats in general is 22-38% (Fielder, 2016) and most of the goats were within this range. Some had higher PCV most likely due to dehydration. None of the goats were anaemic according to PCV.

Body Condition Score, FAMACHA© Score and Packed Cell Volume

Mean BCS were lower in Modipane than in Kopong and Gakuto. Since all the goats grazed on communal grazing grounds it is expected that goats from the same village would have similar BCS. None of the flocks had a mean value lower than the optimal score 2-3. More information about the condition and availability of the grass and bushes in the grazing area would be needed to evaluate these results.

Mean FAMACHA© scores were higher and mean PCV were lower in Kopong than in Gakuto and Modipane, but none of the goats were classified as anaemic. This could be due to different degrees of hydration in the different villages due to a difference in accessibility to water.

Higher FAMACHA© score could not be correlated to a higher rate of infection in this study. This is most likely because the goats did not seem to develop clinical signs even though they were infected. There was a correlation between FAMACHA© and PCV, as expected, since FAMACHA© is a method of evaluating if the animal has anaemia. In this study the FAMACHA© score did not prove valuable to determine if the goats were anaemic since 9 goats were classified as anaemic on FAMACHA© but not according to PCV. This could have been caused by lack of experience of the person performing the FAMACHA© readings.

Diagnostic tests

Blood smears

Some animals had inclusion bodies in the erythrocytes but were then negative for the PCR. Of these, some had single inclusion bodies while others had several. This is most likely due to misinterpretation of the inclusion bodies in the light microscope. An inclusion body can resemble both Howell-Jolly bodies, other intra-erythrocytic parasites and staining artefacts.

ELISA

Some animals were PCR positive but ELISA negative (data not shown) which can be due to a weak immune response, source of error in the test or that the goat has been recently infected and not yet seroconverted. Other animals were ELISA positive but PCR negative (data not shown) which would have been expected since most animals develop a chronic infection after the acute phase. This might not be the case for goats in the same extent as for cattle since the *A. ovis* seems less prone to cause clinical disease in goats than *A. marginale* in cattle.

PCR

PCR with the specific primers showed that none of the goats carried *A. marginale*. This result can be questioned since no positive control was used in the procedure. There is however no reason to believe that this result is false since *A. marginale* usually infects cattle even though it has been found in goats previously (Shompole *et al.*, 1989). Instead, all goats positive for *Anaplasma* spp. were also positive for the PCR detecting *A. ovis*. The negative control used in this assay was slightly positive, which is likely due to a contamination. This means that the test is not valid and the results should be disregarded. However, if the positive samples would be

positive due to contamination they should have a weaker band in the gel, like the negative control. Since this is not the case, these samples are believed to be true positives.

Conclusion

In Botswana, *Anaplasma* in goats seems to be a lesser problem even though the pathogen is well spread among the population. For some individuals with a suppressed immune system it could cause clinical disease but mostly it is a subclinical infection in goats. No correlation between *Anaplasma* infection and PCV could be found and this is probably due to the infection being in the persistent phase of disease or the disease being subclinical.

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REFERENCES

Adogla-Bessa, T, & Aganga, A.A. (2000). Responses of Tswana goats to various lengths of water deprivation. *South African Journal of Animal Science*, vol. 30 (1), pp. 87-91.

Aganga, A.A., Omphile, U.J., Chabo, R.G., Kgosimore, M. & Mochankana, M. (2005). Goat production under traditional management in Gaborone agricultural region in Botswana. *Journal of Animal and Veterinary Advances*, vol. 4 (5), pp 515-519.

Bell-Sakyi, L., Koney, E.B.M., Dogbey, O. & Walker, A.R. (2004). Incidence and prevalence of tick-borne haemoparasites in domestic ruminants in Ghana. *Veterinary Parasitology*, vol. 124, pp. 25-42.

