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

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Wildlife-livestock interface at and around LMNP

Anna Schischke

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En tvärsnittsstudie för prevalensen av *Babesia bigemina* i Uganda

Kontakten mellan vilda djur och boskap runt LMNP

Anna Schischke

***Supervisor**: Prof. Johan Höglund, Department of Biomedical Sciences and Veterinary Public Health, Section of Parasitology*

***Assistant Supervisor**: Dr. Immaculate Nabukenya, Department of Biosecurity, Ecosystems and Veterinary Public Health, Section of Parasitology*

***Examiner**: Dr. Maja Malmberg, Department of Biomedical Sciences and Veterinary Public Health, Section of Virology*

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SUMMARY

Ticks and the diseases they transmit are of major importance throughout the world. In Uganda, cattle are the most important livestock from an economic point of view. Livestock keepers fear bi-directional transmission of tick-borne pathogens between their livestock and wild animals. This cross-sectional study was conducted to establish and compare the sero-prevalence of the tick-borne pathogen *Babesia bigemina* among randomly selected Ankole Long-horned cattle and European crossbred cattle on 30 farms in Kiruhura district, in two sub-counties near Lake Mburo National Park in South-western Uganda. Half of the farms were situated in close proximity to the park and thereby housed cattle with more frequent wildlife-livestock interface (Sanga), whereas the other half had less frequent contact (Kikatsi). The sero-prevalence was established by detection of *Babesia* antibodies using a commercial Indirect Enzyme Linked Immunosorbent Assay (ELISA), Svanova Biotech AB, Uppsala Sweden. Blood smears from the same animals were also examined by microscopy. A structured questionnaire was applied to all participants with related questions to this study and ticks were collected for tick-burden estimation and tick species identification. A total of 130 animals were sampled, 63 in Sanga and 67 in Kikatsi, respectively. Only one animal was detected as positive by microscopy. The overall sero-prevalence was 26.9 ± 7.63 % and comparison showed a significant difference ($P < 0.05$) between the sub-counties of Sanga (44 ± 12.26 %) and Kikatsi (10 ± 7.18 %). This indicated that the wildlife-livestock interface may have a role in the epidemiology of *B. bigemina,* even if previous studies suggest the opposite. Confounders, such as management system, breed of the animal or tick burden did not show a significant difference when comparing the sero-prevalence of *B. bigemina* to the two sub-counties Sanga and Kikatsi. The different results from the present and a previous studies and also that confounders did not affect the sero-prevalence implies that more studies are needed.

SAMMANFATTNING

Fästingar och sjukdomarna som de bär på finns över hela världen och utgör ett stort problem fram för allt i tropiska och subtropiska områden. Ur en ekonomisk synvinkel är nötkreatur det viktigaste boskapet i Uganda. Det oroar djurägare att vilda djur kan agera som reservoarer för fästingburna sjukdomar och därmed smitta deras djur. Denna tvärsnittsstudie genomfördes på 30 gårdar i Kiruhura distriktet i sydvästra Uganda. Syften var att fastställa seroprevalensen av den fästingburna parasiten *Babesia bigemina* hos slumpmässigt valda europeiska korsningar (Holstein Friesian * Ankole boskap) och den lokala Ankole boskapen. Hälften av gårdarna var lokaliserade i Sanga nära Lake Mburo National Park och djuren från dessa hade därmed mer frekvent kontakt med vilda djur än den andra halvan från Kikatsi. Seroprevalensen etablerades genom att påvisa *Babesia* antikroppar med hjälp av ett indirekt serologiskt ELISA test. Alla blodprover undersöktes även med mikroskopi. Alla medverkande i studien svarade på ett frågeformulär anknutet till studien. Fästingar plockades för uppskattning av fästingbördan samt artbestämdes. Totalt provtogs 130 nötkreatur, 63 i Sanga och 67 i Kikatsi. Endast ett djur påvisades som positivt med mikroskopi. Den totala seroprevalensen var 26.9 ± 7.63 % och vid en jämförelse visade det sig att det förelåg en signifikant skillnad ($P < 0.05$) mellan Sanga och Kikatsi, där fler av de som testade positivt provtogs i Sanga. Detta indikerar, till skillnad från tidigare studier, att frekvent kontakt mellan tamboskap och vilda djur kan spela en viktig roll i *B. bigeminas* epidemiologi. Andra påverkande faktorer såsom djurhållningssystem, ras eller fästingbördan visade ingen signifikant skillnad när dessa jämfördes mot seroprevalensen. De olika resultaten från denna och tidigare studie samt att andra påverkande faktorer ej påverkade seroprevalensen antyder att fler studier behövs.

CONTENT

INTRODUCTION

Tick-borne diseases and their importance

Ticks and tick-borne diseases (TBDs) are of major importance throughout the world but are most prevalent and exert their greatest impact in the tropical and sub-tropical regions (Norval *et al.*, 1992). In Uganda, cattle are, from an economic point of view, the most important livestock (Uganda Investment Authority, 2009). Ticks are considered to be the most important vector of disease-causing pathogens both in wild and domestic animals (Antunes *et al.*, 2012). Thus, TBDs constitute one of the major constraints to cattle productivity and are thus of major importance (Kiara *et al.*, 2014; Perry and Young 1995).

B. bigemina is a protozoan parasite and is one of the most important TBDs in eastern and central Africa (Uilenberg 1995). This parasite is widely distributed in Africa, Asia, Australia and central and south America (Bock *et al.*, 2004). The principal vectors are *Rhipicephalus microplus* and *R. decoloratus* which are widespread in the tropics and subtropics (Magona *et al.*, 2008). In Uganda the most common vector for *B. bigemina* is *R. decoloratus* (Okello-Onen *et al.*, 1998).

In a previous study where the effect of wildlife proximity was investigated close to Queen Elizabeth National Park in Uganda, no significant variation in the prevalence of *Babesia bigemina* was found with closeness to wildlife-livestock interface ($P = 0.2$). There was however, a significant increase in the prevalence of *Theileria parva* and *Anaplasma marginale* in cattle living in close proximity to wildlife ($P < 0.01$) (Kabuusu *et al.*, 2013). The study was based on microscopy and recommended that the same question also should be investigated using serology as a diagnostic tool.

Aim of the study

The purpose of this study was to identify if there was an increased risk in prevalence of *B. bigemina* with increasing wildlife-livestock interface. We tested the hypothesis that the prevalence is higher in cattle from an area with more frequent contact with wildlife as compared to cattle from an area with less frequent contact with wildlife. The prevalence was determined by using an Enzyme Linked Immunosorbent Assay (ELISA) for *B. bigemina*, Svanova Biotech AB, Uppsala, Sweden (Katende *et al.*, 1998) and also by microscopical investigation of haemoparasites in thin Giemsa stained blood smears. Furthermore, tick species and burden was estimated as well as a structured questionnaire was administered to all participants. This study constitutes my Master thesis on the veterinary program, SLU, Uppsala, Sweden and the field work was carried out during a Minor Field Study.

