Title: Molecular epidemiology of *Babesia* species, *Theileria parva*, and *Anaplasma marginale* infecting cattle and the tick control malpractices in central and eastern Uganda

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Molecular epidemiology of *Babesia* species, *Theileria parva*, and *Anaplasma marginale* infecting cattle and the tick control malpractices in central and eastern Uganda

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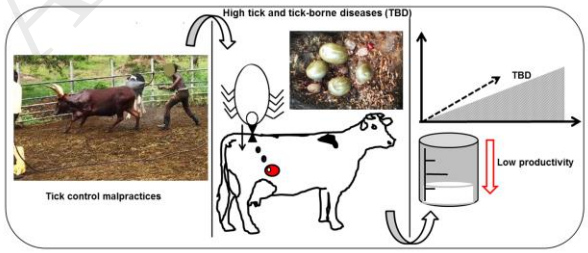
Graphical abstract



Abstract

East Coast fever, babesiosis, and anaplasmosis are the major tick-borne diseases affecting cattle productivity in Uganda. The emergence of acaricide-resistant ticks is suspected to have caused a rise in hemoparasites. This study sought to detect and characterize hemoparasites among farms in acaricide-failure hotspots of central as compared to the acaricide-failure naïve areas in eastern Uganda. Nested PCR assays were performed to determine the prevalences of *Babesia bovis, Babesia bigemina, Theileria parva,* and *Anaplasma marginale* in cattle blood samples sourced from randomly selected farms. Randomly selected isolates were sequenced to determine the genetic diversity of the parasites using the following marker genes: *B. bovis* spherical body protein 4, *B. bigemina* rhoptry-associated protein 1a, *T. parva* 104 kDa microneme-rhoptry antigen, and *A. marginale* major surface protein 5. Furthermore, partially and fully engorged adult ticks were collected for taxonomy, and tick-control practices were assessed using a semi-structured questionnaire. The prevalences of *B. bigemina, T. parva,* and *A. marginale* in cattle were 17.2, 65.1, and 22.0%, and 10.0, 26.5, and 3% in the central and eastern region, respectively. Whilst, *B. bovis* was not detected in the farms involved. The sequences for *B. bigemina*, *T. parva,* and *A. marginale* from the central region showed 99% identity with those from the eastern region. Of the 548 ticks collected, 319, 147, 76, and 6 were *Rhipicephalus* (*Boophilus*) *decoloratus, Rhipicephalus appendiculatus, Amblyomma variegatum*, and *Rhipicephalus evertsi evertsi*, respectively. The *Rhipicephalus* ticks were more abundant in the central, whereas *A. variegatum* ticks were more abundant in the eastern region. Tick control malpractices were found in both central and eastern Uganda, and 42 of the 56 surveyed farms lacked appropriate restraining facilities and so they utilized either ropes or a 'boma' (enclosure). In summary, *B. bigemina*, *T. parva, A. marginale* and their co-infections were more prevalent in the central than eastern region; even though, tick control malpractices were observed in both regions. Therefore, an urgent tick and TBD control strategy is needed.

Keywords: Acaricide failure; Cattle; Molecular epidemiology; Tick-borne infections; Tick control malpractices; Uganda.

