Molecular epidemiology of anaplasmosis in small ruminants along a human-livestock-wildlife interface in Uganda

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ABSTRACT

Background: Information as regards the epidemiology of the Anaplasmataceae in small ruminants in several low- and middle-income countries is scarce.

Methods: In this study a total of 712 DNA samples collected from small ruminants were analyzed for Anaplasmataceae and Anaplasma ovis using the 16s rRNA and MSP4 genes respectively. Infection risk was assessed by location, sex and age of the animals and qGIS\textsuperscript{®} was used to construct spatial maps.

Results: The prevalence of Anaplasmataceae spp was 89.1% (95% CI: 77.5–95.9) and 79.1% (95% CI: 75.9–82.1) in ovines and caprines respectively (RR = 1.1, 95% CI: 1.0–1.3); higher than those previously reported in other eastern African countries. The prevalence of A. ovis was 26.1% and 25.4% for both ovines and caprines respectively with ovines showing significantly higher levels of infection than caprines (P < 0.05). The risk of Anaplasma ovis infections was not affected by age (OR = 1.2, 95% CI: 0.9–1.7) or sex (OR = 1.1, 95% CI: 0.6–2.0). Small ruminants located at the forest edge (<0.3 km) showed higher A. ovis prevalence than those found inland with infections present in the midland regions associated with increased agricultural activity.

Conclusion: Anaplasma ovis remains a major challenge for small ruminant husbandry in Uganda and infections are under-reported. Policy efforts to prioritize management of Anaplasmataceae for small ruminant health would promote livestock productivity in vulnerable communities, improving livelihoods and ecosystem health.

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1. Introduction

Across Africa and Asia, small ruminants often maintained in mixed farms, are a major source of animal protein and raw materials for the fragile food industry (Devendra, 1994). In communities where poverty is endemic, small ruminants contribute essential revenue (Peacock, 2005), assets (Inn, 2003), and sources of food security (Herrero et al., 2013; Laljiie et al., 2019). Small ruminants are an important pathway towards the realization of the 2030 Sustainable Development Goal (SDG) targets for food and agriculture (Organization, 2018) in vulnerable communities, contributing to: zero poverty (goal 1), good health and well-being (goal 3); decent work and economic growth (goal 8). Small ruminants are considered key to fighting poverty in Uganda (Akejo and Otto, 2017; Inn, 2003) but the increasing burden of hemoprotozoan infections threatens their productivity and community livelihoods due to the risk of zoonotic disease transmission (Kasozi et al., 2019). Most small ruminants that are kept are indigenous, having advantages over exotics in terms of climate resilience and being considered low maintenance (Monau et al., 2020). Tick-borne infections are however a major productivity challenge (Bilgic et al., 2017).

Several studies have been conducted on the epidemiology of tick-borne infections in cattle due to their high economic value (Bardosh et al., 2013; Vudriko et al., 2016; Weny et al., 2017). In Uganda, major Ixodid tick species identified include Rhizophytopus species (especially Rhipicephalus appendiculatus), Amblyoma species (especially Amblyomma variagatum) and Hyalomma species (Hyalomma rufipes and Hyalomma truncatum) continue to be common in bovines (Balinandi et al., 2020) and these species continue to be present nationwide (Muhanguzi et al., 2020).

A polymerase chain reaction (PCR) based study (Ikwap et al., 2010) identified new and unknown Anaplasma genotypes in bovines from Uganda. Rather less emphasis has been placed on small ruminants. A ovis is responsible for the majority of anaplasmosis cases in small ruminants (Bilgic et al., 2017; Torina et al., 2010) and there is a need to prioritize studies in eastern Africa to affect improvements in small ruminant health. Since many countries in eastern Africa rely on small ruminants for food and income (Herrero et al., 2013; Inn, 2003; Laljiie et al., 2019; Peacock, 2005), attainment of the SDGs in the region will require national governments to revise their disease control policies.