LITERATURE REVIEW

The importance of livestock in Uganda

The national livestock census estimated the number of cattle in Uganda at 11.4 million in 2008 and having almost doubled since 2006 when there were 6.3 million cattle (MAAIF, 2009). Cattle are the most important livestock considered from its economic value. Most of the cattle are indigenous cattle breeds (93.6 %), whereof 29.6 % are Ankole Long-horned

cattle and 70.4 % are Zebus/Nganda. Of the remaining parts 5.6 % are Holstein Friesian dairy cross-breeds and 0.8 % European cross-breeds used for beef production (UIA, 2009). The indigenous cattle in Uganda are predominantly kept under communal grazing management and are thereby exposed to continuous tick challenge and tick-borne diseases (Magona *et al*., 2000; Okiria *et al.*, 2002). A dairy development project in Uganda led to favoring of European cattle (*Bos taurus*) (Holstein Friesian) over indigenous breeds (e.g. Ankole Long-horned cattle and Nkedi Zebu). Despite a low dairy production potential of indigenous breeds (*Bos indicus*), important undiscovered traits of the Nkedi Zebu and Ankole Long-horned cattle in response to different diseases have been documented (Magona *et al.*, 2011). Mugisha *et al.* (2007) investigated different intra-household dynamics and how these influenced decision-making in vector-borne disease control in the pastoralist system in South-western Uganda. It was evident that cattle health care was priority expenditure. In terms of scoring and ranking, more than twice of the budget was allocated to cattle health care than to human medical care. This demonstrates how important cattle are to livestock keepers.

Wildlife-livestock interface

Conflicts between livestock keepers and wildlife conservation authorities characterize the wildlife-livestock interfaces, and relates to the bi-directional transmission and prevention of diseases common to both domesticated animals and wildlife (Bengis *et al.*, 2002). Wildlife, especially Cape buffalo (*Syncerus caffer*), are thought to act as reservoir for many of the most important tick-borne pathogens infecting also cattle. Cattle and wildlife in East Africa are exposed to a wide range of TBDs, including babesiosis (Oura *et al.*, 2011b).

Lake Mburo National Park (LMNP) is located in Kiruhura district in South-western Uganda. The park is known for a high level of wildlife-livestock interaction. In the ranch and pastoral areas adjacent to the park, a large number of wild ungulates, mainly impala (*Aepyceros melampus*) and zebra (*Equus quagga*), regularly graze and browse together with the domestic livestock. In this wildlife-domestic interface, there have been fears of bi-directional disease transmission. The ranchers have complained that TBDs may be transmitted between wildlife and livestock. Several studies have therefore been conducted in this and other wildlife-livestock interface situations in Lake Mburo to investigate the scope of disease problems and its prevalence's (Ocaido *et al.*, 2009a; b). Development of serological and molecular diagnostic methods, such as ELISA, reverse line blotting (RLB) and indirect fluorescent antibody test (IFAT), have allowed us to investigate an important question in the matter that wildlife act as reservoirs of infection for co-grazed cattle (Morzaria *et al.*, 1999).

According to Kabuusu *et al.* (2013), proximity between wildlife-livestock does not explain the variation in prevalence of *B. bigemina* in cattle. This conclusion was made after a cross-sectional study in Queen Elizabeth National Park, comparing the prevalence as it was done in this study, but using only microscopy as a diagnostic tool. That study recommended further research using serological analyzing methods.

Several studies have concluded that the management system is an important parameter to decrease risk of infection by means of restricting wildlife interface. Restricted grazing and zero grazing were among the most important factors to decrease infection with TBDs

(Muhanguzi *et al.*, 2010). In another study by Rubaire-Akiiki *et al.* (2006), a ten times higher risk of sero-conversion for *T. parva* was seen, compared to animals managed by zero grazing.

Tick-borne diseases

Throughout the world, there are ticks and tick-borne pathogens, but these are most prevalent and exert their greatest impact in tropical and sub-tropical regions. Ticks do not only act as vectors for pathogens, the infection itself may also cause direct problems with milk production, weight loss and predispose animals to other bacterial and fungal infections (Jongejan and Uilenberg 2005; Norval *et al.*, 1992). The full impact of TBDs in general has not been accurately and comprehensively quantified, but it is believed that they cause enormous losses through mortality, morbidity, productive losses and the cost of control (de Castro 1997; Kiara *et al.*, 2014). TBDs are the major cause of cattle mortality and morbidity and present a major economic burden to communities and acaricides must be used to target ticks and prevent transmission across East Africa (Muhanguzi *et al.*, 2014). Cattle and wildlife in East Africa are exposed to a range of tick-borne pathogens of the genera *Theileria, Ehrlichia, Anaplasma* and *Babesia* (Oura *et al.*, 2011b) and of the death attributable to TBDs in Uganda, babesiosis is responsible for 4.4 % (Magona *et al.*, 2004).

Ticks in Uganda

R. decoloratus (Figure 1) (formerly *Boophilus*) is the most common and widespread one-host cattle tick in Uganda. It transmits several pathogens including *B. bigemina*. It is often called the blue tick due to the color of the engorged female but is also characterized by its short mouthparts and legs. Males of this species are rarely seen as they are minute in size (Walker *et al.*, 2003). It can be difficult to distinguish from *R. microplus*, but the latter species is not as common in Uganda (Okello-Onen *et al.*, 1998). In a study by Rubaire-Akiiki *et al*. (2006), the distribution of ticks varied depending on the altitude and a relation to agro-ecological zones (AEZ). *R. decoloratus* was the most common tick in the upland AEZ (1575-4368 m). In the lowland AEZ (altitude of 428-1275 m), *R. appendiculatus* was the most abundant tick. It is known as the brown ear tick, because of its color and for having the ear as one of its main predilection sites. *R. appendiculatus* vectors *T. parva* causing East Coast Fever in cattle, which is a widely distributed disease in Uganda (Kivaria *et al.*, 2004).

R. evertsi evertsi (Figure 1) is also known as the red-legged tick, due to the prominent red color of the appendages. Another prominent feature is pronouncing convex dark eyes. *R. evertsi evertsi* is widely distributed and commonly found on livestock throughout Africa, but is not as widespread in Uganda as the other ticks. In Uganda it is most frequent in the South-western parts of the country (Walker *et al.*, 2003).

One of the most common and widespread ticks on livestock in Africa is *Amblyomma variegatum*. This tick is the most predominant vector of heartwater caused by *Ehrlichia ruminantium,* which is widely distributed through Sub-Saharan Africa. *A. variegatum* can be found almost everywhere in Uganda. It is easily recognized by a very colorful pattern on the scutum, striped legs and characteristic long mouthparts (Walker *et al.*, 2003).

In previous studies where tick challenge was estimated, adult ticks were counted from one side of the body of each individual animal sampled (Kaiser *et al.*, 1982; Magona *et al.*, 2011; Rubaire-Akiiki *et al.*, 2004).

Figure 1. *Ticks under stereo microscope. R. decoloratus, female. to the left. R. evertsi evertsi, male to the right. Photo: Anna Schischke.*

Tick control

Ticks are one of the most important vectors of different livestock pathogens (Ghosh *et al.*, 2006) and cause huge economic losses (Rajput *et al.*, 2006). Actions need to be taken to control tick infestations, whereas chemical acaricides have traditionally played an essential role (Rodríguez-Vivas *et al.*, 2014). European cattle breeds (*Bos taurus*) were observed to carry up to 2.5 times more ticks than local *Bos indicus* crosses under natural conditions (Seifert, 1971). Indigenous African breeds (*Bos taurus africanus*) have also been shown to be more resistant to ticks than imported and local crossbred cattle (Fivaz *et al.*, 1992).