1. Introduction

Tick-borne diseases (TBD) such as East Coast fever (ECF), babesiosis, and anaplasmosis are a threat to the cattle industry, especially in tropical and subtropical countries that have a high distribution of the tick vectors (Jongejan and Uilenberg, 2004; Ocaido et al., 2009; Oura et al., 2011; Uilenberg, 1995). Hemoparasites are transmitted when an infected tick, taking a blood meal, inadvertently injects hemoparasites into the host's blood along with saliva (Antunes et al., 2015; Šimo et al., 2017). The hemoparasites such as *Babesia bovis*, *Babesia bigemina*, *Theileria parva* and *Anaplasma marginale*, then infect the red blood cells and induce hemolysis, fever, and death of the susceptible host (Uilenberg, 2006). *T. parva*, which causes ECF, is the most pathogenic among *Theileria* species affecting cattle (Morrison, 2015). It is widely spread in Uganda due to the distribution of the brown ear tick, *Rhipicephalus appendiculatus* (Rubaire-Akiiki et al., 2006). *B. bigemina* and *B. bovis* are transmitted by *Rhipicephalus* (*Boophilus*) *decoloratus* and *R.* (*B.*) *microplus*, respectively, and cause bovine babesiosis (Jongejan and Uilenberg, 2004). *A. marginale* is transmitted by several tick species, biting flies, or through fomites (Kocan et al., 2010) and causes anaplasmosis. In Uganda, ECF, babesiosis, and anaplasmosis are among the most common TBDs of cattle, affecting cattle productivity (Byaruhanga et al., 2016; Kasozi et al., 2014; Magona et al., 2008; Ocaido et al., 2009). Therefore, farmers use drugs, vaccines, and acaricides for tick and TBD prevention and control (Mugisha et al., 2005). The drugs available to treat ECF include parvaquones and buparvaquones (Musoke et al., 2004), whereas diminazene aceturate and imidocarb dipropionate are used against babesiosis and anaplasmosis (Mosqueda et al., 2012). As a prevention tool, cattle farmers in Uganda vaccinate cattle against ECF, using the infection-treatment method with the live attenuated Muguga Cocktail vaccine (Patel et al., 2016; Perry, 2016). However, it is costly, labor intensive, and requires a steady supply of liquid nitrogen for storage of the vaccine straws. Such difficulties make it less adaptable, applicable, and affordable for grassroots farmers. Additionally, no vaccine is available against babesiosis and anaplasmosis. As a result, farmers in Uganda rely more on acaricides to control ticks. Currently more than 25 acaricide brands have been registered by the National Drug Authority and are readily available to farmers (Vudriko et al., 2016). Nonetheless, ticks have become resistant to all conventional acaricides available on the market in Uganda (Vudriko et al., 2016). Prior to the emergence of acaricide-resistant ticks in Uganda, cattle-farming communities reared indigenous cattle breeds such as the Ankole and Zebu that were known to be TBD tolerant (Kivaria et al.,

2004). However, in the 1960s, the Ministry of Agriculture imported exotic cattle (Holstein Friesian) and encouraged crossbreeding to improve dairy production (Balikowa, 2011; Ndambi et al., 2007). Unfortunately, the exotic cattle breeds and their crosses were known to be more susceptible to TBDs (Ndungu et al., 2005). Therefore, farmers were compelled to use high acaricide pressure to control ticks, which led to the development of acaricide failure (Vudriko et al., 2016; 2017a). Additionally, the breakdown in acaricide zoning due to political strife later in the 1960s; farmers' unregulated access to acaricides due to the liberalization of the pharmaceutical market; coupled with the breakdown of the extension infrastructure, are among the factors that have accelerated the development and spread of acaricide resistance in Uganda (Vudriko et al., 2016). Following the rise in resistant ticks, farmers' complaints of cattle mortalities due to suspected TBDs have been registered, particularly in areas hit by acaricide failure in Uganda's cattle corridor. This study sought to determine the molecular prevalence and genetic characteristics of hemoparasites, including *B. bovis*, *B. bigemina*, *T. parva*, and *A. marginale* from cattle blood samples collected from farms in acaricide failure hotspots of central Uganda and farms in the acaricide-naive region of eastern Uganda. Farm management practices were also assessed.

2. Materials and methods

2.1. Study area

A cross-sectional study was conducted in May and June 2017 to collect blood from cattle in farms located in selected districts, namely, Gomba, Mityana, Budaka, and Iganga. Mityana (0.4455° N, 32.0837° E) and Gomba (0.2230° N, 31.6739° E) districts are located in the cattle corridor in central region. The cattle corridor in Uganda stretches from the southwest to the northeast and comprises more than 40 districts. It has the highest population of cattle, and livestock is the mainstay of the population in this area. However, multi-acaricide-resistant *Rhipicephalus* ticks have been reported in here. Budaka (1.1016° N, 33.9304° E) and Iganga (0.6600° N, 33.4832° E) districts are located in eastern region, just outside the cattle corridor. Livestock production in eastern region is largely subsistence, and no reports of acaricide resistance have been documented.

