Cross-sectional study of bovine anaplasmosis in South-western Uganda
The impact of wildlife-livestock interface

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Tvärsnittstudie av anaplasmos hos nötkreatur i sydvästra Uganda
Hur närhet till vild djur påverkar prevalensen

Mikael Palmfjord

SUMMARY

The tick-borne disease bovine anaplasmosis is primarily caused by *Anaplasma marginale*. A variety of wild animals act as reservoirs for *A. marginale*, but the understanding of their role in the epidemiology of *A. marginale* is yet poor. This cross-sectional study was conducted to establish if proximity of wildlife affect the prevalence of bovine anaplasmosis in cattle. A total of 130 cattle were randomly sampled from two sampling areas within Kiruhura district, South-western Uganda. Sampling sites were chosen due to their distinct proximity to Lake Mburo National Park, resulting in one area with frequent and one with infrequent wildlife-livestock interface. Both sero-prevalence, using an indirect ELISA (Svanova Biotech AB Uppsala, Sweden) and microscopically determined prevalence, based on examination of Giemsa-stained blood smears, were established. Agreement between both diagnostic tests was compared using microscopy as golden standard. To estimate the wildlife-livestock interface and to rank the role of tick-born diseases (TBD) as a constraint to livestock production a questionnaire was used. Furthermore, ticks were collected to estimate the relationship between tick burden and the *A. marginale* prevalence. Statistical analyses was performed using Pearson's χ^2-test and Fisher's exact tests at a significance level of $\alpha = 0.05$.

There was a significant difference in how frequent the livestock interacted with wildlife in the two sampling areas, which confirms the choice of sampling areas. A significantly higher sero-prevalence was found in the area with a more frequent wildlife-livestock interface. This difference was not found microscopically. Sero-prevalence reflects the situation over time, rather than the momentarily picture obtained by microscopy, which could explain the difference seen. In relation to our hypothesis, we think that sero-prevalence provides more reliable results. Confounders, such as age of the animal, grazing system and tickburden, that potentially could explain the observed difference between the sampling areas did not significantly influence sero-prevalence. Concluding, *A. marginale* sero-prevalence increases with a more frequent wildlife-livestock interface.

There was a disagreement between the diagnostic methods, which was confirmed by a low sensitivity and specificity comparing these tests. Further validation of the ELISA test is needed, preferably by use of molecular methods. No correlation between tick burden and *A. marginale* prevalence was found, which could be due to the use of an unstandardized tick sampling method. The overall low sero-prevalence of 24.6 (± 7.4) % and relatively high microscopical prevalence of 30.8 (± 7.29) % indicates endemic instability to bovine anaplasmosis in Kiruhura district. These findings are consistent with recent studies in Uganda showing a growing problem of TBD, including *A. marginale* and should serve as an indicator for possible future interventions in the area.

SAMMANFATTNING

Anaplasmos är en fästingburen sjukdom hos nötkreatur, primärt orsakad av *Anaplasma marginale*. Ett flertal vilda djur fungerar som reservoarer för patogenen, men förståelsen för deras epidemiologiska roll för *A. marginale* är fortfarande begränsad. Denna tvärsnittstudie genomfördes i syfte att undersöka om närheten till vilda djur påverkar prevalensen av anaplasmos hos nötkreatur. I studien provtogs 130 nötkreatur från två områden i Kiruhura distriktet i sydvästra Uganda. Provtagningsområdena valdes utifrån deras geografiskt skilda positioner i förhållande till Lake Mburo National Park. I området som gränsar till Lake Mburo National Park förväntades boskapen interagera mer frekvent med vilda djur jämfört med området längre bort från nationalparken. Prevalens av *A. marginale* analyserades dels med en indirekt ELISA (Svanova Biotech AB Uppsala, Sweden), dels genom mikroskopisk undersökning av Giemsa-färgade blodutstryk. Analysmetodernas överensstämmelse jämfördes med mikroskopi som referensmetod. Ett frågeformulär tillhandahölls djurhållarna för att kvantifiera hur ofta boskapen interagerade med vilda djur samt för att uppskatta hur stort problem djurhållarna uppfattade att fästingburna sjukdomar var i Kiruhura distriktet. Dessutom insamlades fästingar från blodprovstagna djur för att undersöka sambandet mellan fästingbörda och *A. marginale* prevalens. Skillnader mellan den båda undersökningsområdena testades med Pearsons χ^2-test alternativt Fischers exakta test vid signifikansnivån $\alpha = 0.05$.

Det var en signifikant skillnad mellan de två provtagningsområdena i hur ofta boskapen interagerade med vilda djur, vilket bekräftar valet av provtagningsområden. Dessutom påvisades en signifikant skillnad i sero-prevalens mellan de båda provtagningsområdena, där djuren från området med en högre interaktion med vilda djur uppvisade en högre sero-prevalens. Denna skillnad detekterades inte med den mikroskopiska undersökningen. Vi anser likväl att sero-prevalens ger ett pålitligare resultat, eftersom denna metod bättre speglar exponeringen för *A. marginale* över tid. Andra faktorer, så som ålder på djuret, djurhållningssystem och fästingbörda, som likväl potentiellt kan förklara skillnaden i sero-prevalens mellan de båda områdena, visade sig inte påverka sero-prevalens. Sammantaget tyder studien på att sero-prevalens av *A. marginale* ökar vid ökad kontakt med vilda djur.

Överensstämmelsen mellan de båda analysmetoderna var dock svag. Vidare validering av ELISA metoden är önskvärd och då förslagsvis med molekylära detektionsmetoder. Inget samband förelåg mellan fästingbördan och en högre *A. marginale* prevalens, vilket skulle kunna förklaras av användandet av en icke standardiserad metod för att uppskatta fästingbördan. Jämfört med tidigare studier i Uganda var sero-prevalensen låg, 24.6 (± 7.4) %, medan den mikroskopiska prevalensen för *A. marginale* var förhållandevis hög, 30.8 (± 7.29) %. Detta indikerar endemisk instabilitet för anaplasmos i Kiruhura distriktet, vilket överensstämmer med en nyligen publicerade studie i Uganda som visat att fästingburna sjukdomar, inklusive anaplasmos, är ett ökande problem i Uganda. Klassificeringen av området som endemiskt instabilt bör användas för att utvärdera om framtida insatser mot anaplasmos i området behövs, t.ex. immunisering.

CONTENT

INTRODUCTION

Tick-borne disease and wildlife-livestock interface

Ticks and tick-borne diseases (TBDs) are presented globally, but are most prevalent, numerous and exert the greatest impact in tropical and subtropical areas. In many countries TBDs are the major economic and health impediments to livestock production (Bram, 1982). *Anaplasma marginale,* causing bovine anaplasmosis (Theiler, 1910), is the most prevalent tick-borne pathogen of cattle worldwide and is endemic in regions of Asia, America, Australia and Africa (Brayton *et al*., 2009; Aubry & Geale 2011). This is predominantly a result of the high tick burden in these areas (Walker *et al*., 2003.). The presence of wildlife in the National parks is one of the contributing factors, as wildlife may act as reservoirs for the tick population and TBDs (Kuttler, 1984).

Wildlife-livestock interfaces are often characterized by conflict between livestock keepers and wildlife conservation authorities especially as it relates to the opportunities for transmission of diseases common to both wildlife and domesticated animals. These disease problems are frequently bi-directional at the wildlife-livestock interface and thus effects both domesticated animals and wildlife (Bengis *et al*., 2002). The understanding of the susceptibility in wildlife and their role in the epidemiology of *A. marginale* is yet poorly understood (Aubry & Geale, 2011).