Acaricides

In areas where ticks are endemic, cattle develop natural immunity, which is promising for genetic tick control strategies to reduce the use of acaricides (FAO, 2004). However, the major method of tick control is currently the use of chemical acaricides (Antunes *et al.*, 2014). In South-western Uganda, cattle keepers mainly use spraying as a main control strategy. According to Mugisha *et al.* (2007) most farmers applied acaricides on their livestock once weekly but used the drugs in a lower dose than recommended by the manufacturer**.** In a study by Okello-Onen *et al.* (2003) the impact of tick control on the productivity was investigated and showed that dipping twice weekly increased the milk production by 21 % and also prolonged the duration of lactation. Use of acaricides also increased the pre-weaning growth rate by 39 %, but had no significant effect on the post-weaning growth rate. However, these substances are toxic chemicals, and may leave residues in meat, milk and also cause environmental pollution. Another problem is that acaricide resistance will develop faster if the use is incorrect and poses a threat to livestock production (Antunes *et al.*, 2014; Florin-Christensen *et al.*, 2014; Jongejan and Uilenberg 2005). Thus continued acaricide use is unsustainable and in addition, prolonged exposure of acaricides to indigenous cattle may also reduce the level of resistance to ticks and endemic stability to TBDs (Okello-Onen *et al.*, 1998). There is a growing concern on acaricide resistance and research efforts are devoted to the design of anti-tick vaccines and antiprotozoal drugs (Florin-Christensen *et al.*, 2014). Many studies investigate the use and evaluate different vaccines, but this subject is beyond the scope of this study.

Babesia bigemina

B. bigemina is a protozoan parasite within the genus *Babesia*, phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, suborder Piroplasmorina, family Babesiidae. The parasite is widely distributed on the southern hemisphere in Africa, Asia, Australia and Central and South America where it is of major importance (Bock *et al.*, 2004; Geleta 2005; Uilenberg 1995). It was described in 1888 by Victor Babes, who correlated the presence of an intra-erythrocytic microorganism with the appearance of haemoglobinuria in cattle (Babes, 1888). The genus later received the name *Babesia* and today incorporates more than 100 different species (Florin-Christensen *et al.*, 2014). It is one of the most important TBDs in eastern and central Africa (Uilenberg 1995) and from an economic point of view considered to be the most important arthropod-transmitted disease in cattle (Bock *et al.*, 2004). According to Uilenberg (1995) the financial costs of babesiosis depends on regional factors, such as the type of livestock, the availability and cost-effect ratio of control measures. The costs in general are connected to the decrease in milk and meat production, abortions, mortalities and loss of productive potential of endemic areas.

Vectors

The distribution of *B. bigemina* is restricted by the distribution of suitable vectors. It has been proposed that any existing vertebrate species may turn into a *Babesia* carrier host as long as there is a transmitting tick vector available (Florin-Christensen *et al.*, 2014). The main vectors of *Babesia* are ticks in the genus *Boophilus*, which recently have been reclassified as *Rhipicephalus,* sub-genus *Boophilus*. The principal vectors are *R. microplus* and *R. decoloratus,* which are widespread in the tropics and subtropics. *B. bigemina* is also documented to be transmitted by *R. annulatus, R. geigyi* and *R. evertsi evertsi,* making it the most widespread bovine *Babesia* species (Bock *et al.*, 2004; Florin-Christensen *et al*., 2014; Uilenberg 1995).

Life cycle

Infected tick bites and attaches to a host and then transfer infectious sporozoites in tick saliva, which invade the erythrocytes of the host (Florin-Christensen *et al.*, 2014). By binary fission the sporozoites transforms into two merozoites causing lysis of the erythrocyte (asexual reproduction). Each merozoite then invades a new erythrocyte and thereby successfully completes the process called merogony (Suarez and Noh, 2011). When babesia-infected erythrocytes are ingested by ticks, most of the parasites degenerate and are destroyed. However, a specific stage of the parasite occurring after becoming merozoites, called pre-gametocytes, survive and undergo further development to devolve into gametocytes inside the tick. The gametocytes elongate in the midgut of the tick and form so called “ray bodies”. The gametes fuse in the lumen of the digestive tract of the tick to form an elongated zygote, which facilitates cell penetration. The zygote with its arrowhead touches the midgut cell membrane which then invaginates the zygote. Once the zygote has been internalized, it transforms into a motile kinete, which escape the midgut and invades the tick’s body tissue. In the female, the zygote invades the ovaries, resulting in many *Babesia*-infected eggs (Jonsson *et al.*, 2008). This step is called transovarial transmission. The larvae are infected with kinetes and sporogony takes place in all development stages and thus being able to infect a host with

infective sporozoites in all stages. The fact that *Babesia* persist in all tick stages, means that both the tick and the host act as reservoirs and thereby facilitate a long-term persistence in the ecosystem (Chauvin *et al.*, 2009). Transstadial transmission occurs, meaning that the parasite follows the tick even when the tick evolves from larvae to nymph or from nymph to adult tick. Even when the ticks feed on non-susceptible hosts, *B. bigemina* can pass from one generation to the next (Bock *et al.*, 2004). This is a very important adaptation in the life cycle as the vectors are one-host ticks, meaning that it only feeds on one host throughout all three life stages (Bock *et al.*, 2006; Chauvin *et al.*, 2009; Florin-Christensen and Schnittger 2009; Florin-Christensen *et al.*, 2014).

Pathophysiology and clinical signs of babesiosis

The percentage of infected erythrocytes in circulating blood often exceeds 10 % and may in case of *B. bigemina* be as high as 30 % in the acute phase of the infection (OIE 2010; Magona *et al.*, 2008). The major clinical signs include fever, anaemia, anorexia, lethargy and haemoglobinuria (Bock *et al.*, 2004, 2006; Brown and Palmer 1999). The pathogeneses is almost entirely related to rapid and sometimes massive intravascular haemolysis. Haemoglobinuria is present early in the process and fever is less frequent compared to in *B. bovis.* However in some cases the disease can develop suddenly and severe anaemia, jaundice and death may occur with little warning (Bock *et al.*, 2004).

Diagnosis of babesiosis

A tentative diagnosis is made on clinical signs as previously mentioned. To confirm the diagnosis, blood and/or organ smears stained with Giemsa can be examined for search of intraerythrocytic parasites (Böse *et al.*, 1995). Sero-prevalence however is not used in clinical stages but is an important tool in research purpose and epidemiological studies (Bock *et al.*, 2004; Böse *et al.*, 1995) as well as molecular diagnostics (Gubbels *et al.*, 1999).

Inverse age resistance

The severity of clinical signs as well as the speed of recovery and mortality rates are inversely related to the age of the host (Florin-Christensen *et al.*, 2014). It is believed that calves are initially protected by means of the passive immunity from maternal antibodies received through colostrum. However, maternal antibodies to babesiosis disappear after the age of 9-12 months (Jongejan *et al.*, 1988; Zintl *et al.*, 2005). Inverse age resistance is not only due to passive immunity conferred by maternal antibodies. It has been observed that the resistance in calves is irrespective of immune status of their mother and also that calves remain resistant longer than passively transferred antibodies persist (Magona *et al.*, 2008). The levels of parasitaemia and anaemia in calves are lower compared to adults and the fever is milder and haematocrit recovers more rapidly. The protection from babesiosis is abrogated by removal of the spleen, indicating an involvement of cellular mechanisms (Löhr 1973). Inverse age resistance is unusual thus most other infectious diseases affect juveniles more severely than adults and yet little is known about the mechanisms that control innate immunity against *B. bigemina* and further studies are needed to identify the background to inverse age resistance (Zintl *et al.*, 2005).