2.2. Sample collection and processing

In each of the districts involved, the District Veterinary Officer (DVO) and the extension officers generated a list of farms by random selection. With the farmer's consent, a questionnaire was administered. The questions assessed the tick control practices that could influence the prevalence of hemoparasites. Animals were selected as follows: all animals were sampled if a farm had less than 10 head of cattle, 10 animals were randomly selected if the farmer kept up to 20 head of cattle, and 30% of the herd was sampled for farms with more than 20 head of cattle. The selected animals were restrained and parameters such as palpable lymph nodes, rectal temperature, mucous membrane color, and body condition score, were recorded. Partially and fully engorged adult ticks were carefully handpicked from the animals and transferred into 50 ml tubes that were then covered with a perforated top to maintain air circulation. By puncture of the caudal vein, approximately 4 ml of blood was collected into a vacutainer tube. The vacutainer tube was gently inverted 4–5 times to mix the blood with the anticoagulant. The vacutainer was labeled with details of the farm, packed into a cool box, and transported to the Research Center for Tick and Tick-borne Diseases Control (RTC) for DNA extraction. The genomic DNA was extracted from the collected blood samples using a commercial DNA extraction kit in accordance with the manufacturer's instructions (QIAamp DNA Blood Mini Kit, Germany). The ticks collected were enumerated under a stereo microscope and taxonomy was performed as previously described (Walker et al., 2003).

2.3. Polymerase chain reaction assays

Nested polymerase chain reaction (nPCR) assays targeting *B. bovis* spherical body protein 4 (Terkawi et al., 2011), *B. bigemina* rhoptry-associated protein 1a (Terkawi et al., 2011), *T. parva* 104 kDa microneme-rhoptry antigen (Konnai et al., 2006), and *A. marginale* major surface protein 5 (Ybañez et al., 2013) were conducted in accordance with the previously described method (Adjou Moumouni et al., 2015). Briefly, the reaction was performed in a 10 µl mixture containing 0.5 µM of the forward and reverse primers, 2 µl of 5× SuperFi™ buffer, 0.2 mM dNTP mix, 0.1 µl of Platinum SuperFi™ DNA polymerase (Thermo Fisher Scientific, Japan), 1 µl of DNA template, and 4.9 µl of double-distilled water. After an initial denaturation at 98ºC for 30s, the PCR amplifications were performed with 35 cycles each contained a denaturation step at 98ºC for 10s, an annealing step at appropriate temperature for 30s and an extension step at 72º C for 30s. The annealing temperature for each primer (Table S1) was determined using the Tm calculator (online tool, Thermo Fisher Scientific). Subsequently, 1 µl of DNA template from the first PCR was used for the nPCR assays. The *B. bovis* (Texas strain), *B. bigemina* (Argentina strain), *T. parva* (Muguga G6, ILRI strain)

and *A. marginale* (previously identified and confirmed from infected cattle blood sample by Adjou Moumouni et al. (2015) were used as positive controls, and double-distilled water was used as a negative control. The PCR products were then resolved by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under the UV transilluminator. The nested PCR amplicons with the 503, 412, 277 and 195 bp were considered as a positive for *B. bovis*, *B. bigemina*, *T. parva* and *A. marginale*, respectively.

2.4. Cloning and sequencing

A total of 16 samples were randomly selected from the positive samples, from the central and eastern regions. Of these, 6, 6, and 4 samples were for *B. bigemina*, *T. parva*, and *A. marginale*, respectively. Sequencing was performed as described previously (Adjou Moumouni et al., 2015). Briefly, the nPCR amplicons were extracted from the agarose gel and purified with the QIAquick Gel Extraction Kit (QIAGEN GmbH, Germany). The amplicons were inserted into TOPO® TA vector (Invitrogen, Thermo Fisher Scientific, Japan) and then transfected in competent *E. coli* (TOP10, Invitrogen, Thermo Fisher Scientific, Japan), following the manufacturer's instructions. For each sample, three colonies with the expected insert were multiplied and purified with the QIAGEN Plasmid Purification Kit, in accordance with the manufacturer's instructions. Sequence analysis was performed using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) for both the forward and reverse primers of the M13 plasmid.

2.5. Blast analysis, sequence alignment, and phylogenetic analysis

Bioinformatics and phylogenetic analyses for *B. bigemina*, *T. parva*, and *A. marginale* sequences obtained in the present study were performed using the basic local alignment search tool, (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the genetic relationship with query sequences obtained from the NCBI GenBank database. The identity was determined using the EMBOSS Needle online tool (https://www.ebi.ac.uk/Tools/psa/emboss_needle/). Phylogenetic analyses were done using the maximum likelihood method with the Kimura 2-parameter (K2) model for *B. bigemina* and *A. marginale*, and the Jukes-Cantor model with Gamma distribution and invariable (JC+G+I) for *T. parva* sequences using MEGA 7.0 software. *B. bovis rap 1* (Accession number L77326), *T. annulata* (Accession number 965955), and *A. phagocytophilum* (Accession number EF185293) were used as out-groups for *B. bigemina*, *T. parva*, and *A. marginale*, respectively.