Recent surveys show increasing evidence of Anaplasma spp in small ruminants. The prevalence of Anaplasma spp was found to be 40.8% in ovines from central and western Kenya by PCR (Ringo et al., 2019). In northeastern Uganda, a prevalence of 19.5% for Anaplasma spp in small ruminants was reported by microscopy (Lolli et al., 2016). In Sudan, a prevalence of 60.1% for A. ovis with prevalence of infection being higher in ovines than caprines following PCR analysis (Lee et al., 2018). In Tunisia, Anaplasma spp. and A. ovis prevalence were 95.0% and 93.8% in ovines and 69.6% and 65.3% in caprines, respectively while no A. phagocytophilum was detected by PCR (Said et al., 2015). In Senegal, prevalence of Anaplasmataceae infection was 41.1% and in these, Anaplasma ovis 55.9% in ovines, A. marginale and A. centrale 19.4% and 8.1% in bovines and putative new species of Anaplasmataceae were found by PCR (Dahmani et al., 2019). A study from Turkey, Iraq, Sudan, and Portugal on Anaplasma ovis established a prevalence of 31.4%, 66.6% 41.6% and 82.5%, respectively, indicating that the high prevalence of A. ovis in these countries, calling for action to stop the neglect of these bacterial infections in small ruminants (Reneker et al., 2013). This showed that molecular studies estimating the prevalence of anaplasmosis in small ruminants of Uganda are scarce, this is despite mounting epidemiological evidence of their importance as maintenance hosts for Anaplasmataceae in Ugandan livestock communities.

Molecular diagnosis of Anaplasma bacteria is by detection of the 16S rRNA gene (Ringo et al., 2019; Shi et al., 2020; Yang et al., 2016). This forms the basis of phylogenetic analysis in the Anaplasmataceae (Seo et al., 2018) for insight into genotypic variation and diversity. In Xinning, northwest China, a prevalence of 17.6% for A. phagocytophilum, 4.8% for A. bovis and 40.5% for A. ovis shows the importance of these bacteria in small ruminant health (Yang et al., 2015). For A. ovis diagnosis, the MSP4 gene is the a major target (Ali et al., 2017).

This study aimed to assess the prevalence of Anaplasmataceae in small ruminants of Uganda Prior to this study, examination of A. ovis in small ruminants had never been conducted in Uganda.

2. Material and methods

2.1. Study design

This cross sectional study was conducted on 712 DNA samples obtained from small ruminants at the fringes of Budongo Conservation Forest in Masindi district, Western Uganda (Figure 1). Small ruminants included were only ovines and caprines sampled since these were the prevalent (90%) animal species in the community using records from the veterinary officer at the local subcounty office. Sampled villages were chosen randomly using a random assortment algorithm in MS Excel 2019 in consultation with the local community leaders (Kasozi et al., 2019).

2.2. Blood collection and DNA extraction

Blood samples were aseptically collected from the jugular vein of the survey animals, placed in a redtop vacutainer and stored at 4 °C. DNA was extracted using Himedia® HiPura™ blood genomic DNA Mini-prep purification spin kit with minor modifications (Jaswal et al., 2014). In an Eppendorf, 300 μl of blood was added to 900 μl of RBC lysis solution, incubated for 3 min at room temperature. This was then centrifuged at max speed (16,000 relative centrifugal force) for 1 min and the supernatant was discarded. The pellet this was resuspended in the residual fluid. To the tube containing the residual fluid, 150 μl of cell lysis solution was added, followed by 50 μl of protein precipitation solution. This was vortexed vigorously for 20 s, spun for 2 min at max speed. In a clean Eppendorf tube with labels transferred, 150 μl of isopropanol (100%), and the supernatant after the spin was added to the isopropanol. This was then mixed gently by inverting the tube 50 times, then centrifuged at max speed for 2 min. The supernatant was then discarded, leaving only the pellet. Finally, 150 μl of ethanol was added to wash the pellet and this was centrifuged at max speed for 2 min. The supernatant was then discarded and the tube was drained and left in the PCR hood to dry. To the dried tube, 50 μl of DNA hydration solution was added and the mixture was incubated at 65 °C for 10 min, mixed gently by pipetting and stored at 4 °C for routine use and -20 °C for future reference.

2.3. Molecular identification of Anaplasmataceae in small ruminants

PCR targeting the Anaplasmataceae 16S rRNA gene was used to detect Anaplasma spp (Ringo et al., 2019) with minor modifications. A 10 μl reaction containing 1x My Taq mix (Bioline®), about 1 μg of genomic DNA 0.2 μl each of the forward and reverse primers. Primer nucleotide sequences used were: forward primers, 5’GGTTTTATCTGATGCAACGC- GA-3’, and reverse primers 5’CTTATTCAACCTTGCGAT-3’ (Bekker et al., 2002; Ringo et al., 2019). Anaplasma centrale DNA was used as a positive control (Ikwap et al., 2010). After an initial denaturation step at 95 °C for 5 min, the PCR amplifications were performed for 45 cycles, each contained a denaturation step at 95 °C for 30 s, an annealing step at 74 °C for 30 s and an extension step at 72 °C for 45s. PCR products were then resolved by gel electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under a UV transilluminator, the PCR amplicons of 430 bp were considered as positive for Anaplasmataceae after visualization alongside the 1000 bp DNA ladder.
2.4. Molecular identification of Anaplasma ovis in small ruminants