When cattle were infected at the age of 5 – 7 months and kept under tick-free conditions, they became carriers of *B. bigemina* for two years, but remained immune for four years (Mahoney *et al.*, 1973).

Endemic stability to bovine babesiosis

The principle of endemic stability is that when the inoculation rate of *Babesia*, from tick to cattle, is sufficiently high to infect all calves while they are still protected by innate immunity, clinical disease will be minimal. Conversely, if the inoculation rate in calves is too low, endemic instability and clinical cases will result (Mahoney & Ross 1972). Resistance of young animals to the disease is the basis of endemic stability (Zintl *et al.*, 2005), defined as the state where host, agent, vector and environment live in such relationship that clinical cases rarely occur or not at all (Bock *et al.*, 2004). Norval *et al.* (1983) defined endemic stability as follow:

- Endemic stable area (81-100 % sero-positivity)
- Approaching endemic stability (61-80 % sero-positivity)
- Endemic instable area (21-60 % sero-positivity)
- Minimal disease situation (1-20 % sero-positivity)
- Disease-free area (0 % sero-positivity)

B. bigemina in wildlife

Many studies have investigated the question whether wild animals act as a reservoir for *B. bigemina*, but with contradicting results (Berggoetz *et al.*, 2014; Kabuusu *et al.*, 2013; Oura *et al.*, 2011b).

In South Africa, *B. bigemina* was detected in cattle, impala and for the first time in greater kudu (*Tragelaphus strepsiceros*) using RLB and it was suggested that transmission of tick-borne pathogen species remain mainly restricted to genetically related host species, except for impalas which may represent a bridge species between several transmission routes (Berggoetz *et al.*, 2014).

B. bigemina is also found in Cape buffalo but clinical cases are rarely seen due to long co-evolutionary adaptation with *Babesia* parasites (Bock *et al.*, 2004; Uilenberg 1995). However, when buffaloes were investigated by RLB in four national parks in Uganda (Lake Mburo, Queen Elizabeth, Murchison Falls and Kidepo Valley), none of the sampled buffaloes were carriers of *B. bigemina*, even when *R. microplus* was present. This indicates absence of *B. bigemina* in these areas (Oura *et al.*, 2011a). In another study the prevalence of tick-borne haemoparasites in cattle as well as wildlife such as buffalo, impala, eland (*Taurotragus oryx*) and bushbuck (*Tragelaphus scriptus*), grazing inside and neighboring LMNP in Uganda was investigated with RLB. The results showed that neither cattle nor wildlife hosts were carriers for *B. bigemina*. The only findings related to *Babesia* were that all 12 impala were strongly positive in the RLB assay with the *Theileria/Babesia* catch-all probe. However, none of the individual species were positive (e.g. *B. bigemina* or *T. parva*), indicating that these must represent other uncharacterized species (Oura *et al.*, 2011b).

Breed differences

There are differences in susceptibility to babesiosis caused by *B. bigemina* between indigenous and European cattle (Jongejan & Uilenberg 2005), suggesting that local cattle in babesia-endemic regions have a certain degree of natural resistance. The consequences of infection are not as serious as when European breeds are affected (Bock *et al.*, 2004). In Australia, there are ten times more outbreaks of *B. bigemina* in European *Bos taurus* than in local *Bos indicus* cattle. It has also been reported that indigenous cattle and, to a lesser extent, crossbred cattle are more resistant to *B. bigemina* than European cattle (Bock *et al.*, 1999). However, when the *B. bigemina* challenge is mild, the differences in between breeds are not obviously seen (Bock *et al.*, 1997).

Differences in susceptibility to *B. bigemina* have also been demonstrated between indigenous Ugandian cattle such as Nkedi Zebu and Ankole Long-horned cattle. In a cross-sectional study in the Soroti district in Uganda, Nkedi Zebu had higher antibody levels against *B. bigemina* than the Ankole Long-horned cattle, suggesting that the Nkedi Zebu are capable of mounting a significantly stronger immunological response against *B. bigemina* infection than the Ankole Long-horned cattle. Generally, both breeds had similar antibody profiles which increased with age, but the Nkedi Zebu showed a higher degree of resistance, due to the higher antibody levels detected in the study (Magona *et al.*, 2011).

However, in a similar cross-sectional study by Kabi *et al.* (2008), there was no significant difference in sero-prevalence between these two breeds for *B. bigemina* in the same district (100 % sero-positivity respectively). The high sero-prevalence implies that tick challenge of TBDs to both breeds was similar in the Soroti district. Communal grazing and the climatic conditions in this area favors the rapid multiplication of ticks, which leads to high tick burdens on cattle and consequently higher occurrence of the diseases they transmit (Rubaire-Akiiki *et al.*, 2004).

In a study by Oura *et al.* (2004), none of the tested crossbred cattle (n = 46) were positive for *B. bigemina* and only one (2.3 %) of the indigenous cattle (n = 44), was positive by RLB in central Uganda. In another study in Western Uganda, only 12.5 % of a total of 930 calves were found to have antibodies to *B. bigemina* (Okello-Onen *et al.*, 1998). In another study made to compare the antibody titres and sero-prevalences of TBDs in Nkedi Zebu and the Ankole Long-horned cattle, no difference could be seen regarding *B. bigemina* (Kabi *et al.*, 2008).

Diagnostic methods

Sensitive and specific diagnostic tests for tick-borne pathogens are necessary for correct identification of the pathogen so that appropriate therapy can be given.

Microscopy

The traditional method of identifying blood parasites is by microscopic examination of thick and thin blood smears stained with Giemsa. The technique is widely used for the diagnosis of babesiosis, but the sensitivity of this technique is low (Buling *et al.*, 2007; Oura *et al.*, 2004). Parasitaemias as low as 1 parasite in 10^6 red blood cells can be detected on thick blood films

but species differentiation is better performed from thin smears. Microscopy is good for detection of acute infections, to confirm clinical cases (Zintl *et al.*, 2003), but not for detecting carriers where parasitaemia usually is low (OIE 2010).

Serology

Enzyme-linked immunosorbent assay (ELISA) is one of the most widely used serological tests because of its superior sensitivity and ease of use (Tebele *et al.*, 2000). The advantage of ELISA over indirect fluorescent antibody test (IFAT) is that interpretation of results is less subjective and it is easily automated for a large number of samples (Zintl *et al.*, 2003).

The SVANOVIR *B. bigemina*-Ab indirect-ELISA (I-ELISA) is an improved serological assay based on a standardized recombinant immunodominant antigen, developed by International Livestock Research Institute (ILRI) in Nairobi, Kenya (Tebele 1996, Tebele *et al.*, 2000). Tebele (1996) estimated the sensitivity and specificity at 96 % and 97.5 % respectively. In the same study, it was also shown that this ELISA test has a high agreement with IFAT (SVANOVA).