2.6. Descriptive data analysis

Data from the questionnaires were analyzed using SPSS version 21 (IBM SPSS, Statistics for Windows, version 21.0. Armonk, NY: IBM Corp.). Pearson's chi-squared analysis was performed to determine the factors associated with the prevalence of hemoparasites at a 95% confidence interval. A *p*-value ≤ 0.05 was considered statistically significant. The upper and lower limits of the confidence intervals for the infection rate of each pathogen species were calculated using the EpiTools online epidemiological calculators (http://epitools.ausvet.com.au). A *t*-test analysis was performed to compare the means of tick populations collected in this study using Excel (Microsoft Office 2010).

2.7. Ethical statement

All sampling and experimental procedures were carried out in accordance with ethical guidelines for the use of animal samples as permitted by the College of Veterinary Medicine, Animal Resources and Biosecurity (experiment number: VAB/REC/15/104) and Obihiro University of Agriculture and Veterinary Medicine (Animal experiment number: 280082).

3. Results

3.1. Farm characteristics

A total of 56 farms, including 18 (32%) from the central and 38 (68%) from the eastern region, were involved. All respondents from the central region were male, whilst 71% were male and the rest (29%) were female. The farm management system varied based on the region. Majority (94%) of the farmers in the central region practiced either paddocking or ranching, whereas 96% of farmers in the eastern region practiced tethering. Similarly, the herd size differed based on the region; for instance, 82% of farmers in the eastern region kept small herds (< 10 animals). In contrast, 94 % of farmers in the central region kept medium to large herds.

3.2. Tick control practices on farms

The tick control practices used in central and eastern regions are shown in table 1. All farms in the central region applied acaricides for tick control, whereas only 76% in the eastern region applied acaricides. The frequency of acaricide application varied among the two regions. Farmers in the central region applied acaricides once or twice week, whilst majority

(61%) of farmers in the eastern region applied acaricides monthly or handpicked ticks. Farmers in the central region were better equipped for tick control than those in the eastern region. The restraint of animals during acaricide application was assessed. Noteworthy, 94% of farmers who used ropes were from the eastern region. In contrast, 80 % of the farmers using a crush were from the central region. Unfortunately, most of the crushes were either too wide (poorly constructed) or in poor condition (Fig. S1). At the time of the study, 72% and 13% of the farms involved were experiencing acaricide failure in the central and eastern regions, respectively. The farmers responded to acaricide failure by changing the acaricide, increasing the concentration of acaricides, and using ivermectin-injectable to replace the acaricide wash.

3.3. Animal characteristics

A total of 409 head of cattle were involved in this study. Of these, 209 and 200 animals were from farms in the central and eastern region, respectively. Female cattle were the most sampled, accounting for 80.9% and 72.5% of the total animals sampled from the central and eastern regions, respectively. The breeds of the cattle sampled varied based on the region. Exotic cattle (Holstein Friesian) were predominant in the central region (71.8%), while indigenous cattle were the predominant breed (65%) in the eastern region. Of the total number of cattle sampled, 79.9% and 80% were adult and the rest were calves from the central and eastern regions, respectively. In the central region, 19.6% were vaccinated, as compared to only 2.0% from the eastern region. Among the sampled animals, 18 and 6 animals from the central and eastern regions, respectively, exhibited clinical signs such as fever and pale mucous membranes.

3.4. Prevalence of hemoparasites

The overall prevalences of *B. bigemina*, *T. parva*, and *A. marginale* diagnosed in this study are shown in table 2. The overall burdens of hemoparasites in the central and eastern regions were 75.6% and 36.5%, respectively. The infection rates for *B. bigemina*, *T. parva*, and *A. marginale* were 1.7, 2.5, and 7.3 times higher, respectively, in the central region than in the eastern region. No positive sample for *B. bovis* was detected in this study. Co-infection was more prevalent in the central region than in the eastern region (Table 2).