The MSP4 gene of Anaplasma ovis with GenBank no. HQ456350.1 was amplified using forward primer 5’TGAAGGGAGCGGGGTCATGGG3’ with reverse primer 5’GTAATTGCAGCCAGGGACTCT 3’ were used (Ali et al., 2017). The PCR mix consisted of the primers each at a final concentration of 0.2 μM, 1x My Taq mix (Bioline®) and about 1 μg of genomic DNA. The PCR was done following a cycling program that consisted of an initial denaturation step of 5 min at 95 °C, each cycle consisting of a denaturing step of 30 s at 95 °C, an annealing temperature for 30 s at 60 °C and an extension step of 45 s at 72 °C for 35 cycles. PCR was completed with the additional extension step for 10 min at 72 °C. The positive control for A. ovis from the study was used. PCR products were run on a 1.5% agarose gel and visualized under UV transilluminator to detect a PCR product of 347 bp.

2.5. Ecological distribution of Anaplasmataceae in the study area

Georeferenced village survey points were imported into quantitative geographical system (qGIS®) open-source software. Using an image acquired from the United States Geographical Surveys (USGS) Aster global Dem Satellite image number ASTG02_N01E031_dem land elevation analysis was done and vegetation cover analysis was conducted using an image acquired from USGS Sentinel-2 satellite, image number L1C_T36NUG_A013088_20171224T091914. The satellite image files were modified to show 4 levels of classifications, band 1 (Red) with rendering type of single band pseudocolor linear using settings in qGIS®. Unit 1 was lake vegetation (purple), 46.3 units for budongo forest vegetation (blue), 91.7 units for sugarcane and community agricultural cultivation (green) and 137 units for bare soil (yellow). Digital land elevation was divided into 4 categories i.e. 592 units (color deep blue) for lake level, 1055 units (light blue) low land, 1095 units (brown color) midlands and 1440 and above (red color) on highlands. Vegetation land cover satellite images were divided into 3 categories i.e. 1-unit (purple color) for Budongo forest, 69 units (blue color) community crops and sugarcane plantation and 137 units (yellow color) for soil with no vegetation were used to define risk areas for infection in the study area and presented on maps.

2.6. Ethical approval

Ethical approval from the Ethical Review Board at the University of Edinburgh and clearance from the Uganda National Council of Science and Technology with reference numbers OS5-17 and A570 respectively were acquired.

2.7. Statistical analysis

Data were recorded in Microsoft Excel and descriptive statistics were conducted using WinPepi® to determine the disease risks and their 95% confidence interval, for infections in small ruminants. Significance was reported when the 95% confidence interval didn’t contain the null value.

3. Results

3.1. Prevalence of Anaplasmataceae in small ruminants of Uganda

The study showed an Anaplasmataceae prevalence of 79.1% (95% CI: 75.9–82.1) and 89.1% (95% CI: 77.5–95.9) in caprines and ovines respectively. Infections in small ruminants due to Anaplasmataceae were significantly higher in ovines than caprines (RR = 1.1, 95% CI: 1.0–1.3). Prevalence of Anaplasma ovis was 25.4% (95% CI: 22.2–28.8) and 26.1%, (95% CI: 15.6–40.3) for both caprines and ovines respectively and there were no significant differences between infections in caprines and ovines (RR = 1.0, 95% CI: 0.6–1.7) as shown in Table 1.

3.2. Major risk factors for anaplasmosis in small ruminants of Uganda

Anaplasmataceae infections in adults and juveniles were not observed to be significantly different (RR = 1.0, 95% CI: 1.0–1.1) and similar observations were found amongst males and females and location. No difference (OR = 1.2, 95% CI: 0.9–1.7) was found in the prevalence of vegetation.
Table 1. Prevalence of Anaplasmataceae in small ruminants of Uganda.