With an I-ELISA antibodies are detected in serum by adding the serum into capture antigen coated wells. Specific antibodies will bind and unbound antibodies will be washed away with a buffer solution. A secondary anti-antibody antibody is added and that in turn will bind to the primary specific antibody. The secondary antibody has an enzyme attached that after addition of a substrate it will change color indicating the presence of primary antibodies. The higher the concentration of the primary antibody, the stronger the color change will be, and this absorbance can be measured in a micro plate reader giving an optical density (SVANOVA).

Indirect fluorescent antibody test

Another common serological antibody assay for *B. bigemina*, is the IFAT. It is suitable for epidemiological surveys both to distinguish between *Babesia* species and to demonstrate the presence of *Babesia* antibodies in a population (Zintl *et al.*, 2003). An unlabelled antibody attaches to the parasites antigen and a conjugate is added with a fluorescein-labelled antibody.

Molecular diagnostics

Reverse line blot

Reverse line blot (RLB) assay is a molecular diagnostic tool and can simultaneously detect and differentiate between all known *Theileria* and *Babesia* species (Gubbels *et al.*, 1999). It has been applied in field studies in Uganda and has shown to be a useful tool to establish and monitor the prevalence of TBDs (Oura *et al.*, 2004). Molecular diagnostics were not used in this study and are thus not further discussed.

MATERIALS AND METHODS

Study design

The study was conducted in Kiruhura district, due to its proximity to Lake Mburo National Park (Figure 2). Out of 12 sub-counties, Sanga (animals grazing close to the park) and Kikatsi (animals without frequent contact with wildlife) were selected. Inclusion criteria of the farms were the locations in either of the two sub-counties; availability of at least four cattle in the

herd and consent from the farmer to participate. With this criterion, the District Veterinary Office in the sub-counties selected the farms and the sampled animals were selected using a simple random sampling method.

Figure 2. *Map of Uganda, showing Kiruhura district in black and Lake Mburo National Park circled in red. (Modified from Google maps).*

Sample size

A total number of 30 farms were selected, 15 farms in each of the sub-counties. Sanga is located close to the park and cattle graze with wildlife very frequently. The number of 30 farms is based on the epidemiological consideration that a large sample size ($\geq$ 30) and sampling distribution for the sampling mean most likely gives a normal distribution.

A *B. bigemina* prevalence of 6.7 % (rounded up to 7 %) from a recent published study done in a nearby area was used to determine the individual sample size at 95 % level of confidence using the equation (Dohoo *et al.*, 2003).

$$n = \frac{Z\alpha^2 PQ}{\Delta^2}$$

Where:

n	Calculated sample size
Zα	Standard normal deviation at 95 % confidence interval, corresponding to 1.96
P	Prevalence of *B. bigemina* based on 6.7 % by (Matovu *et al.*, 2014)
Q	(1-P) the probability of not being the true prevalence
Δ	95 % Confidence level = 0.05

Calculated sample size:

$$n = \frac{1.96^2 * 0.07(1-0.07)}{0.05^2} = 100$$

A large sample size gives a higher power and thus likelihood to detect a difference.

Field sampling

Blood was collected from the *vena coccygea* or *vena jugularis* from a total of 130 cattle with a closed vacutainer system (BDH, UK). About 8 ml of blood was collected in plain vacutainers and about 4 ml in EDTA coated vacutainer tubes and then stored in iceboxes at ≈ 4ºC. The samples were transported to the laboratory at Makerere University, Kampala, where blood smears were prepared and serum separated from clotted blood by centrifugation at 5000 rpm for 5 minutes and later stored in the freezer at -20ºC until measurement of antibody levels was performed. Ticks (5 – 6 per animal) were collected mainly from ears, rump and udder from all cattle where ticks could be found, and put into eppendorf tubes. The ticks were collected by a simple random sampling method. On all animals the breed, age and sex was recorded, as well as the number of ticks. At each farm a structured questionnaire with a majority of close-ended questions was administered to the farmer or farm manager with questions related to this study.

In the Central Diagnostic laboratory at Makerere University, the tick species, sex and state of feeding was determined as previously described (Walker *et al.*, 2003).

Analysis

Enzyme-linked immunosorbent assay

Serological analysis was carried out using the indirect antibody detection ELISA kits (Svanova Biotech AB, Uppsala, Sweden) using a recombinant immunodominant antigen for *B. bigemina* as the capture antigen (Tebele *et al.*, 2000). Reagents were equilibrated to room temperature before use. Samples were diluted 1:100 using a PBS-tween buffer. The negative and positive controls were diluted according to manufacturers manual and loaded in duplicate on each micro-titer plate as well as the pre-diluted serum samples. All the steps were followed according to manufacturer's instruction. The absorbance was measured at 405 nm using BioTek EL-800 micro plate reader and Gen-5 software. Optical density (OD) values were obtained from the readings and transferred to a Microsoft Excel spread-sheet. Percent sero-positivity (PP) was calculated and cut-off values were used according to manufacturer's instructions and samples were denoted as positive or negative. Only when both duplicates gave a PP ≥ 40 an animal was considered as positive.

$$PP = \frac{MeanOD_{Negative\ controls}}{MeanOD_{Positive\ controls}} * 100$$

Cut-off PP-values:

- PP ≤ 25 = Negative
- PP 26-39 = Doubtful
- PP ≥ 40 = Positive

Microscopy

One thin blood smear was prepared for each sample, using blood from the EDTA vacutainer tubes. The slides were stained with Giemsa for examination of haemoparasites. *B. bigemina* is a large piroplasm, meaning its length can reach almost the full diameter of an erythrocyte (Bock *et al.*, 2004). It is characteristically pear shaped and lies in pairs forming an acute angle in the red blood cell (Bock *et al.*, 2006). Round, oval or irregular shaped forms may occur depending on the stage of the development of the parasite in the red blood cells. Microscopic examination was assisted by technicians at Central Diagnostic laboratory and Molecular Science laboratory at Makerere University, Kampala.

Statistical analyses

Data entry from questionnaires and laboratory examinations was done in Microsoft Excel version 2007 and analyzed using SPSS statistics version 17.0 (IBM SPSS software), at a confidence level of 95 % and a significant level of $\alpha = 0.05$. Chi-square test was used to evaluate significant differences in sero-prevalence between cattle from Sanga and Kikatsi sub-counties. When data was unbalanced and one value in the contingency table was less than five, Fisher's exact test was used.

Tick identification

All collected ticks were examined under the stereo microscope. Species, sex and state of feeding were determined. Due to a limited time frame tick collection could not be done as in previous studies (Kaiser *et al.*, 1982; Rubaire-Akiiki *et al.*, 2004), instead a goal of six ticks per animal was set and this target compliance was feasible. Tick identification was done according to the descriptions by Walker *et al.* (2003).

RESULTS

Clinical observations

One animal sampled had symptoms of babesiosis, showing signs with severe haemoglobinuria (Figure 3) since one week, fever (40.2ºC) and lethargy. Another animal showed clinical symptom for theileriosis with enlarged lymph nodes and fever. All the remaining 128 animals collected showed no clinical symptoms of any disease.