3.5. Ticks identified in this study

A total of 548 partially and fully engorged adult ticks, including 273 from the central and 275 from the eastern regions, were collected in this study. The most dominant tick species was *Rhipicephalus* (*Boophilus*) *decoloratus*, of which 178 and 141 were from the central and eastern regions, respectively. Among the *R.* (*B.*) *decoloratus* ticks collected from the central region, 53 were males and 125 were females, whereas in the eastern region, 7 males and 134 females were collected. The second most dominant tick species was *Rhipicephalus appendiculatus*, of which 83 and 64 were from the central and eastern regions, respectively. Among the *R. appendiculatus* collected from central region, 9 were males and 74 were females, whereas from the eastern region, 12 males and 52 females were collected. The third dominant tick species was *Amblyomma variegatum*, which accounted for 6 and 70 ticks collected from the central and eastern regions, respectively. All 6 *A. variegatum* from the central region were females, while 58 males and 12 females were collected from the eastern region. The least dominant tick species was *Rhipicephalus evertsi evertsi*, with only 6 males collected from the central region.

3.6. Phylogenetic analysis

The accession numbers for the 16 sequences for *B. bigemina*, *T. parva*, and *A. marginale* are shown in table S2. The *B. bigemina rap 1a* isolates from the central and eastern regions showed 99% identity score and clustered in one clade together with the isolates from Kenya, Egypt, Thailand, Syria, and Mexico (Fig. 2). The *T. parva p104* isolates from central and eastern Uganda showed 99% identity score and clustered in one clade with isolates from other countries in Africa, such as Kenya, Zimbabwe, Zambia, and Tanzania (Fig. 3). The *A. marginale msp5* isolates from central and eastern regions clustered in two separate clades. Sequences (MG426193 and MG426194) from the central region clustered together with sequences from Kenya, Brazil, China, and Australia, while the sequences (MG426195 and MG426196) from eastern region were related to the strains from India and China (Fig. 4).

4. Discussion

Tick-borne diseases such as ECF, babesiosis, and anaplasmosis are endemic in Uganda (Jongejan and Uilenberg, 2004; Ndambi et al., 2007; Ocaido et al., 2009). Recent studies in Uganda have reported the emergence of acaricide-resistant ticks, which is a major concern for livestock industry (Vudriko *et al*., 2016, 2017a). The emergence of acaricide-resistant

ticks is likely to increase the TBD burden. Therefore, the current study aimed to document the molecular prevalence, the genetic characteristics of *B. bigemina*, *T. parva*, and *A. marginale* infecting cattle, and the tick control practices on farms in the acaricide-failure hotspots in central region, and the acaricide-failure naïve areas in eastern region of Uganda (Fig. 1).

In this study, *B. bigemina*, *T. parva*, and *A. marginale* infections and their co-infections were more prevalent in the central than in eastern Uganda. Alarmingly, 10 cattle sampled from the central region were co-infected with all three hemoparasites diagnosed, of which 2 cattle showed clinical signs such as fever, pale mucous membranes and loss of weight. Regrettably, the four districts involved in this study lacked up-to-date laboratory diagnostic facilities to guide rational prescription. This implied that farmers and veterinarians treated cattle based on clinical signs. However, the clinical signs of most TBDs are overtly similar (Jongejan and Uilenberg, 2004). To cater for this blind treatment, farmers administer a cocktail of antibiotics and antiprotozoal drugs (Fig. S1) to animals showing clinical signs (personal communication with a herdsman), which may be unethical (Nabukenya et al., 2014). Although the blind treatment might work for mono infections, co-infection are complex (Musoke et al., 2004; Thumbi et al., 2014; Van Wyk et al., 2014). This might explain why reports on TBD-related cattle mortalities have increased in central Uganda.

In line with the ticks and TBD challenge in Uganda, vaccination might be a reliable control measure for TBD. Regrettably, only a small proportion (19.6% and 2%) of the total cattle sampled in central and eastern were vaccinated against ECF. The low adoption of vaccine use could be due to the high cost (Mukhebi et al., 1990). More to that, livestock is not a commercial priority commodity in eastern Uganda and the farmers kept indigenous breeds that are known to be more tolerant to TBD (Kivaria et al., 2004; Muhanguzi et al., 2014b). This possibly might explain why vaccinated animals were comparably fewer among the surveyed animals in the eastern region than in the central region.