Objectives

The main objective of this observational study was to compare the prevalence of *A. marginale* in cattle in two areas; one with a high and another with a limited level of wildlife interface. The aim was to test the hypothesis that prevalence of this pathogen in cattle is influenced by proximity to wildlife. The study was conducted around Lake Mburo National Park (LMNP) in South-western Uganda.

Specific research objectives;

- Investigate and compare the prevalence of *A. marginale* based on serology and Giemsa-stained blood smears in cattle with high and limited level of wildlife-livestock interface around LMNP.

- Estimate the tick-burden and presence of different tick species on sampled cattle to investigate the association with prevalence of *A. marginale*.

- Estimate the wildlife-livestock interface and identify and rank the impact of ticks and TBDs as a constraint to livestock production in the different farms by a questionnaire.

LITERATURE REVIEW

Anaplasma marginale

Description of the pathogen

A. marginale is an obligate intraerytrocytic microbe, found exclusively in membrane-bound vacuoles in the host cells cytoplasm. *A. marginale* belongs to the genus *Anaplasma*, family Anaplasmataceae im the order Ricketsialles. The organisms in this order were recently reclassified by Dumler *et al*. 2001 based on genetic analysis of 16S rRNA, groELS and surface protein genes. The genus *Anaplasma* include three species that can infect ruminants, *A. marginale, A. centrale* and *A. ovis* (Dumler *et al*., 2001). *A. marginale* and *A. centrale* infect cattle, but differ in morphology, virulence and geographical distribution. *A. marginale* is the pathogen primarily causing bovin anaplasmosis (Ristic, 1968; Bram, 1982).

Disease transmission

Transmission of *A. marginale* occurs mechanically, either by blood-contaminated fomites or by biting arthropods including flies (Hawkins *et al*., 1982; Scoles *et al*., 2005; Potgieter *et al*., 1981) and biologically by ticks (Dikmans, 1950). Transmission by biting flies is presumed to be purely mechanical and directly dependent on the levels of rickettsemia during their feeding (Scoles *et al*., 2008). This is in contrast to the biological transmission by ticks in which *A. marginale* can replicate within the gut-epithelium and salivary glands acini regardless of the level of rickettsemia in the acquiring host. For this reason ticks are more competent of acquiring and transmit *A. marginale* than biting flies and can thus transmit infection from persistently infected animals with a low-level of rickettsemia (Löhr *et al*., 2002; Scoles *et al*., 2008; Futse *et al*., 2003). Transmissibility of *A. marginale* is quite complex and differs among different *Anaplasma* strains as well as various vectors (Ueti *et al*., 2007).

As reviewed by Kocan *et al*. 2004, more than 20 species of ticks have been identified as vectors of *A. marginale*. In general, *A. marginale* is spread by *Rhipicephalus* spp., *Dermacentor* spp. and *Ixodes ricinus*, but has not been proved to be transmitted by *Amblyomma* spp. (Kocan *et al*., 2004). Despite the fact that *A. marginale* is globally distributed, the prevalence is highest in tropical and subtropical areas where *R. microplus* (the tropical cattle tick) is endemic (Futse *et al*., 2003). In Uganda *R. evertsi evertsi* (Figure 1) and *R. decoloratus* (Figure 1) are abundant and can thereby transmit *A. marginale* (Walker *et al*., 2003; Rubaire-Akiiki *et al*., 2004; Okello-Onen *et al*., 1999). *R. decoloratus* is the most widespread one-host tick in Africa and it feed only on ruminates including cattle, sheep, goats as well as on wild ungulates. *R. evertsi evertsi*, commonly known as the red-legged tick, is a two-host tick, meaning that the larvae and nymphs infect the same host and after moulting the adult tick attaches to a final host. Among the domestic hosts are cattle and sheep, but a widely variety of wild animals can also be infected (Walker *et al*., 2003).

Figure 1. Rhipicephalus evertsi evertsi *and* Rhipicephalus decoloratus *under stereo microscope. Photo by Anna Schischke.*

Lifecycle and clinical symptoms

When cattle are exposed to *A. marginale*, an incubation period of 7-60 days follows depending on the infective dose. *A. marginale* is a strictly intra-erythrocytic microbe and the infected erythrocyte contains a membrane-bound inclusion, called initial body, that each contains four to eight rickettsias (Kocan *et al*., 2003). In the erythrocytes *A. marginale* undergoes a cycle of replication, and subsequently they are phagocytized by the reticuloendothelial system to further reinvade other erythrocytes. During this acute phase as many as 10^9 erythrocytes per millilitre of blood, corresponding 70 % of all erythrocytes, can be infected (Kocan *et al*., 2003; Kieser *et al*., 1990). The phagocytosis of erythrocytes results in mild to severe haemolytic anaemia and icterus without hemoglobinemia or hemoglobinuria (Kocan *et al*., 2010; Aubry & Geale, 2011). Clinical signs may include fever, weight loss, lethargy, abortion and in some clinical cases even death (Kocan *et al*., 2010; Palmer *et al*., 1999).

The severity of the disease is age-dependent. Mainly animals over two year of age develop acute disease associated with a high mortality, contrary to calves that are less susceptible. Calves under the age of 6 months rarely develop clinical signs and when older than 6 months they usually only suffer from mild clinical disease. Cattle between one and two years of age may have more acute signs of disease, which seldom is fatal (Kocan *et al*., 2010).

Regardless of the age, cattle that survive the acute phase become persistently infected. The persistently infected cattle are usually free from clinical signs and usually have a rickettsemia that fluctuate, in relatively constant cycles of 5 week interval, between 10^3 and 10^7 infected erythrocytes per ml of blood (Kieser *et al*., 1990; Eriks *et al*., 1989; Eriks *et al*., 1993).

Persistently infected cattle develop life-long immunity to bovine anaplasmosis, but act as reservoirs (Kocan *et al.*, 2003).

Humoral immune responses

Calves can be diagnosed as sero-positive for *A. marginale* after intake of maternal antibodies via colostrum. This antibody level usually decreases from birth until the age of 16 weeks. Sero-conversion data indicates that calves may be infected with *A.marginale* from their first week in life and the number of calves in a herd that sero-convert thereafter gradually increase. Circulating antibodies in persistently infected cattle have been detected with a competitive enzyme-linked immunosorbent assay (C-ELISA) as long as 6 years after inoculation (Knowles *et al.*, 1996).

Epidemiology

Geographic distribution

Bovine anaplasmosis is on the list of notifiable diseases by the Organisation for Animal Health (OIE). Local outbreaks are reported to the African Union – Interafrican Bureau for Animal Resources (AU-IBAR). In 2011, anaplasmosis was reported from 14 countries, and the geographical distribution of the disease shows that it was mainly recorded in the southern parts of Africa (AU-IBAR , 2013).

Host occurrence

Among domestic livestock, *A. marginale* infects ruminants, but is principally pathogenic only in cattle. The pathogen has a wide host occurrence including various wild animals. In regards to the epidemiology of *A. marginale,* the knowledge of the contribution of domestic and wild animals towards the prevalence of the disease is incomplete due to lack of published research, validation of tests and cross reactivity of *Anaplasma* spp. antibodies (Aubry & Geale, 2011; Kuttler, 1984). Clinical anaplasmosis does not seem to appear in wild animals, with the exception of two reported clinical cases in giraffe (*Giraffa camelopardalis)* (Lohr & Meyer, 1973; Gustyn, 1974).