Daramola, J.O., Adeloye, A.A., Fatoba, T.A. & Soladoye, A.O. (2005). Haematological and biochemical parameters of West African Dwarf goats. *Livestock Research for Rural Development*, vol. 17 (8), art. #95.

de la Fuente, J., Atkinson, M.W., Naranjo, V., Fernández de Mera, I.G., Mangold, A.J., Keating, K.A. & Kocan, K.M. (2007). Sequence analysis of the *msp4* gene of *Anaplasma ovis* strains. *Veterinary Microbiology*, vol. 119, pp. 375-381.

Dossa, L.H., Wollny, C. & Gauly, M. (2007). Smallholders' perceptions of goat farming in southern Benin and opportunities for improvement. *Tropical Animal Health and Production*, vol. 39, pp. 49-57.

Fielder, S.E. (2016). *Hematologic reference ranges*. Available at: http://www.merckvetmanual.com/appendixes/reference-guides/hematologic-reference-ranges (Accessed 2017-01-07).

Fry, M.M. & McGavin, M.D. (2012). Bone marrow, blood cells, and the lymphatic system. In: Zachary, J.F. & McGavin, M.D. (eds), *Pathologic Basis of Veterinary Disease*. Fifth edition. St. Louis, Missouri: Elsevier Mosby, pp 720.

Hillström, A., Jones, B., Ström Holst, B., Strage, E., Tvedten, H., Lilliehöök, I. & Falkenö, U. (2013). *Kompendium i klinisk kemi*. Unpublished manuscript. Department of Clinical Sciences, SLU.

Jalali, S.M., Khaki, Z., Kazemi, B., Bandehpour, M., Rahbari, S., Razi Jalali, M. & Yasini, S.P. (2013). Molecular detection and identification of *Anaplasma* species in sheep from Ahvaz, Iran. *Iranian Journal of Veterinary Research, Shiraz University*, vol. 14 (1), pp. 50-56.

Kaplan, R.M., Burke, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Larsen, M. & Vatta, A.F. (2004). Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in southern United States. *Veterinary Parasitology*, vol. 123, pp. 105-120.

Kocan, K.M., de la Fuente, J., Guglielmone, A.A. & Meléndez, R.D. (2003). Antigens and alternatives for control of *Anaplasma marginale* infection in Cattle. *Clinical Microbiology Reviews*, vol. 16 (4), pp. 698-712.

Kocho, T., Abebe, G., Tegegne, A. & Gebremedhin, B. (2011). Marketing value-chain of smallholder sheep and goats in crop-livestock mixed farming system of Alaba, Southern Ethiopia. *Small Ruminant Research*, vol. 96, pp. 101-105.

Kosgey, I.S., Rowlands, G.J., van Arendonk, J.A.M. & Baker, R.L. (2008). Small ruminant production in smallholder and pastoral/extensive farming systems in Kenya. *Small Ruminant Research*, vol. 77, pp. 11-24.

Kubelová, A., Mazancová, J. & Široký, P. (2012). *Theileria, Bebesia,* and *Anaplasma* detected by PCR in ruminant herds at Bié province, Angola. *Parasite*, vol. 19, pp. 417-422.

Liu, Z., Ma, M., Wang, Z., Wang, J., Peng, Y., Li, Y., Guan, G., Luo, J. & Yin, H. (2011). Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. *Applied and Environmental Microbiology*, vol. 78 (2), pp. 464-470.

Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., Bødker, R., Fach, P. & Moutailler, S. (2014). High-throughput screening of tick-borne pathogens in Europe. *Frontiers in Cellular and Infection Microbiology*, vol. 14, art #103.

Mushi, E.Z., Isa, J.F.W., Proctor, J., Machete, J.B. & Kapaata, R.W. (1997). Seasonal fluctuations of ixodid ticks on a herd of indigenous goats at Oodi, Kgatleng district, Botswana. *Tropical Animal Health and Production*, vol. 29, pp. 29-30.