| Species          | Frequency (prevalence; 95% CI) | Risk of infections |
|------------------|---------------------------------|-------------------|
|                  | Positive | Negative | Total | RR (95% CI) | OR (95% CI) | aR (95% CI) |
| Anaplasmataceae  |          |          |       |             |             |             |
| Ovines (n = 46)  | 41 (89.1; 77.5-95.9) |          |       | 1.1 (1.0-1.3) | 2.2 (0.9-6.3) | 7.0 (0.7-20.7) |
| Caprines (n = 666) | 527 (79.1; 75.9-82.1) |          |       |             |             |             |
| Total (n = 712)  | 566 (79.8; 76.7-82.6) |          |       |             |             |             |
| Anaplasma ovis   |          |          |       |             |             |             |
| Ovines (n = 46)  | 12 (26.1; 15.0-40.1) |          |       | 1.0 (0.6-1.7) | 1.0 (0.5-2.0) | 0.7 (-13.6-15.0) |
| Caprines (n = 666) | 169 (25.4; 22.8-28.8) |          |       |             |             |             |
| Total (n = 712)  | 181 (25.4; 22.3-28.7) |          |       |             |             |             |

KEY: RR = relative risk, OR = odds ratios and aR = attributable risk.

A. ovis between juveniles and adults. There was also no difference (OR = 1.1, 95% CI: 0.6-2.0) between sex of male prevalence (27.8%; 95% CI: 18.8-39.1) and female prevalence (25.2%, 95% CI: 22.0-28.7) of small ruminants (Table 2). Anaplasmataceae prevalence was highest in communities <0.3 km from the forest edge than those far away i.e. 82.6% vs 79.1%. A. ovis prevalence was higher in communities closer to the forest edge than those more than 0.3 km from the forest edge (24.9%) although no significant differences were found (OR = 1.2, 95% CI: 0.7-1.8).

3.3. Anaplasmataceae prevalence in the surveyed villages amongst small ruminants

The prevalence in small ruminants attributable to Anaplasmataceae was in the order of Rwempisi > Kapeka 3 > Rwentale 1 > Rwengabi > Simba > Ewafula > Nyabuye > Maraam > Nakafunj > Kapeka 1 > Kapeka 2 > Kanyege > Kalongo while Anaplasma ovis prevalence was in the order of Kalongo > Kanyege > Nyabuye > Maraam > Ewafula > Nakafunj > Simba > Rwengabi > Kapeka 1 > Kapeka 2 > Rwentale 1 > Kapeka 1 > Kapeka 3 > Rwempisi and significantly higher differences in Anaplasmataceae than Anaplasma ovis prevalence were found (P < 0.05).

The prevalence of Anaplasma ovis ranked in this order Kalongo > Kanyege > Nyabuye > Maraam > Ewafula > Nakafunj > Simba > Rwengabi > Kapeka 2 > Rwentale 1 > Kapeka 1 > Kapeka 3 > Rwempisi (Table 3). The odds ratio following infection with other Anaplasmataceae parasites other than A. ovis was 28 times in Kalongo, 22 times in Kapeka 3, 21 times in Rwentale 1, 15 times in Rwengabi and Simba respectively.

3.4. Visual distribution of Anaplasmataceae in the study area of Uganda

Within the community, prevalence of Anaplasmataceae was in the range of 70%–86% while A. ovis prevalence was 12%–36% showing the importance of Anaplasmataceae in small ruminants in the community. Prevalence and risk of infection was greatest in Rwempisi, Kapeka 3, Ewafula, Nyabuye 2 and Rwengabi, Rwentale 1, Kapeka 1 and all of these villages were in the midland level elevation (Figure 2A) with sparse vegetation cover (Figure 2B) as a result of human agricultural activities and commercial sugarcane production. Villages with high Anaplasma ovis prevalence (Kalongo, Kanyege, Nyabuye 2, Marram and Ewafula) were in lowland areas (Figure 2C) with relatively heavy vegetation due (Figure 2D) to their close proximity to the forest edge (less than 0.5km).

Table 2. Factors precipitating anaplasmosis infections in small ruminants of Uganda.