Figure 3. *Haemoglobinuria collected from a sick cow in the sub-county of Sanga. Photo: Dr. Dickson S. Tayebwa.*

Farm characteristics

A total of 130 cattle were sampled from 30 farms whereof 63 animals in Sanga (48.5 %) and 67 animals in Kikatsi (51.5 %). In the sub-county of Sanga, all 15 farmers (100 %) stated that their animals interacted with wildlife every day in contrast to Kikatsi where two farms (13.3 %) stated that interface occurred once weekly, eight farms (53.3 %) stated once a month and five farms (33.3 %) never observed any interaction (Table 1). The major wildlife species interacting with cattle was zebra (50 %) whereas each of the remaining five farms (16.7 %) stated contact with buffalo, impala or no interaction.

Table 1. *Wildlife-livestock interface between the sub-counties of Sanga and Kikatsi*

	Wildlife-livestock interaction			
Sub-county	Every day (%)	Once a week (%)	Once a month (%)	Never (%)
Sanga	100 %	0 %	0 %	0 %
Kikatsi	0 %	13.3 %	53.3 %	33.3 %

Of all cattle sampled, 49 animals were Ankole Long-horned cattle (37.7 %) and 81 (62.3 %) were European crossbreds (crosses between Ankole Long-horned cattle and European breeds). Only five animals under one year of age were examined. Most of the animal keepers (46.7 %) had small animal households with < 30 animals. Nine farms (30 %) were medium sized (31-90 animals) and seven (23.3 %) were large farms (> 91 cattle). The main management system used was communal grazing on 26 farms (86.7 %), meaning animals are supervised by a pastoralist leading them to good grazing areas and water holes during daytime. The remaining four farms (13.3 %) used a fenced system with clear paddocks where the animals were grazed and accessed water. The educational level among the participants in the questionnaire was in general low. In total 14 farmers (46.7 %) had no education, whereas 12 farmers (40 %) had finished primary school and four (13.3 %) secondary school.

Prevalence of *B. bigemina*

The total prevalence of *B. bigemina* was calculated by the results of the indirect ELISA analysis. A total of 35 animals out of the 130 sampled animals were tested positive, giving a sero-prevalence of 26.9 ± 7.63 %. A significant difference was detected ($\chi^2 = 19.1$, $P < 0.01$) when comparing the sero-prevalence between the two sub-counties (Figure 4). No significant ($\chi^2 = 0.08$, $P = 0.8$) difference was found when comparing the sero-prevalence and the management system. Out of the 26 farms (87 %) using communal grazing system, 15 farms tested positive and 11 tested negative and out of the four farms (13 %) using a fenced system two tested positive and negative respectively.

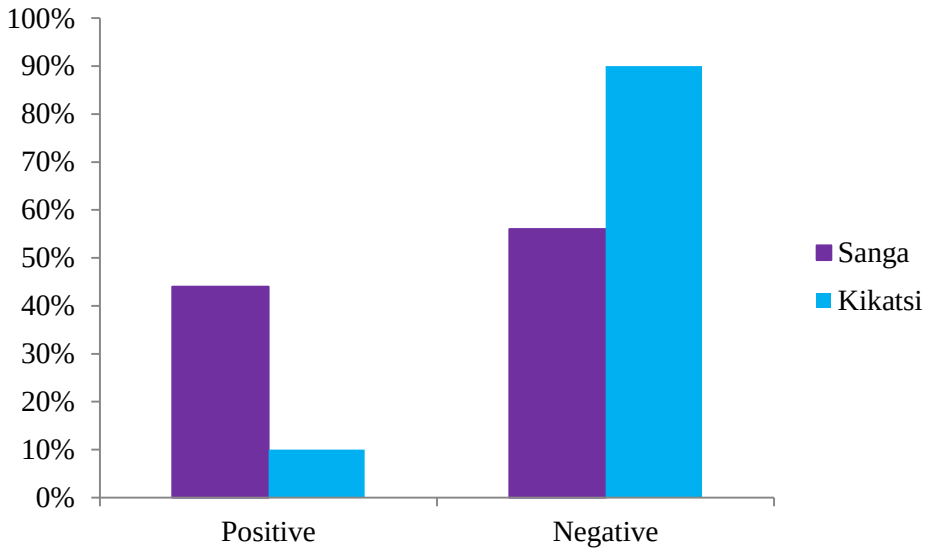

Figure 4. *Prevalence of B. bigemina, showing a higher prevalence in Sanga, where frequent wildlife-livestock interface is seen.*

No significant ($\chi^2 = 0.54$, $P = 0.46$) difference could be seen comparing the sero-prevalence between the two breeds. Of all Ankole Long-horned cattle (n = 49), 15 animals were sero-positive (30.6 % ± 12.95) and of all European crossbred cattle (n = 81), 20 cattle were sero-positive (24.7 % ± 9.43). However, a significant ($\chi^2 = 23$, $P < 0.001$) difference could be seen comparing the two sub-counties to the two breeds. Out of all Ankole Long-horned cattle (n = 49), 76 % were in Sanga and of all Europeans crossbred cattle (n = 81), 68 % were in Kikatsi.

Microscopy

There was only one slide detected as positive with *B. bigemina* (Figure 5). It was the animal showing clinical symptoms for babesiosis.

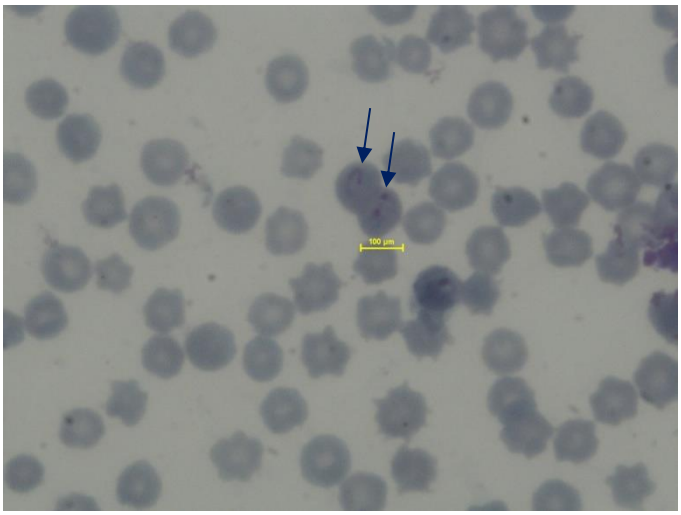

Figure 5. *Babesia bigemina under light microscope, blue arrows showing two infected erythrocytes. Photo: Dr. Dickson S. Tayebwa.*

Constraints to livestock production

In this study, 24 out of 30 farms (80 %), found ticks and TBDs to be the major constraint to livestock production. Other constraints considered were draught, other animal diseases, acaricide or drug failure and limited access to veterinary services. Most of the farms (n = 22, 73.3 %) did not have any confirmed cases of babesiosis during the last year, seven farms had 1-5 cases (23.3 %) and one farm had more than five cases (3.3 %).

Tick sampling and identification

In 43 animals the goal to collect six or more ticks was achieved (33.1 %). In 51 animals less than six ticks were sampled (39.2 %) and in the remaining 36 no ticks could be found (27.7 %). There was a significant ($\chi^2 = 32.1$, $P < 0.001$) difference in tick burdens between sub-counties. Out of the 36 animals where no ticks could be found, 32 were from Kikatsi (89 %) and out of the 43 animals where six or more ticks could be found, 32 were from Sanga (74 %). *R. appendiculatus* was present in 78 cattle (60 %), *R. decoloratus* (Figure 6) in 19 (14.6 %), *R. evertsi evertsi* in 22 animal (16.9 %) and *A. variegatum* in six animals (4.6 %).