Mushi, E.Z., Binta, M.G., Chabo, R.G. & Tautsagae, J.S. (2002). Detection of *Anaplasma* parasites in the blood of Tswana goats from Oodi, Kgatleng district, Botswana. *UNISWA Research Journal of Agriculture, Science and Technology*, vol. 6 (1), pp. 33-36.

Mushi, E.Z., Isa, J.F.W. & Sakia, R.M. (1996). Monthly fluctuations of Ixodid tick infestation on indigenous goats in Botswana. *Small ruminant research*, vol. 21, pp. 277-279.

Ndung'u, L.W., Aguirre, C., Rurangirwa, F.R., McElwain, T.F., McGuire, T.C., Knowles, D.P. & Palmer, G.H. (1995). Detection of *Anaplasma ovis* infection in goats by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, vol. 33 (3), pp. 675-679.

Ngeranwa, J.J.N., Shompole, S.P., Venter, E.H., Wambugu, A., Crafford, J.E. & Penzhorn, B.L. (2008). Detection of *Anaplasma* antibodies in wildlife and domestic species in wildlife-livestock interface areas of Kenya by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *Onderstepoort Journal of Veterinary Research*, vol. 75, pp. 199-205.

Nsoso, S.J., Aganga, A.A., Moganetsi, B.P. & Tshwenyane, S.O. (2003). Body weight, body condition score and heart girth in indigenous Tswana goats during the dry and wet seasons in southeast Botswana. *Livestock Research for Rural Development*, vol 15 (4).

Obi, T.U. & Anosa, V.O. (1980). Haematological studies on domestic animals in Nigeria. *Zentralblatt für Veterinärmedizin Reihe B*, vol. 27, pp. 789-797.

Palmer, G.H., Abbott, J.R., French, D.M. & McElwain, T.F. (1998). Persistence of *Anaplasma ovis* infection and conservation of the msp-2 and msp-3 multigene families within the genus *Anaplasma*. *Infection and Immunity*, vol. 66 (12), pp. 6035-6039.

Panin, A. & Mahabile, M. (1997). Profitability and household income contribution of small ruminants to small-scale farmers in Botswana. *Small ruminant research*, vol. 25, pp. 9-15.

Panin, A. (2000). A comparative economic analysis of smallholder cattle and small ruminant production systems in Botswana. *Tropical Animal Health and Production*, vol. 32, pp. 189-196.

Potgieter, F.T. & Van Rensburg, L. (1987). Tick transmission of *Anaplasma centrale*. *Onderstepoort Journal of Veterinary Research*, vol. 54, pp. 5-7.

Renneker, S., Abdo, J., Salih, D.E.A., Karagenç, T., Bilgiç, H., Torina, A., Oliva, A.G., Campos, J., Kullmann, B., Ahmed, J. & Seitzer, U. (2013). Can *Anaplasma ovis* in small ruminants be neglected any longer? *Transboundary and Emerging Diseases*, vol. 60 (2), pp. 105-112.

Rymaszewska, A. & Grenda, S. (2008). Bacteria of the genus *Anaplasma* – characteristics of *Anaplasma* and their vectors: a review. *Veterinarni Medicina*, vol 53 (11), pp. 573-584.

Shompole, S., Waghela, S.D., Rurangirwa, F.R. & McGuire, T.C. (1989). Cloned DNA probes identify *Anaplasma ovis* in goats and reveal a high prevalence of infection. *Journal of Clinical Microbiology*, vol. 27 (12), pp. 2730-2735.

The World Bank. 2016. *Botswana overwiew.* Available at: http://www.worldbank.org/en/country/botswana/overview (Accessed 2016-11-30).

Vatta, A.F., Letty, B.A., van der Linde, M.J., van Wijk, E.F., Hansen, J.W. & Krecek, R.C. (2001). Testing for clinical anaemia caused by *Haemonchus* spp. in goats farmed under resource-poor conditions in South Africa using an eye colour chart developed for sheep. *Veterinary Parasitology*, vol. 99, pp. 1-14.