Recent studies based on polymerase chain reaction (PCR) and reverse line blot hybridization (RLB) have recognized a wide host occurrence for *A. marginale* in wild animals. In sub-Saharan Africa, *A. marginale* is known to infect various wild animals including African buffalo (*Syncerus caffe*r), common Eland *(Taurotragus oryx)* and gemsbok *(Oryx gazella gazella)*. All have been proven to be carriers using these molecular techniques. (Berggoetz *et al.*, 2014; Tonetti *et al.*, 2009; Oura *et al.*, 2011a; Oura *et al.*, 2011b). Based on serological analyses, the occurrence of *Anaplasma* spp. has also been reported in impala *(Aepyceros melampus)*, waterbuck (*Kobus ellipsiprymnus)* and plain zebra (*Equus quagga)* (Ngeranwa *et al.*, 2008). All of these wild animals are present around LMNP.

Breed differences in susceptibility

Several studies have been carried out to determine if there is a difference in susceptibility for *A. marginale* infection between local breeds (*Bos indicus*), European breeds (*Bos taurus*) and

their crosses (Jonsson *et al.*, 2008; Carter, 2011). *B. indicus* have been shown to be susceptible to anaplasmosis and may also develop clinical symptoms (Wilson, 1979). Under experimental inoculation *B. taurus*, *B. indicus* and their crosses were equally susceptible to *A. marginale* and developed similar responses in packed cell volume (PCV)-depression and maximum rickettsemia detected microscopically (Bock *et al.*, 1997; Carter, 2011). Bock *et al.* (1997) showed that 50 % of *B. indicus* needed treatment to recover in contrast to 100 % of the pure *B. taurus*. When comparing *B. indicus* and crosses after artificial infection with *A. marginale* via *R. microplus* there were no significant breed differences (Bock *et al.*, 1999).

Breed related susceptibility is documented in other hemoparasites such as *Theileria parva* and *Babesia bigemina,* where *B. indicus* are more resistant to infection compared to *B. taurus* (Paling *et al.*, 1991; Jonsson *et al.*, 2008; Carter, 2011). Matovu *et al.* 2014 recently showed a significantly difference in prevalence of hemoparasites between *Bos indicus* and *Bos taurus* in Central and South-western Uganda, but they did not specify how the prevalence differed for *A. marginale.*

Endemic stability and instability

Endemic stability is characterized, among several things, by a high sero-prevalence (> 70 %) resulting in little fluctuation in disease incidence over time (Rubaire-Akiiki *et al.*, 2004). In an endemic stable area, the young animals become infected at an early age, when there is a significant passively acquired immunity and consequently they usually do not develop severe symptoms. This way the young animals become immune to challenge later in life and the incidence of clinical cases becomes low despite high level of infection (de Vos, 1992). In contrast, in endemic instable areas the young animals do not encounter the pathogen frequently enough and subsequently do not become immune, which is reflected in a low sero-prevalence (> 30 %) (Alonso *et al.*, 1992). For anaplasmosis the sero-prevalence has been used as an indicator of the existents of endemic stability and it thus serve as a biological indicator for the need of interventions, as for example immunization (Perry & Young, 1995). There are more factors influencing endemic stability including tick-challenge. If there is a low tick transmission rate the young animals challenge to the disease is low and immunity will not be boosted (de Vos, 1992).

Wildlife-livestock interface

In settings with wildlife-livestock interface disease problems are often bidirectional (Bengis *et al.*, 2002). Ticks and TBDs are one of the major conflicts in areas with a high wildlife-livestock interface and wild animals are often blamed to serve as reservoirs (Ocaido *et al.*. 2009a; Wesonga *et al.*, 2006). For *A. marginale,* as reviewed above, wild animals can be carriers of *A. marginale* (Oura *et al.*, 2011a; Berggoetz *et al.*, 2014).

There is limited knowledge of how and if wildlife-livestock interface affects the occurrence of *A. marginale*. However, Kabuusu *et al.* (2013) showed that *A. marginale* in livestock significantly increased with close proximity to a wildlife-livestock interface.

Diagnosis of *Anaplasma*

During the acute phase of infection bovine anaplasmosis can be diagnosed microscopically in Giemsa-stained blood smears by presence of intraerythrocytic initial bodies. Even if *Anaplasma* infections usually are persistent, it may be undetectable by microscopy after the acute phase. Thus, for detection of pre-symptomatic and persistently infected animals serological method seems more reliable. The only way to diagnose of the presence of the causative organism is to demonstrate it, either by presence in blood smears or by molecular diagnostics. The golden standard is to inoculate blood from a suspected animal into a splenectomized calf. This procedure is followed by multiple blood-smear examination every 2nd–3rd week for the presence of the pathogen. This method is very expensive and raises welfare issues (OIE, 2012).

Giemsa-stained blood smears

For identification of the agent during the acute phase of the disease, thin Giemsa-stained blood smears can be inspected. Due to the rather indistinct morphology of *A. marginale* it is crucial that the smears are thin and free from foreign material. The experience of the reader is as well an important bias. *A. marginale* appears as a dense rounded and deeply stained intraerythrocytic initial body, approximately 0.3–1.0 µm in diameter (Figure 2). It is normally distinguished from *A. centrale* due to its peripheral position in the erythrocyte. The differentiation of these two species is difficult especially in low-level rickettsemia. The infection becomes visible approximately 2-6 weeks after transmission and subsequently during clinical disease. However, the percentage of infected erythrocytes varies with the stage and the severity of disease (OIE, 2012). During acute phase the rickettsia levels can reach above 10^9 infected erythrocytes per ml blood (Kieser *et al.*, 1990). Less than $10^{7.2}$ infected erythrocytes per ml of blood is not detectable in microscopy and persistently infected animals usually fluctuate in between 10^4 and 10^7 infected erythrocytes per ml of blood (Eriks *et al.*, 1993; Kieser *et al.*, 1990).
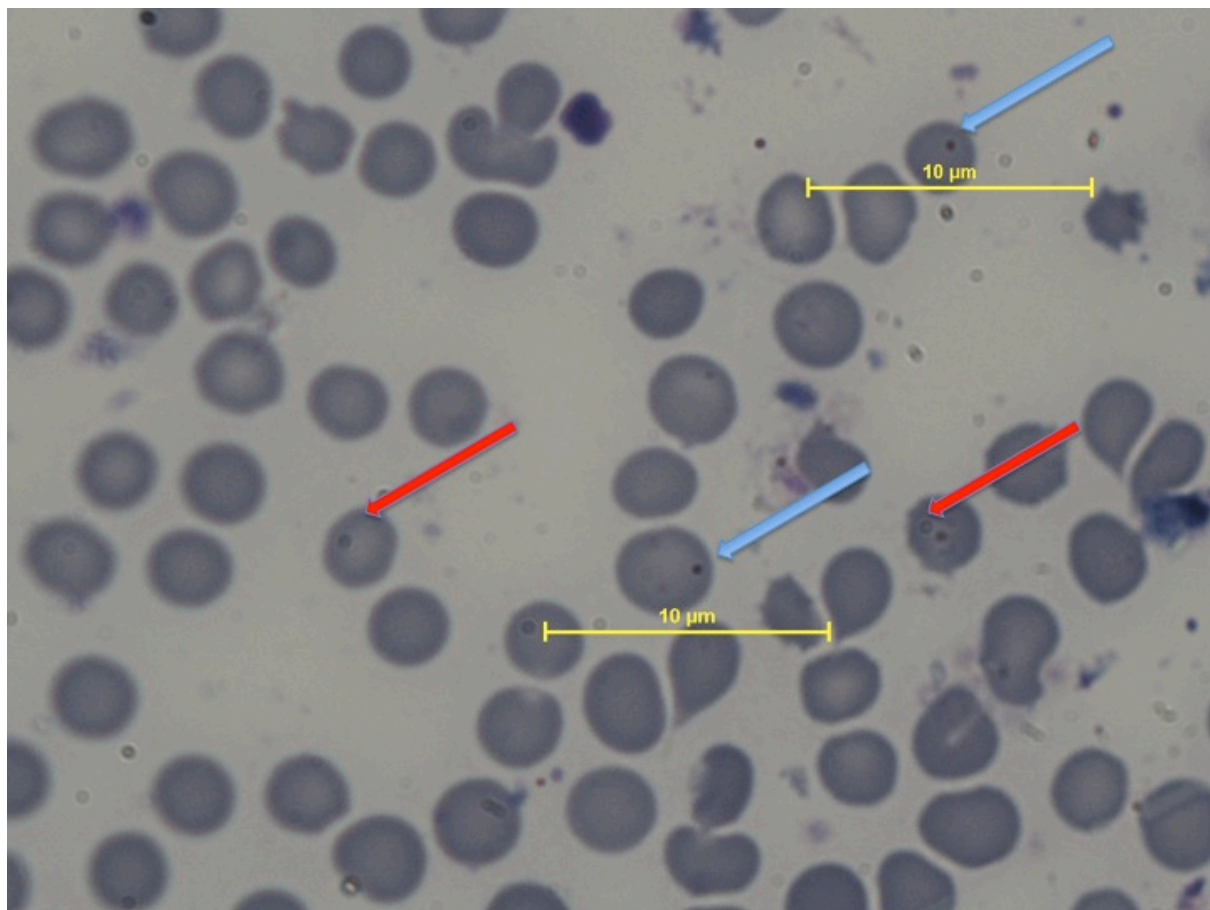
Figure 2. Anaplasma marginale *under light microscope ×1000 magnification. Blue arrows pointing at* A. marginale *and red arrows pointing at artefacts. Photo by Dr. Dickson S. Tayebwa.*