Among the hemoparasites diagnosed, *T. parva* was the most prevalent compared to *B. bigemina* and *A. marginale* infections in both central and eastern Uganda. This result was consistent with previous studies that reported ECF as the most economically important TBD of cattle in Uganda (Byaruhanga et al., 2016; Chenyambuga et al., 2010; Kabi et al., 2014; Muhanguzi et al., 2014a; Rubaire-Akiiki et al., 2006). *B. bovis* was not detected in the cattle samples from central and eastern Uganda, which could be justified by the fact that *R.* (*B.*)

microplus, the vector for *B. bovis* was not among the ticks collected. Even though, *B. bovis* has been reported in neighboring countries such as Kenya (Adjou Moumouni et al., 2015; Njiiri et al., 2015) and Tanzania (Woodward et al., 1990). Therefore, it is imperative that cattle imported from neighboring countries be subjected to routine screening for presence ticks and hemoparasites.

The phylogenetic analysis showed that *B. bigemina* and *T. parva* infecting cattle in the central and eastern regions were related to isolates from other countries around the world. On the contrary, *A. marginale* isolates from the central region clustered with isolates from Kenya, Brazil, China, and Australia, whereas, the isolates from the eastern region clustered with isolates from India and China. The findings indicate that two distinct strains are present in cattle in central and eastern region. One assumption is that the cross-border movement of cattle between Uganda and the neighboring countries (Regional Integration Support Programme, 2013), has played a role to introduce different isolates of *A. marginale* in Uganda. This could explain the close relationship between *A. marginale* isolates from the central to isolates from Kenya (Adjou Moumouni et al., 2015). However, the isolates from the east clustered with isolates from India and China which remains unsolved. Since the data presented in this study are based on the *A. marginale msp5* partial gene, the conclusions derived can only be suggestive. In addition to characterizing the complete *msp5* gene, future studies should consider analyzing the diversity of other marker genes such as 16S ribosomal RNA (*16S rRNA*) and heat-shock protein genes (*GroEL*).

The burden of *B. bigemina*, *T. parva* and *A. marginale*, diagnosed in central and eastern Uganda is justified by the burden of the tick species detected. In the current study, *R.* (*B.*) *decoloratus, R. appendiculatus*, *A. variegatum* and *R. evertsi* tick species were detected as the major tick species infecting cattle (Fig. S2), consistent with previous studies (Rubaire-Akiiki et al., 2004; Vudriko et al., 2016; Walker et al., 2003). Noteworthy, the burden of ticks was comparable in the central and eastern regions despite the difference in acaricide pressure. The farmers in central Uganda that were involved in the current study applied acaricides either weekly or twice a week, whereas the majority of farmers in the eastern region applied acaricides monthly or no acaricide at all. The increased intensity of acaricide application in the central region might be due to farmers response to the prevalence of acaricide-resistant ticks (Vudriko et al., 2016). This finding is consistent with previous reports that documented a high tick burden concomitant with the emergence of acaricide resistance (Vudriko et al., 2016, 2017a). Of the tick species identified, *A. variegatum* was comparably lower in the

central region than the eastern region (P=0.04). Although this could be attributed to the low acaricide pressure in the eastern region, it should be noted that several factors such as geographical location, climate and management/acaricide use influence the tick burden. As in the case for this study, the samples from both regions were collected at the end of the rainy season (end of May and early June). Therefore, future studies focusing on the questing ticks as opposed to handpicked ticks, might provide crucial information on the epidemiology of ticks in the different regions of Uganda.

Several tick control malpractices were observed in farms surveyed in the central and the eastern regions (Fig. S1), *inter alia*, the use of a knapsack sprayer, the use of ropes and a "boma" for restraint during acaricide application, a poorly constructed crush, the use of muddy water to mix acaricides, underexposure of acaricide and poor record keeping. Distinctively, farmers in central were injecting ivermectin for tick control, whilst selective-acaricide application to exotic cattle, use of hand sprays, handpicking of the ticks and purchasing acaricide aliquots in syringes were practiced in the eastern. Such malpractices contribute to the acaricide failure as previously documented (Abbas et al., 2014; Vudriko et al., 2016). The malpractices observed in the central and east shows the need for sensitization of farmers in Uganda on appropriate tick control practices (Byaruhanga et al., 2017; Kasozi et al., 2014; Vudriko et al., 2016; Vudriko et al., 2017b).