There was a significant ($P < 0.001$) difference in where the different tick species were most prevalent with an exception for *A. variegatum* (Table 2). *R. appendiculatus* was present in 59 (94 %) of the animals in Sanga, compared to 19 (28 %) in Kikatsi. *R. evertsi evertsi* was also more common in Sanga where 19 (30 %) were infected with this species, whereas it was only found in 3 (4 %) of the animals in Kikatsi. In contrast, cattle found infected by *R. decoloratus* were more common in Kikatsi (25 %) compared to Sanga (3 %).

Table 2. *Comparison of the prevalence of tick species between the sub-counties of Sanga and Kikatsi*

	Sanga (63)	%	Kikatsi (67)	%	Chi-square	*P*-value
R. appendiculatus						
Present	59	94 %	19	28 %	57.7	0.000*
Not present	4	6 %	48	72 %		
R. evertsi evertsi						
Present	19	30 %	3	4 %	15.2	0.000*
Not present	44	70 %	64	96 %		
R. decoloratus						
Present	2	3 %	17	25 %	12.8	0.000*
Not present	61	97 %	50	75 %		
A. variegatum						
Present	5	8 %	1	1.5 %		0.107**
Not present	58	92 %	66	98.5 %		

* Significant if $P < 0.05$

** Fisher's exact test was applied

There was no significant association between numbers of ticks observed per animal and prevalence of Babesia sero-positivity ($\chi^2 = 1.6$, $P = 0.485$) (Table 3). The only value of significance identified was when the presence of *R. decoloratus* was compared to the sero-prevalence ($P < 0.05$). Of those animals having ticks of this species, 95 % tested negative, meaning that the presence of *R. decoloratus* is negatively correlated to the sero-prevalence.

Table 3. *Results of ELISA compared to tick burden and the prevalence of the different tick species with a confidence level of 95 %*

	ELISA-positive	%	ELISA-negative	%	Chi-square	*P*-value
Number of ticks						
None (36)	7	19 %	29	81 %	1.6	0.485
Less than 6 (51)	16	31 %	35	69 %		
6 or more (43)	12	28 %	31	72 %		
R. appendiculatus						
Present (78)	25	32 %	53	68 %	2.6	0.157
Not present (52)	10	19 %	42	81 %		
R. evertsi evertsi						
Present (22)	9	41 %	13	59 %	2.6	0.119
Not present (108)	26	24 %	82	76 %		
R. decoloratus						
Present (19)	1	5 %	18	95 %		0.024*/**
Not present (111)	34	31 %	77	69 %		
A. variegatum						
Present (6)	3	50 %	3	50 %		0.342**
Not present (124)	32	26 %	92	74 %		

* Significant if $P < 0.05$

** Fisher's exact test was applied

Tick control

Spraying with acaricides was used on all farms as a prophylactic measure against ticks and TBDs. Most farmers sprayed their animals once weekly (80 %) whilst the remaining 20 % sprayed twice every week. Nearly all farms (n = 28; 93 %) had made changes in what acaricide class they used the past year and only two out of 30 farms (6.7 %) had used the same. Five farms (16.7 %) have used four classes of acaricides the past year and some farmers stated that they alternate between different substances. Out of the 28 farms where changes in acaricide class have been made, 17 have changed 1-2 times and 11 have changed ≥ 3 times. The main reason to why there have been changes in the choice of acaricide was, as demonstrated in Figure 6, that the ticks do not die (86.7 %). Other reasons for change are that the veterinary has recommended a change (3.3 %) or that another farmer has recommended a change (3.3 %).

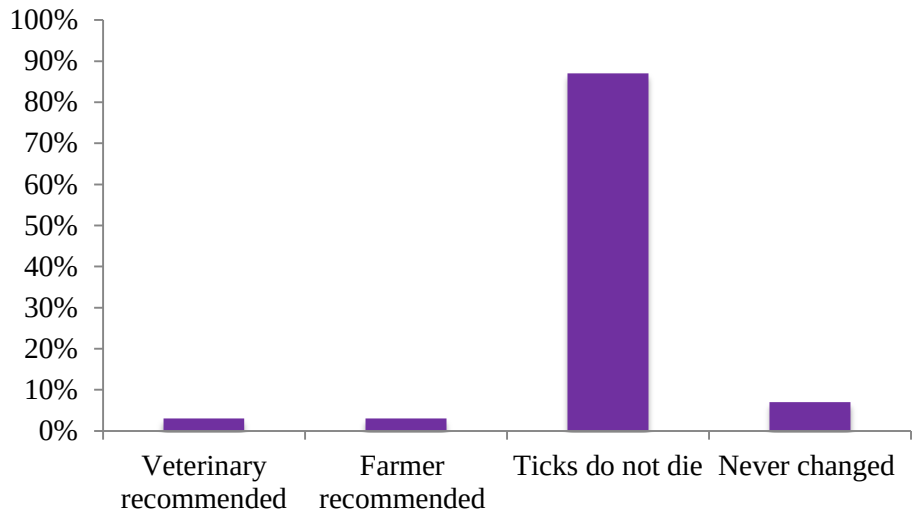

Figure 6. *Reason why farmers changed acaricide the past year.*

DISCUSSION

The sero-prevalence of *B. bigemina* in cattle around Lake Mburo National Park in Uganda was 26.9 ± 7.6 %. In earlier studies based on RLB from the same region, *B. bigemina* was not detected neither in cattle nor buffalo and it was even suggested that the parasite was absent (Oura *et al.*, 2011a; b). In contrast, the present study proved that *B. bigemina* is a rather common tick-borne haemoparasite. This is surprising as the sero-prevalence of *B. bigemina* is generally quite low in Uganda. In previous studies the sero-prevalence ranged between 2.3 % (Oura *et al.*, 2004) and 12.5 % (Okello-Onen *et al.*, 1998) whereas the microscopical prevalence for *Babesia* spp. was 6.7 % in another study (Matovu *et al.*, 2014). According to the classification of endemic stability by Norval *et al.* (1983), the present study indicates such instability in the investigated areas.

There was a difference comparing the sero-prevalence in the two sub-counties (Figure 4), which was significantly higher in Sanga than Kikatsi ($P < 0.01$). Sanga also was the area with an increased opportunity for interaction between cattle and wildlife (Table 1). This suggests that wildlife-livestock interface is an influencing factor for the sero-prevalence of *B. bigemina* and thus support the hypothesis that cattle interacting with wild animals are exposed to an increased risk of this tick-borne infection. There is to our knowledge only one study in Uganda that has investigated the role of wildlife interaction for *B. bigemina* infection in cattle before. However, it was conducted in another national park (Queen Elizabeth National Park) with microscopy as the diagnostic tool and concluded that proximity of livestock to wildlife does not explain variations in prevalence (Kabuusu *et al.*, 2013). These contradictory results could partly be explained by local differences but also in the use of partly different diagnostic methods. Microscopy is mainly useful for detection of the acute infection (Zintl *et al.*, 2003), whereas serology is more suitable for detection of carrier animals in epidemiological surveys where antibodies persist but the parasitaemia is low (OIE 2010; Böse *et al.*, 1995). An alternate explanation could be related to differences in the kind of wildlife-livestock interaction. In our study, zebra (*Equus quagga*) was the major wildlife, which confirms with