Serological tests

Serological analyses have the ability of detecting persistently infected animals and are suitable for epidemiological studies (OIE, 2012). A potential problem with serological diagnosis is the risk for cross-reactions between closely related species. Concerning serological analyses for *A. marginale* cross-reactions have been reported with other *Anaplasma* spp. including *A. marginale, A. centrale, A. ovis, A. phagocytophilum* (Visser *et al*., 1992; Fuente, *et al*., 2005) as well as *Ehrlichia* spp (Al-Adhami *et al*., 2011).

Still ELISA has the advantage that it is a cost-effective diagnostic method which allows the run of large samples sizes and an objective interpretation of results (Morzaria *et al*., 1999). An indirect antibody detection ELISA kit for *A. marginale* has recently been developed at Boehringer Ingelheim Svanova Uppsala, Sweden, in collaboration with International Livestock Research Institute (ILRI), Nairobi, Kenya (Svanovir, 2014). ILRI developed an assay based on a recombinant 19 kD protein as capture antigen and according to in house experiments the sensitivity and specificity of this ELISA are >90% for detection of *A. marginale* antibodies in experimentally infected cattle (Morzaria *et al*., 1999). Boehringer Ingelheim Svanova modified the assay and used a recombinant immunodominat antigen as capture antigen (Svanovir, 2014).

Molecular detection methods

Molecular detection methods is useful in finding persistently infected animals with a low level of rickettsemia (Echaide *et al*., 1998; Carelli *et al*., 2007). A reverse line blot assay (RBL) allows simultaneously detection of all known tick-borne parasites of *Theileria* spp., *Babesia* spp., *Ehrlichia* spp. and *Anaplasma* spp. in ruminants (Gubbels *et al*., 1999; Bekker *et al*., 2002; Oura *et al*., 2004). The RBL for *Ehrlichia* spp. and *Anaplasma* spp. has not yet been evaluated concerning its detection limits, but has proven to be specific for each pathogen (Bekker *et al*., 2002).

Tick burden and breed-related resistance to ticks

Tick burden, defined as the numbers of ticks infecting an animal, is influenced by several factors. It principally reflects the tick density in the grazing environment, which shows a seasonal pattern with rainfall (Wesonga *et al*., 2006). There is an association between the presence of certain wildlife-species and the abundance of ticks on pasture. One example is that presence of plain zebras significantly increased the amount of *R. appendiculatus* instars found in pastures around LMNP (Ocaido *et al*., 2009b).

Concerning anaplasmosis, differences in the tick burden of *R. microplus* do not seem to be reflected in the disease response. Both heavily and lightly infected calves showed a similar incubation period, raise of body temperature, decrease of PCV and duration of rickettsemia (Corrier & Kuttler, 1983).

Generally, *B. indicus* cattle have proven to possess a higher resistance to ticks, in terms of limiting the tick burden and their pathological consequences, than *B. taurus* cattle and its crosses. Breed-related resistance varies to different tick species, but is also influenced by age of the animal, sex and season (Mattioli *et al*., 2000; Wambura *et al*., 1998; Fivaz *et al*., 1992).

Anaplasmosis in Uganda

Cattle production in Uganda

Livestock production is an important sub-sector, where cattle are the most important livestock from an economic point of view. The amount of cattle increases steadily and according to the most recent available results for 2008, the national cattle herd was estimated to be 11.4 million in Uganda. The dominant cattle breed in Uganda is the indigenous cattle, *B. indicus* (93.6%), including Ankole longhorn and Zebu/Nganda. The western part of Uganda has the highest proportion of *B. taurus*, European breeds (Holstein Friesian) and their crosses reaching up to 12.1 %. Kiruhura district has among the highest proportion of European breeds and their crosses in Uganda (National livestock census, 2008).

Reported prevalences in Uganda

In serological analyses of *Anaplasma* spp. in Uganda sero-prevalences have ranged between 30-60 % (Table 1). One recently published study in Central and Western Uganda showed an overall microscopical prevalence of *Anaplasma* spp. of 14.4 % (Matovu *et al*., 2014).

Table 1. *Recorded sero-prevalence and microscopical prevalences of* Anaplasma *spp. in Uganda*

Study	Study area	Method	Recorded prevalence	*Anaplasma* spp
Magona & Mayende, 2001	Tororo and Soroti, Central eastern Uganda.	Giemsa-stained blood smears	13.3%	*A. marginale*
Rubaire-Akiiki *et al.*, 2004	Mbale district Central eastern Uganda	I-ELISA (Morzaria *et al.* 1999)	~ 30 % in fenched in lowland < 60 % in free-range in lowland	*Anaplasma* spp.
Kabi *et al.*, 2008	Soroti district Central eastern, Uganda	I-ELISA (Svanova Biotech AB Uppsala)	58 % Ankole cattle 57 % Zebu/Nakidi cattle	*Anaplasma* spp.
Angwech, 2011	Gulu district, Nothern Uganda	Giemsa-stained blood smears	10.4 %	*Anaplasma and A.centrale*
Kabuusu *et al.*, 2013	Queen Elisabeth National park, Western Uganda	Giemsa-stained blood smears	15%	*A. marginale*
Matovu *et al.*, 2014	Central and Western Uganda	Giemsa-stained blood smears	14.4 % overall 24.8 % western 6.0 % central	*A. marginale* and *A. centrale*

Lake Mburo National Park

Livestock production around Lake Mburo National Park

LMNP is a small national park situated in South-western Uganda. It has a reported high wildlife-livestock interface both inside and in areas bordering to the park (Ocaido & Siefert 1996; Ocaido *et al.*, 2009a). The predominant wildlife in the wildlife-livestock interface are impala *(Aepyceros melampus)*, zebra *(Equus burchelii)* and Cape buffaloe *(Syncerus caffer)* although the latter are not found as frequently in the mixed grazing areas as the other two species. Cattle are mainly indigenous Ankole longhorn and their crosses with European breeds. The cattle holder systems in this area are mainly ranching and communal grazed systems. Most of cattle (76%) are kept under smallholder communal grazing system (<40 cattle) (Ocaido *et al.*, 2009a).