Although acaricide failure has not been documented in eastern Uganda, 5 farmers reported suspected cases of acaricide failure during the survey. They had recently acquired exotic animals from the acaricide hotspots of south-western Uganda under an ongoing agro-livestock-based poverty alleviation program (James et al., 2015). Previously, Vudriko et al. (2016) documented resistant ticks on cattle in a slaughterhouse in northern Uganda. The cattle had been purchased from the acaricide-resistant hotspots in southwest and taken for slaughter in northern Uganda. This suggests that acaricide-resistant ticks are already spreading across the country. Therefore, strict measures on cattle movement are needed to secure the areas where it has not yet spread.

5. Conclusion

Acaricide failure perpetuated by tick control malpractices have led to the high burden of ticks in tandem with a rise in TBDs in the central region. In frustration, farmers facing acaricide failure are taking aggressive measures such as increasing the acaricide concentrations, mixing different acaricides together, and the extra-label use of ivermectin. Although such measures

may have a direct effect on tick reduction, they may have adverse effects on the health of animals, humans, and the environment. Hence the impact of acaricide resistance should not be underestimated and demands an urgent solution. The genetic characteristics showed that *T. parva* and *B. bigemina* isolates from central and eastern were related which could guide mitigation strategies that include vaccine and chemotherapeutics development. On the other hand, *A. marginale* isolates from the central and eastern were distinct and necessitates further in-depth studies.

Conflict of interest

The authors have none to declare.

Authors' contributions

DST, II, NY, PV: Conception and design of the study; DST, MK, JSB, JB: Performed sample collection; DST, AG, ABN, SG, GEB: Guided and performed laboratory experiments; DST, PFAM: Performed data analysis and sequence analysis; DST, II, NY, BT, TS, JOA, PV: Wrote the first draft of the manuscript; SPM, RT, EMW: Provided critical advice and review of the intellectual content of the draft of the manuscript. All authors read and approved the final manuscript.

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Figure captions

Fig. 1. Map of Uganda, showing the districts involved in this study

Mityana and Gomba from central Uganda are indicated by red circles, while Budaka and Iganga districts are indicated by black circles. The light green shading shows the demarcation of Uganda's cattle corridor (Map credit, ArcGIS https://www.arcgis.com/home/index.html).

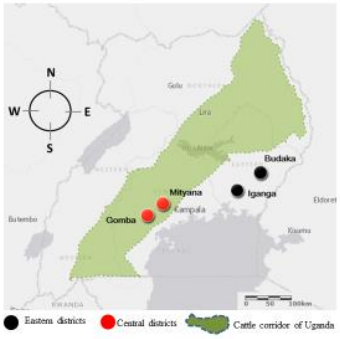

Fig. 1.

Fig. 2. Phylogenetic analysis of *B. bigemina rap 1a* sequences

Sequences from the central region are indicated by squares, while those from the eastern region are indicated by circles. The numbers on the branches show the percentages of 1000 bootstrap replications.

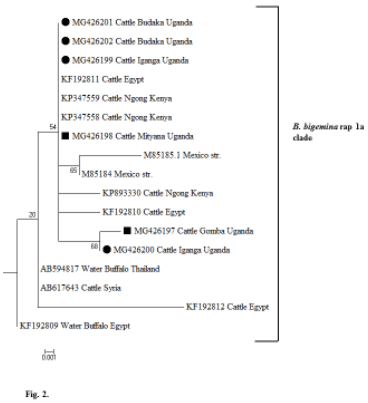

Fig. 2.

Fig. 3. Phylogenetic analysis of *T. parva p104* sequences

Sequences from the central region are indicated by squares, while those from the eastern region are indicated by circles. The numbers on the branches show the percentages of 1000 bootstrap replications.

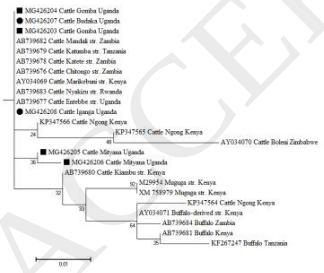

Fig. 3.

Fig. 4. Phylogenetic analysis of *A. marginale msp5* sequences

Sequences from the central region are indicated by squares, while those from eastern region are indicated by circles. The numbers on the branches show the percentages of 1000 bootstrap replications.