earlier reports (Ocaido & Siefert 1996; Ocaido *et al.*, 2009a). However in the study by Kabuusu *et al*. (2013) the dominant wildlife in Queen Elizabeth was not assigned, although Cape buffalo (*Syncerus caffer*) is a keystone species in this park. According to Moghari and Talbot (2009), Ugandan kob (*Kobus kob thomasi*), followed by Cape buffalo are the major wildlife in Queen Elizabeth but how they interact with livestock is unclear. Although, *B. bigemina* has been described from Cape buffalo (Bock *et al.*, 2004; Uilenberg 1995), when they were investigated for *B. bigemina* in four national parks in Uganda, none of the 83 animals were infected (Oura *et al.*, 2011a). In South Africa, *B. bigemina* have been diagnosed in impala (*Aepyceros melampus*) (Berggoetz *et al.*, 2014), but neither cattle nor in wildlife including impala were carriers in Lake Mburo (Oura *et al.*, 2011b). Whether zebra or Ugandan kobs are carriers of *B. bigemina*, has to our knowledge not been investigated. These contradictory results indicate that more studies are required.

Confounders, such as management system for livestock, age and breed differences did not significantly influence the sero-prevalence of *B. bigemina.* In a previous study, management system influenced the sero-prevalence and restricted or zero grazing were among the two most important factors associated with a decreased risk of infection with TBDs (Muhanguzi *et al.*, 2010). Likewise the risk of sero-conversion for *T. parva* was ten times higher in communally grazed cattle compared to zero-grazed animals (Rubaire-Akiiki *et al*., 2006). As for *B. bigemina*, few studies have investigated how the management system influences sero-prevalence. In a study by Rubaire-Akiiki *et al.* (2004), the sero-prevalence for *B. bigemina* was associated with zone, age and grazing system. Higher risk of infection was seen in the lowland agro-ecological zone (AEZ) and free ranged system (Odds Ratio 2.89) and in the upland AEZ and tethered system (OR 2.41). In the present study, management system was not a key factor but none of the investigated farms used a zero-grazed or tethered system, which could explain that no association was observed. There was a lower risk of infection for animals 0 – 12 month of age according to Rubaire-Akiiki *et al.* (2004). In the present study, the age of the animal showed no association with sero-prevalence, most likely due to an uneven distribution of sampled animals among age classes (definition of a calf was < 1 year of age). A significant difference between the breeds and the two sub-counties was seen. Most of the Ankole Long-horned cattle were in Sanga and most of the European crossbred cattle were in Kikatsi. Local cattle have been suggested to have a certain degree of natural resistance against *B. bigemina* (Bock *et al.*, 2004), however in the present study the kind of breed did not seem to influence the sero-prevalence. This is in agreement with the statement that when the challenge of *B. bigemina* is mild, breed differences are hard to find (Bock *et al.*, 1997). Overall, in the present study, confounders discussed above were not inclusion criteria and no significance to the sero-prevalence were seen. In earlier studies however, these factors were of importance for the sero-prevalence (Rubaire-Akiiki *et al.*, 2004; 2006), suggesting that more studies are recommended for investigation of how management system, breed, age of the animal and AEZ affects the sero-prevalence of *B. bigemina*.

Microscopically there was only one animal detected with *B. bigemina* in the present study, showing the typical clinical signs such as haemoglobinuria and fever that are associated with babesiosis. This is consistent according to Böse *et al.* (1995), who suggested that microscopy is adequate for detection of acute infections, but not for carrier animals. In order to detect low

carriers of infection with microscopy, thick films with an increased sensitivity are required. However, high parasitaemia with *B. bigemina* is characterized by up to 30 % infected erythrocytes (OIE 2010; Magona *et al.*, 2008), which means that parasites can be detected in the acute phase even in thin blood films (Böse *et al.*, 1995; Zintl *et al.*, 2003). Overall, these results indicate that the babesia-infection rate was relatively low in the present study.

Although tick burden were also estimated in this study, we did not use the same method as in previous studies (Kaiser *et al.*, 1982; Magona *et al.*, 2011; Rubaire-Akiiki *et al.*, 2004). According to our results *R. appendiculatus* and *R. evertsi evertsi* were more common in Sanga, whereas *R. decoloratus* in Kikatsi. This could be related to differences in agro-ecological zones. According to Rubaire-Akiiki *et al.* (2006) *R. appendiculatus* occur mainly in lowland areas, while the highest number of *R. decoloratus* are present in upland AEZ. However, we did not register the exact locations of our sampling sites with Global Positioning System (GPS), so it will remain speculative if the observed differences in tick composition were related to altitude.

Tick vectors were present in both sub-counties and as proposed by Florin-Christensen *et al.* (2014), suggesting that as long as suitable vectors are present in a region, *B. bigemina* is spread. As indicated earlier, the species composition of ticks differed somehow between sampling areas and we observed that *R. decoloratus* was negatively correlated to the sero-prevalence. This is opposite to what is expected, given that *R. decoloratus* is believed to be the most common vector for *B. bigemina* in Uganda (Magona *et al.*, 2008). One explanation could be that ticks were not counted on half of the body surface as in previous studies and thus our estimates were therefore inaccurate. Another cause could be that this was a cross-sectional study, and thus only give an instantaneous insight in the situation. It would be advised to investigate this further in a longitudinal study.

In this study, 80 % of the farms claimed that ticks and TBDs is a major constraint to livestock production. This is in agreement with Ocaido *et al.* (2009a), where TBDs were regarded as one of the major constraints to cattle production in LMNP. This once again outlines the importance of ticks and TBDs in Uganda.

Frequent spraying of the animals with acaricides was the most widely applied control measure. Dipping twice weekly increased the milk production by 21 % and prolonged the duration of lactation in cows (Okello-Onen *et al.*, 2003). It also increased the pre-weaning growth rate by 39 %, but had no significant effect on the post-weaning growth rate. This suggests that dipping twice weekly is preferable, whilst on the other hand acaricides also involve disadvantages; they are toxic, leave residues in meat, milk and cause environmental pollution. In earlier studies it has been shown that acaricides mostly are used incorrectly and that acaricide resistance pose an increasing threat to livestock production (Antunes *et al.*, 2014; Florin-Christensen *et al.*, 2014; Jongejan and Uilenberg 2005). The situation does not improve by frequent changes in what acaricide chemical animal keepers use as shown in the present study; almost all of the participants had alternated between acaricide classes the past year. The main reason for change of acaricides as stated in Figure 6 is due to that ticks are not dying, indicating a problem with acaricide resistance. Animal keepers should regularly be instructed by their veterinarians on how to use and dose acaricides to prevent further

development of resistance. More studies are also needed to further evaluate the use of anti-tick vaccines.

CONCLUSION

In conclusion, this study revealed that *B. bigemina* is present in areas around Lake Mburo National Park and suggests that wildlife-livestock interface seems to have an impact on the sero-prevalence. The only study that has investigated this question before stated that wildlife interface does not influence the prevalence. Explanations for these contradictious results could for example be due to different analyzing methods used or the differences in the wildlife the animals interact with. Further research is needed to investigate whether wildlife act as reservoir for *B. bigemina* and can spread the parasite to cattle. It would be recommended to sample both wildlife and cattle and analyze samples with serological and molecular methods.

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