Tick born diseases in Lake Mburo National Park

Ocaido *et al*., (2009a) showed that cattle farmers around LMNP fear that wildlife may transmit diseases to their livestock and thus act as a constraint to their livestock production. The economic cost of ticks and TBDs in LMNP has been estimated to USD 308,144 annually. The major costs were constituted from the control of TBDs, including tick control (dipping and spraying) and chemotherapy (Ocaido *et al*., 2009b).

Epidemiology of anaplasmosis in Lake Mburo National Park

In LMNP both *A. marginale* and *A. centrale* have been diagnosed in Cape buffaloes, while impalas only act as reservoirs for *A. centrale*. In cattle, low carrier prevalence for *A. marginale* (<16 %) and absence of *A. centrale* have been reported in calves (3-12 months) both co-grazing with wildlife and without wildlife interface (Oura *et al*., 2011b).

MATERIAL AND METHODS

Sampling areas and study population

This cross-sectional study was conducted on 130 heads of cattle from 30 farms in Kiruhura district, South-western Uganda. Sampling was performed in two sub-counties, Sanga and Kikatsi, due to their distinct difference in proximity to LMNP. Sanga sub-county borders LMNP and was considered to be an area with frequent wildlife-livestock interface. Kikatsi was chosen due to its distance to LMNP, approximately 30-50 km, thus an area assumed to have limited wildlife-livestock interaction. Kikatsi is as well delimited to LMNP by the road between Kampala and Mbarara, which was considered to serve as a natural barrier for movements of wild animals from LMNP to Kikatsi.

The grazing system was predominantly communal grazing; where the pastoralist herds their animals in search for water and feed. In fenced grazing the cattle are grazed in an enclosure without as frequent supervision from a herdsman. In communal grazing there is a constant mix with other herds of livestock. Thus there is a possibility to share grazing areas with wild animals. Among the cattle breeds randomly selected for sampling were both Ankole Longhorn (*B. indicus*) and their crosses with European breeds (*B. taurus*).

Sample size

A total number of 30 farms, were chosen based on the epidemiological consideration that a large sample size (> 30) generates a sampling distribution for the sampling mean that is most likely to be normally distributed. In each sampling area the District Veterinary Office assisted in the selection of farms. In Sanga farms bordering the park were chosen to ensure a frequent wildlife-livestock interface. Farms included had at least four animals available at the moment of sampling and we had their consent to participate in the study. Depending on size of the farm between three to six animals were sampled at each farm. The 130 individual animals sampled were in turn randomly selected from the herds.

Sampling

Blood sampling, storage and preparation

Blood was collected from the coccygeal or the jugular vein using a closed vacutainer system (BDH, UK). Two 4 ml serum tube for serological analyses and one 4 ml EDTA tube for microscopy were collected from each animal. Blood samples were stored in iceboxes and transported to Makerere University in Kampala. Serum samples were separated within 48 h from the sampling, assisted by the Molecular Science laboratory, Makerere University and transferred to 2 ml cryovial tubes for storage at -20°C. EDTA blood was used for preparation of thin Giemsa-stained blood smears for microscopic examination. For every animal sampled metadata including farm, breed, age and sex were recorded.

Estimation of tick burden

Ticks were collected from all animals that were blood sampled. Whenever possible, six or more (at most 11) ticks were randomly collected from the predilection areas including ears,

rump and udder. Animals were categorized to have either no ticks, less than 6 ticks and 6 or more ticks. The ticks were stored in eppendorf tubes at room temperature before identification.

Questionnaire

Farm descriptive data was obtained using a questionnaire. The questionnaire was addressed to the person normally in charge of the livestock. A field assistant performed the interviews in their local language Ankole and recorded the answers in the questionnaire. Most questions where designed in a closed format, to maintain consistency. However, when a numerical answer was sought an open-ended question was used. The questions were designed to estimate the wildlife-livestock interface and identify and rank the impact of ticks and anaplasmosis as a constraint to livestock production. Additional data with description of the farm including location, number of animals, management system and if livestock was used for milk or meat production was sought, as well as socio-demographic factors.

Analyses

Enzyme-linked immunosorbent assay

Serological analyses, for the detection of antibodies to *A. marginale,* were performed using the Svanovir ELISA for *A. marginale* (Svanova Biotech AB Uppsala, Sweden) (Morzaria *et al*. 1999). The analyses were performed according to manufactures instruction. All reagents were equilibrated to room temperature before use. Samples, as well as positive and negative controls, were diluted 1:40 in Phosphate Buffered Saline (PBS)-tween buffer. Controls and samples were loaded in duplicates into the pre-coated ELISA micro-titre plate. All following steps, including incubation, washing and adding of HRP-conjugate monoclonal anti-species-Ig specific antibodies, substrate solution as well as stop solution, were performed following the kit protocol precisely. The micro-plate was read at 405 nm wavelength using Biotek EL-800 micro plate reader and Gen-5 software to generate a Microsoft Excel- sheet with measured optical densities (OD). Results were expressed as percent positivity (PP) values based on the following formula:

$$PP = 100 \times (\text{mean OD}_{sample})/\text{mean OD}_{positive\ control}$$

If all the criteria for test validity was fulfilled, samples with a PP-value ≥ 25 were interpret as positives, according to the manufactures recommended cut-off value.

Microscopy

To investigate the presence of *A. marginale* and *A. centrale* microscopically assistance in preparation and the reading of the slides were provided by Molecular Science laboratory and Central Diagnostic laboratory at Makerere University. Briefly, preparation was performed using EDTA blood to make thin blood smears, followed by fixation in ethanol and staining with Giemsa. The slides were examined microscopically at $\times 1000$ magnification with oil

immersion. Positive slides for either *A. marginale* or *A. centrale*, as well as negative slides, were connected to the sample-id and recorded in a Microsoft Excel-sheet.

Tick identification

Ticks collected from the animals were identified to species level using a stereo microscope, according to the descriptions by Walker *et al.* (2003).

Statistical analyses and test evaluation of ELISA

Data derived from questionnaires and laboratory examinations were entered into Microsoft Excel for Mac 2007 and statistical analyzed using SPSS (version 22) at a confidence level of 95 % and a significance level of $\alpha = 0.05$. Analysis of statistical difference between proportions was done using either Pearson's $\chi2$-test or Fisher's exact test. Fisher's exact test was applied for unbalanced data whenever one observed value in the contingency table was below five. All requirements to use these non-parametric analyses were fulfilled. Serological and microscopical prevalence was calculated at a 95 % confidence interval using GraphPadPrism 6.