| Parameter          | Variable | Frequency (%) in small ruminants | Risk of infections | P-value |
|--------------------|----------|---------------------------------|-------------------|---------|
|                    |          | Positive | Negative | Total | RR (95% CI) | OR (95% CI) | aR (95% CI) |
| Anaplasmataceae    |          |          |          |       |             |             |             |
| Age                |          |          |          |       |             |             |             |
| Adult              | 349 (80.4) | 85 (19.6) | 434 (100) | 1.0 (1.0-1.1) | 1.1 (0.8-1.6) | 1.6 (4.7-8.0) |
| Juvenile           | 219 (78.8) | 59 (21.2) | 278 (100) |             |             |             |
| Sex                |          |          |          |       |             |             |             |
| Male               | 59 (81.9) | 13 (18.1) | 72 (100) | 1.0 (0.9-1.2) | 1.2 (0.6-2.3) | 2.4 (7.8-12.6) |
| Female             | 509 (79.5) | 120 (20.5) | 640 (100) |             |             |             |
| Location (parish)  |          |          |          |       |             |             |             |
| Kabango            | 193 (75.7) | 62 (24.3) | 255 (100) | 3.1 | 1 | 51.4 | 1 |
| Kasemene           | 90 (83.3) | 16 (16.7) | 106 (100) | 5.0 | 1.6 | 66.7 | 2.7 (0.6) |
| Nyabuye            | 186 (81.2) | 43 (18.8) | 229 (100) | 4.3 | 1.4 | 62.5 | 2.2 (0.8) |
| Nyantonzi          | 99 (82.5) | 21 (17.5) | 120 (100) | 4.7 | 1.5 | 65.0 | 2.3 (0.8) |
| Entroachment on forest edge |          |          |          |       |             |             |             |
| <0.3 km            | 114 (82.6) | 24 (17.4) | 138 (100) | 1.0 (1.0-1.1) | 1.3 (0.8-2.1) | 3.5 (4.1-11.1) |
| >0.3 km            | 454 (79.1) | 120 (20.9) | 574 (100) |             |             |             |
| Anaplasma ovis     |          |          |          |       |             |             |             |
| Age                |          |          |          |       |             |             |             |
| Juvenile           | 77 (27.7) | 201 (72.3) | 278 (100) | 1.2 (0.9-1.5) | 1.2 (0.9-1.7) | 3.7 (3.2-10.6) |
| Adult              | 104 (24.0) | 330 (76.0) | 434 (100) |             |             |             |
| Sex                |          |          |          |       |             |             |             |
| Male               | 20 (27.8) | 52 (72.2) | 72 (100) | 1.1 (0.7-1.6) | 1.1 (0.6-2.0) | 2.6 (9.0-14.3) |
| Female             | 161 (25.2) | 479 (74.8) | 640 (100) |             |             |             |
| Location (Parish)  |          |          |          |       |             |             |             |
| Kabango            | 60 (23.5) | 195 (76.5) | 255 (100) | 0.3 | 1 | 52.9 | 1 |
| Kasemene           | 27 (25.0) | 81 (75.0) | 108 (100) | 0.3 | 1.1 | 50.0 | 0.9 (1.0) |
| Nyabuye            | 73 (31.9) | 156 (68.1) | 229 (100) | 0.5 | 1.5 | 36.2 | 4.2 (0.1) |
| Nyantonzi          | 21 (17.5) | 99 (82.5) | 120 (100) | 0.2 | 0.7 | 65.0 | 1.8 (0.5) |
| Entroachment on forest edge |          |          |          |       |             |             |             |
| <0.3 km            | 38 (27.5) | 100 (72.5) | 138 (100) | 1.1 (0.8-1.5) | 1.2 (0.7-1.8) | 2.6 (6.1-11.3) |
| >0.3 km            | 143 (24.9) | 431 (75.1) | 574 (100) |             |             |             |

KEY: RR = relative risk, OR = odds ratios and aR = attributable risk.
4. Discussion

This molecular based study has demonstrated a high-level of *Anaplasma* infection in small ruminants within western Uganda. This was the first molecular study in small ruminants of Uganda and the prevalence reported in this study was much higher than reports from Kenya (40.8%) (Ringo et al., 2019) and Sudan (60.1%) (Lee et al., 2018). Here we targeted only one species of *Anaplasma* (*A. ovis*). The high levels of positivity of 16S is suggestive of other infectious agents within the small ruminant population. New strains of *Anaplasma* bacterial infections