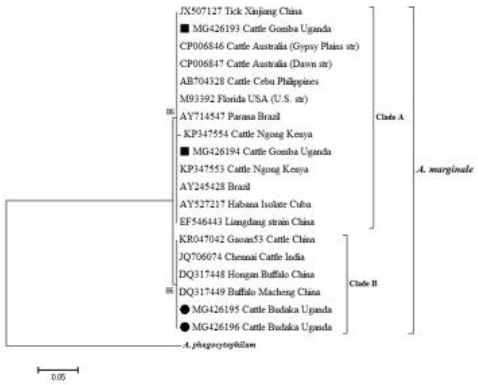

Fig. 4.

Table 1. The tick-control practices on the cattle farms in central and eastern Uganda

Query	Response	Frequency		Total	%
		Central	Eastern		
Method of tick control used	Acaricides	18	29	47	83.9
	None	0	6	6	10.7
	Hand picking	0	3	3	5.4
Method of application	Spraying	17	28	45	80.4
	Pouring on	0	6	6	10.7
	None	0	6	6	10.7
	Hand picking	0	3	3	5.4
	Dipping	1	0	1	1.8
Equipment used	Knapsack sprayer	4	20	24	42.9
	Bucket/foot pump	10	4	14	25.0
	None	0	7	7	12.5
	Hand sprayer	0	4	4	7.1
	Hands for picking	0	3	3	5.4
	Spray race	3	0	3	5.4
	Dip	1	0	1	1.8
Method of restraint	Ropes	2	31	33	58.9
	Crush	8	2	10	17.9
	Boma	4	5	9	16.1
	Spray race	3	0	3	5.4
	Dip	1	0	1	1.8
Who monitors the acaricide application?	Owner	6	18	24	42.9
	Herdsmen	5	10	15	26.8
	Do not use acaricide	0	9	9	16.1
	Farm manager	7	1	8	14.3
Source of water for acaricide mixing	Borehole	0	23	23	41.1
	Well	11	3	14	25
	Valley dam	4	0	4	7.1
	Tap water	2	1	3	5.4
	Swamp	1	1	2	3.6
	No water needed	0	10	10	17.8
Animal sprayed with 20 l	Less than 7	9	17	26	46.4
	More than 7	6	6	12	21.4
	Not sure	3	6	9	16.1

	Do not use acaricide	0	9	9	16.1
Frequency of acaricide application	Weekly	14	9	23	41.1
	Monthly	0	14	14	25
	Twice a week	4	6	10	17.9
	Do not use acaricide	0	9	9	16.1
Class of acaricide used	Not sure and no record	0	17	17	30.4
	Amitraz	9	3	12	21.4
	Synthetic pyrethroid	2	6	8	14.3
	Do not use acaricide	0	9	9	16.1
	Organophosphate	3	2	5	8.9
	Co-formulation	4	1	5	8.9
Duration of use of the current acaricide	No record	3	24	27	48.2
	Less than 1 year	11	3	14	25
	Do not use acaricide	0	9	9	16.1
	More than 1 year	4	2	6	10.7
History of tick resistance	No	5	33	38	67.9
	Yes	13	5	18	32.1
Measures taken to avert tick resistance	Not applicable	5	33	38	67.9
	Change acaricide	8	4	12	21.5
	Use ivermectin injectable and change acaricide	4	0	4	7.1
	Double concentration	1	1	2	3.6
Total		**18**	**38**	**56**	**100**

Total number of respondents (n=56).

Table 2. The prevalence of hemoparasites infecting cattle and the co-infections diagnosed in central and eastern Uganda

Parameter	Hemoparasites	Prevalence % (N)			
		Central region	**CI**	**Eastern region**	**CI**
Single infections	*Babesia bigemina*	17.2% (36)	0.13-0.23	10.0% (20)	0.07-0.15
	Theileria parva	65.1% (136)	0.58-0.71	26.6% (53)	0.21-0.33
	Anaplasma marginale	22.0% (46)	0.17-0.28	3.0% (6)	0.01-0.06
Co-infection	*B. bigemina + T. parva*	12.4% (26)	ND	1.5% (3)	ND
	B. bigemina + A. marginale	6.7% (14)	ND	6.7% (2)	ND
	T. parva + A. marginale	13.9% (29)	ND	0.5% (1)	ND
	B. bigemina + T. parva + A. marginale	4.8% (10%)	ND	0.0 % (0)	ND
	Total	**100% (209)**		**100% (200)**	

CI: Confidence interval, ND: Not done