To decide the agreement between the ELISA and the microscopy the sensitivity, specificity, likelihood ratio of a positive test (LH (+)) and likelihood ratio of a negative test (LH (-)) was calculated using the microscopy results as golden standard. Calculation was performed with three different cut-off values; PP-value ≥ 25, PP-value ≥ 20 and PP-value ≥ 15.

RESULTS

Distribution of sampled animals in the sampling areas

A total number of 130 animals were sampled; 48.5 % in Sanga and 51.5 % in Kikatsi. 37.7 % of the sampled animals were Ankole (*B. indicus*) and 62.3 % were crosses of Ankole and European breeds (*B. taurus*). There was a significant (*P* < 0.001) difference in how the breeds were distributed between the different areas. There were significantly more Ankole in Sanga 58.7 % than in Kikatsi 17.9 % and accordingly more crosses in Kikatsi 82.1 % than in Sanga 41.3 %. Most of the sampled animals were adults (> 2 years), corresponding to 83.8 % of the total sampled animals. Further 3.8 % and 12.3 % of the animals sampled were calves (< 1 year) and heifers (1-2 years), respectively. A total of 97.7 % of the animals were females. For all results see Table 2.

Table 2. *Distribution of sampled animals corresponding to sampling areas*

		Sampling area		Total	%	χ^2	P-value*
		Sanga	Kikatsi				
Breed	Ankole	37	12	49	37.7	23.04	
	Crosses	26	55	81	62.3		< 0.001
Age	Calve (<1 year)	0	5	5	3.8		
	Heifer (1 - 2 years)	10	6	16	12.3		
	Adult (> 2 years)	53	56	109	83.8		
Sex	Female	63	64	127	97.7		
	Male	0	3	3	2.3		0.25**
Total		63	67	130			

* Significant if P < 0,05

** Fisher's exact test applied

Farm characteristics

The farms in both sampling areas were mainly communally grazed small cattle herds (< 30 animals), whereas 87.6 % of the farms used a communal grazing system. There was no statistically significant (*P* = 0.60) difference between uses of grazing systems comparing the two sampling areas. A majority of the farms (60.0 %) held their cattle for both milk- and meat production. A total of 46.7 % of the interviewed cattle holders had no education and 50.0 % had been working with cattle for more than 20 years.

Estimation of wildlife-livestock interface and impact of TBD in different sampling areas

There was a significant (*P* < 0.001) difference between the sampling areas in how often the cattle interacted with the wildlife. All owners in Sanga reported that their cattle interacted

with wildlife every day. In Kikatsi, none of the owners experienced wildlife-livestock interaction every day and 86.7 % observed interaction as infrequently as once every month or never (Figure 3). Moreover there was a significant ($P < 0.001$) difference in what the farmers experienced as the major wildlife their cattle most frequently encountered. In Sanga, 33.3 % of the farmers mentioned Cape buffalo as the major wildlife encountered by their cattle and 77.7 % answered plain zebra. None of the farmers in Sanga experienced impalas as the major wildlife in wildlife-livestock interface, but 33.3 % of the farmers in Kikatsi ranked impalas as the major wildlife their cattle encounter.
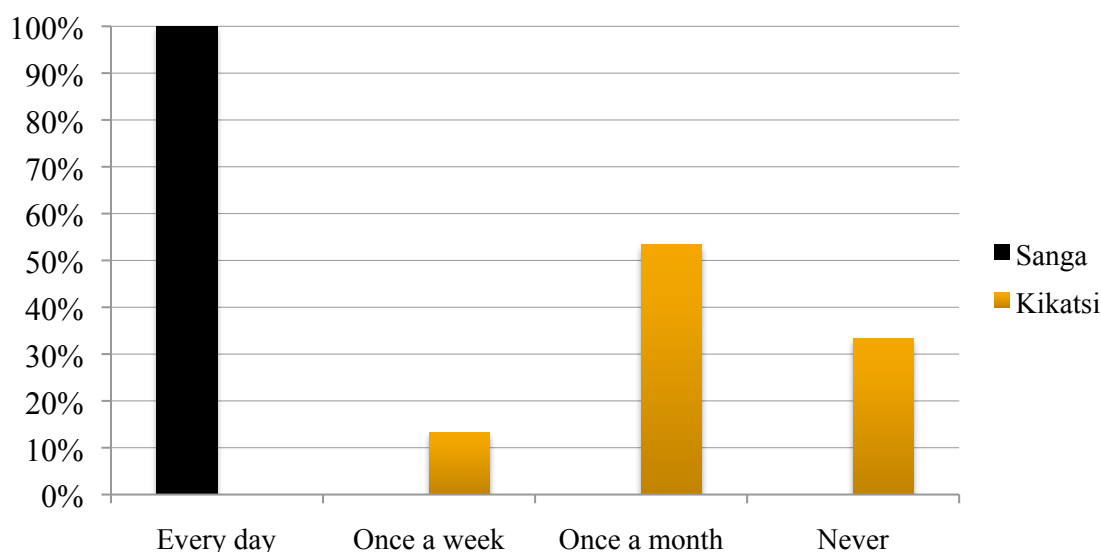

Figure 3. *Frequency of wildlife-livestock interaction in different sampling areas.*

Ticks and TBDs were ranked as the major constraint to livestock production (80 %), but there was no significant ($P = 0.65$) difference in the ranking of the risk between sampling areas. When asked how many cattle the farmers had lost within the last three months due to TBDs (not specified which TBD), all farmers except one had lost animals due to TBDs. Concerning how many cases of anaplasmosis the farmers had experience within the last year, 70 % said they have had none. In Sanga 7 out of 15 farms reported that they have had cases of anaplasmosis within the last year, but only 2 out of 15 reported this in Kikatsi.

Prevalence of *A. marginale*

ELISA

The overall sero-prevalence was 24.6 (± 7.4) %. There was a significant difference in prevalence between the sampling areas. Sanga, with a high level of wildlife-livestock interface, had a significantly ($P < 0.001$) higher sero-prevalence than Kikatsi, with a limited wildlife-livestock interface (Figure 4). The sero-prevalence in Sanga was 38.1 (± 12.0) %, which is significantly ($P < 0.001$) higher than the corresponding sero-prevalence of 11.9 (± 7.8) % in Kikatsi.
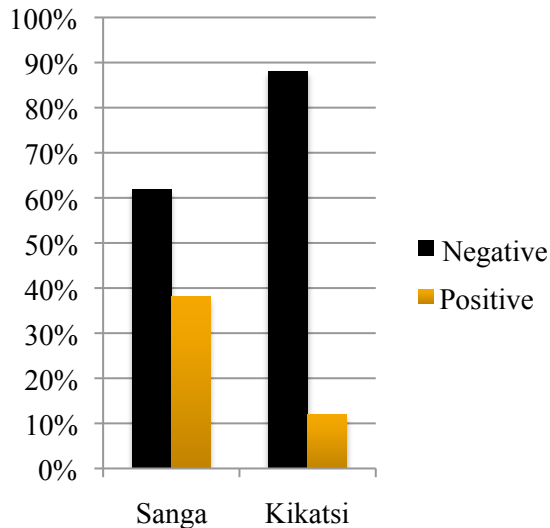
Figure 4. *Sero-prevalence of* A. marginale *in different sampling areas*.

There was a no significant (*P* = 0.83) difference in sero-prevalence between different breeds. Of the Ankole 26.5 (± 12.4) % were sero-positive and 23.4 (± 9.2) % of the crossbreeds. Concerning the age, all sero-positive animals, except two, were adults. The remaining two sero-positive animals were heifers and correspondingly none of the calves were sero-positive. The tick burden on the sampled animals did not significantly (*P* = 0.15) influence the sero-prevalence. The presence of either *R. evertsi evertsi* or *R. decoloratus* was not correlated to a higher sero-positivity (*P* = 0.83). Nevertheless, of all cattle with *R. evertsi evertsi,* 40.9 % were sero-positive in contrast to 21.3 % of those where this tick was not found (*P* = 0.06).

Microscopy

The overall prevalence microscopically was 30.8 (± 7.29) %, for *A. marginale* and 24.6 (± 7.4) % for *A. centrale*. Co-infection of both *A. marginale* and *A. centrale* was observed in 14.6 (± 6.1) % of the sampled cattle. There was no significant (*P* > 0.05) difference in prevalence between the sampling areas, neither for *A. marginale* nor *A. centrale* (Figure 5).
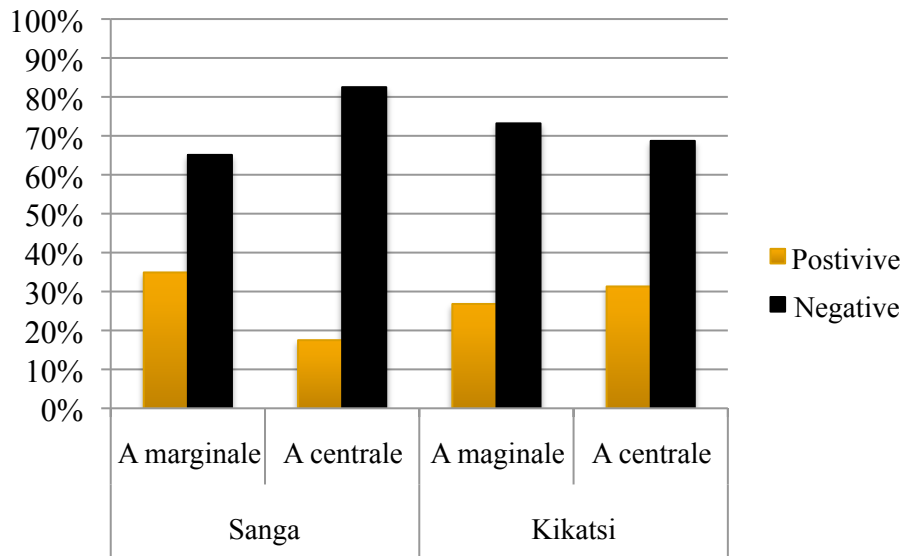
Figure 5. *Microscopical prevalence of* A. marginale *and* A. centrale *in different sampling areas.*

For *A. marginale* there was a significant (*P* = 0.008) difference in microscopical prevalence correlated to the breed. A total of 49.0 (± 14.0) % of the Ankole cattle were positive and only 19.7 (± 8.5) % of the crosses. There was a significant difference between the sampling areas in how many of the positive samples derived from the different breeds. In Sanga, 48.6 % of the Ankole cattle were positive and only 15.4 % of the crosses (*P* = 0.006). In Kikatsi, 50 % of the Ankole cattle were positive and 21.8 % of the crosses (*P* = 0.046). As for sero-prevalence, none of the calves were positive for *A. marginale* microscopically, but 37.5 % of the heifers were positive. The microscopical prevalence for *A. marginale* did not significantly (*P* = 0.538) differ with tick burden. A correlation was found between *R. evertsi evertsi* and microscopical positivity for *A. marginale* (*P* = 0.002). 59 % of the sampled cattle were positive microscopically when *R. evertsi evertsi* was present in contrast to 25 % when *R. evertsi evertsi* was not present. No correlation was found between *R. decoloratus* and microscopical positivity for *A. marginale* (*P* = 0.18).

Test evaluation of ELISA

The sensitivity, specificity, LH (+) and LH (-) using microscopy results of *A. marginale* as golden standard as well as the combined results of *A. marginale* or *A. centrale* are summarized in Table 3 and Table 4, respectively.

Table 3. *Test evaluation of ELISA using microscopy results of A. marginale as golden standard*

	Cut-off value		
	25	**20**	**15**
Sensitivity	22.5%	32.5%	60.0%
Specificity	74.4%	63.3%	41.1%
LR(+)	0.88	0.89	1.02
LR(-)	1.04	1.07	0.97

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Table 4. *Test evaluation of ELISA using the results of the microscopy for* A. marginale *and* A. centrale *as golden standard*

	Cut-off value		
	25	**20**	**15**
Sensitivity	18.9 %	30.2 %	60.4 %
Specificity	71.4 %	61.0 %	41.6 %
LR(+)	0.66	0.77	1.03
LR(-)	1.14	1.14	0.95

Tick burden

The most common tick species recorded in decreasing order of abundance were *R. appendiculatus, R. evertsi evertsi, R. decoloratus* and *A. variegatum.* The general tick burden was significantly ($P < 0.001$) higher in Sanga compared to Kikatsi (Table 5). Concerning the biological vectors for *A. marginale; R. evertsi evertsi* and *R. decoloratus* there was no significant ($P = 0.54$) differences between the sampling areas (Table 5). *R. evertsi evertsi* was significantly ($P < 0.001$) more prevalent in Sanga, in contrast to *R. decoloratus* that was more ($P < 0.001$) abundant in Kikatsi (Table 5). Tick burden was significantly higher in the Ankole cattle compared to crosses ($P < 0.001$) and Ankole were significantly more infected with *R. evertsi evertsi,* 38.8 % compared to crosses 3.7 %.

Table 5. *Tick burden in different sampling areas*

		Sampling area		Total	%	χ^2	*P*- value[*]
		Sanga	Kikatsi				
Amount ticks	None	4	32	36	27.7		
	Less than 6	27	24	51	39.2		<0.001[**]
	6 or more	32	11	43	33.1		
R. evertsi evertsi	Present	19	3	22	16.9		
	Not present	44	64	108	83.1		<0.001[**]
R. decoloratus	Present	2	17	19	14.6		
	Not present	61	50	111	85.4		<0.001[**]
R. evertsi evertsi or R. decoloratus	Present	21	19	40	4.6	0.38	
	Not present	42	48	90	95.4		0.54
Total		63	67	130			

* Significant if P < 0,05

** Fisher's exact test applied

DISCUSSION

This study investigates if wildlife-livestock interface affects the prevalence of *A. marginale* around LMNP. The sero-prevalence (Figure 4) of *A. marginale* was significantly higher in Sanga, with the highest wildlife-livestock interface (Figure 3). The microscopical prevalence of *A. marginale* (Figure 5 and 6) was, on the other hand, not significantly different between investigated areas. Concluding, there are, in this study, conflicting evidence if the overall *A. marginale* prevalence differs between investigated areas and especially if *A. marginale* is influenced by proximity to wildlife. However, we are of the opinion that, for the purpose of assessing our hypothesis, that prevalence of *A. marginale* in cattle is influenced by proximity to wildlife, the sero-prevalence is more reliable than the result from microscopical examination. Sero-prevalence reflects the exposure over time, rather than the momentarily and thus seasonally dependent prevalence shown by microscopy (Matovu *et al.*, 2014). Other confounders previously reported to influence sero-prevalence of *A. marginale* including age of the animal and grazing system (Rubaire-Akiiki *et al.*, 2004), did not differ significantly between the sampling areas. The presence of the biological vectors for *A. marginale, R. evertsi evertsi* and *R. decoloratus*, did not significantly influence the sero-prevalence and their distrubution was not significantly different between the two sampling areas. Even though there was a significant difference in how the breeds were distributed in the two areas, we found no association between breed and sero-prevalence. Thereby, the most reasonable explanation for the observed difference in sero-prevalence between the sampling areas is the difference in wildlife-livestock interface between the sampling areas, indicating that sero-prevalence of *A. marginale* in cattle increases with proximity to wildlife around LMNP.

The only previous study investigating how wildlife-livestock interface affects the prevalence of *A. marginale* in Uganda, showed that proximity of wildlife significantly influenced the microscopical prevalence of *A. marginale* in cattle (Kabuusu *et al.*, 2013). The opposing results compared to the present study, where microscopical prevalence of *A. marginale* was not influenced by proximity to wildlife, could be due to many factors. Possible explanations for the observed difference between the studies could be either differences in season when the studies were conducted or difference in predominate wildlife at the wildlife-livestock interface. Microscopical prevalence of *A. marginale* fluctuated seasonally (Matovu *et al.* 2014) as there are seasonal fluctuations in the transmission of TBDs (Ocaido *et al.*, 2009a). Kabuusu *et al.* (2013) stated that Cape buffalo was the predominant wildlife interacting with livestock. Cape buffaloe is a carrier of *A.marginale* and thus act as a reservoir (Oura *et al.*, 2011a; Oura *et al.*, 2011b). In our study, 77 % of the farmers in Sanga reported plain zebras as the major wildlife interacting with their cattle. Although Ngeranwa *et al.* (2008) reported *A. marginale* in plain zebra based on serology, the knowledge is limited if they can act as reservoirs for *A. marginale*. The epidemiology of anaplasmosis is complicated and incomplete as wildlife are suspected to be carriers of specific strains of *A. marginale,* not transmittable to livestock (Oura *et al.*, 2011b). Further studies based on molecular diagnostics are required to investigate *A. marginale* from different wildlife hosts.

There was a disagreement between the microscopical and ELISA results in the present study, which is confirmed by a low sensitivity and specificity, irrespective of the cut-off level in the ELISA. Using the manufactures cut-off value PP-values ≥ 25, the ELISA obtained a sensitivity of 22.5 % and a specificity of 74.4 % (Table 4). The accuracy of ELISA tests are dependent on a population specific cut-off value (Aubry & Geale, 2011). Thus, a poor agreement could be due to the cut-off value. Lowering the cut-off value in the present study to PP-values ≥ 15 increased the sensitivity to 60.0 % but lowered the specificity to 41.1 % (Table 4), which is expected as there is a trade-off between sensitivity and specificity in relation to the cut-off value. Concluding, using microscopical result for validation of this ELISA gave a poor agreement between these diagnostic tests. This poor agreement could be due to several factors, including the underlying difference between the diagnostic techniques that they analyze different biological markers. Serology detects antibodies that persists over time, whereas microscopy detects the presence of the pathogen momentarily, which differs with season (OIE, 2012, Knowles *et al*., 1996). Secondly, the poor agreement could be due to falsely interpreted results. Microscopically it is difficult to differentiate between pathogens causing TBDs and the experience of the technician is an important bias (OIE 2012). On the other hand, by using serology there is a risk of cross-reactivity with other pathogens including other *Anaplasma* spp. (Visser *et al*., 1992; Fuente, *et al*., 2005), but also with *Ehrlichia* spp (Al-Adhami *et al*., 2011). Further validation of the ELISA is therefore needed, preferably including molecular diagnostics, such as PCR and RBL, which are able to detect low-level rickettsemia in carrier animals (Echaide *et al*., 1998; Carelli *et al*., 2007).

The microscopical prevalence of 30.8 (± 7.29) % corresponds to a 100 % increase compared to previous studies in Uganda (Table 1) (Magona & Mayende, 2001; Kabuusu *et al*., 2013) and provides evidence for endemic instability in the Kiruhura district. When a larger proportion of adult cattle are diagnosed microscopically they are in the acute phase and thus have not encountered the pathogens as calves (Alonso *et al*., 1992). The low sero-prevalence compared to previous studies (Kabi *et al*., 2008; Rubaire-Akiiki *et al*., 2004), could similarly be an indicator of endemic instability. A sero-prevalence < 30% indicates endemic instability (Rubaire-Akiiki *et al*., 2004; de Vos, 1992). Since none of the calves sampled and only 2 out of 16 heifers sampled were sero-positive in the present study, this further strengthens this idea. Many factors, such as tick challenge and presence of natural reservoirs (wild animals) induce endemic stability of anaplasmosis (Alonso *et al*., 1992). Still more research, about influencing factors on endemic stability and how this classification can be used to estimate the need of interventions, is needed.

The fact that the microscopical prevalence of *A. marginale* was significantly higher among the Ankole cattle compared to their crosses is consistent with recent findings by Matovu *et al*. (2014), where a higher hemoparasite-prevalence, including *Anaplasma* spp., was found among *B. indicus* compared to *B. taurus*. Matovu *et al*. (2014) likewise suggested that this indicates an endemic instability among *B. indicus* and further proposed this might be due to the management practices in rural communities. These management practices, including farmers offering medication without consulting a veterinarian, add to a growing drug resistance problem to TBDs. The animal keepers also wait with treatment of TBDs until the

conditions in the animals have deteriorated and thereby the animals may serve as reservoirs for a longer time. These management practices, together with a growing acaricide resistance among tick (SNV, 2013), contributes to an increasing loss of animals due to TBDs and a steadily increase of their prevalence within Uganda.

The method used to estimate tick burden in this study was not according to a standardized method, including scanning and collecting all ticks from one body side (Kaiser *et al*., 1982; Rubaire-Akiiki *et al*., 2004). This limits us in the interpretations of the results. Tick burden is influenced by many factors including seasonal changes (Wesonga *et al*., 2006), availablity of hosts (Norval, 1979), agro-ecological zones (Rubaire-Akiiki *et al*., 2004), grazing system (Rubaire-Akiiki *et al*., 2006) and use of acaricides, which all are factors that were beyond the scope of this study. However, the presence the biological vectors for *A. marginale*, *R. evertsi evertsi* or *R. decoloratus,* did not correlate to a higher prevalence for *A. marginale*. Tickburden was significantly higher among the Ankole cattle, which is contrary to previous reports of breed-related tick resistance among *B. indicus* (Mattioli *et al*., 2000; Wambura *et al*., 1998; Fivaz *et al*., 1992). This may indicate that Ankole cattle have become less resistant to ticks and consequently more exposed to TBDs including *A. marginale.* This observation was supported by the higher microscopical prevalence recorded in Ankole cattle than in crosses.

In conclusion, this study provides evidence that proximity to wildlife affect the sero-prevalence of *A. marginale* in cattle. There seems to be an endemic instability of anaplasmosis in Kiruhura district, due to a high microscopical prevalence and correspondingly low sero-prevalence. These results contribute to the fact that TBDs are a growing problem in Uganda (Matovu *et al*., 2014), including *Anaplasma* spp. and should serve as an indicator for possible future interventions such as immunization and information campaigns to farmers about therapeutical regiments for effective treatment of TBDs. Further research about drug resistance among various pathogens causing TBDs, acaricide resistance among ticks and breed-related susceptibility to *A. marginale* are encouraged.

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