**Table 3. Village prevalence of Anaplasmataceae and infection risk estimates in western Uganda.**

| Village (a) | Distance in km from forest | Anaplasmataceae prevalence | Anaplasma ovis prevalence | Risk estimates |
|-------------|----------------------------|----------------------------|---------------------------|----------------|
| Ewafula (68), 1.0 | 58 (85.3) 75.4–92.3 6 | 19 (27.9) 18.3–39.5 5 | 57.4 (42.3–72.4) 3.0 (2.1–4.6) 15.0 (5.9–39.0) |
| Kalongo (67), 0.5 | 54 (80.6) 69.8–88.8 13 | 24 (35.8) 25.1–47.8 1 | 44.8 (28.4–61.2) 2.3 (1.6–3.2) 7.4 (3.2–17.7) |
| Kyangyege (42), 0.8 | 34 (81.0) 67.0–90.7 12 | 15 (35.7) 22.4–51.0 2 | 45.2 (24.1–66.4) 2.3 (1.5–3.5) 7.7 (2.6–23.7) |
| Kapeka 1 (27), 0.8 | 18 (66.7) 47.6–82.4 10 | 5 (18.5) 7.1–36.4 11 | 48.1 (21.4–74.9) 3.6 (1.6–8.3) 8.8 (2.2–38.5) |
| Kapeka 2 (129), 0.7 | 92 (71.3) 63.1–79.6 11 | 31 (24.0) 17.3–32.0 9 | 47.5 (35.8–58.8) 3.0 (2.1–4.1) 7.9 (4.4–14.3) |
| Kapeka 3 (31), 1.9 | 25 (80.6) 64.0–91.8 2 | 5 (16.1) 6.2–32.2 12 | 64.5 (42.3–86.7) 5.0 (2.2–11.4) 21.7 (5.1–99.6) |
| Maraam (98), 0.2 | 72 (81.8) 72.7–88.9 8 | 25 (28.4) 197.3–38.5 4 | 53.4 (39.9–66.9) 2.9 (2.0–4.1) 11.3 (5.3–24.8) |
| Nyabeya 2 (14), 0.7 | 12 (85.7) 60.3–97.5 7 | 4 (28.6) 9.8–55.5 3 | 57.1 (20.1–94.2) 3.0 (1.3–7.1) 15.0 (1.8–176.1) |
| Nyakafunjara (18), 0.5 | 14 (77.8) 54.7–92.5 9 | 5 (27.8) 11.0–51.3 6 | 50.0 (16.2–83.3) 2.8 (1.3–6.1) 9.1 (1.6–55.1) |
| Rwempisi (31), 6.4 | 25 (80.6) 64.0–91.8 1 | 4 (12.9) 4.2–28.3 13 | 67.7 (46.3–89.2) 6.3 (2.5–15.9) 28.1 (6.1–144.3) |
| Rwengabi (58), 1.3 | 48 (82.8) 71.4–90.9 4 | 14 (24.1) 14.5–16.4 8 | 58.6 (42.2–75.0) 3.4 (2.1–5.5) 15.1 (5.6–41.7) |
| Rwentale 1 (89), 5.5 | 74 (83.1) 74.3–89.9 3 | 17 (19.1) 11.9–28.3 10 | 64.0 (31.6–76.4) 4.4 (2.8–6.7) 20.9 (9.1–48.5) |
| Simba (50), 0.2 | 42 (84.0) 70.9–92.8 5 | 13 (26.0) 15.3–39.4 7 | 58.0 (40.2–75.8) 3.2 (2.0–5.2) 14.9 (5.1–45.7) |
| Total (712) | 568 (79.8) 76.7–82.6 | 181 (25.4) 22.3–28.7 | 54.4 (49.9–58.8) 3.1 (2.8–3.6) 11.6 (9.0–15.0) |

**Figure 2. Geographical distribution of Anaplasmataceae with land elevation and vegetation cover in the study area. A – Digital land elevation showing villages with *Anaplasma* spp. B – Anaplasmataceae with vegetation cover in the villages. C – digital land elevation with *A. ovis* while D – vegetation cover with *A. ovis* in the study area.**
have been reported in China (Yang et al., 2016) and in Uganda (Ikwaph et al., 2010), however, emphasis continues to be placed on large ruminants. Exclusion of small ruminants from policy and control initiatives will compromise community disease control efforts due to small ruminants acting as reservoirs in farming communities.

Anaplasma infections have previously been found higher in ovines than caprines in studies in Sudan and Kenya (El Imam et al., 2016; Lee et al., 2018; Ringo et al., 2019), in agreement with findings shown in this study. There is a need for a more holistic approach in policy for vector control in Uganda given small ruminants, especially ovines are equally infected with Anaplasma bacteria as large ruminants (Kasozi et al., 2019; Matovu et al., 2020; Vudrifo et al., 2016), and are critical to community livelihoods. The presence of Anaplasma parasites in ticks at the forest edge (Punsantsogvoo et al., 2014), increased wildlife-human conflict through increased crop raiding by wildlife (Siljander et al., 2020), disrupts ecosystem health when vectors are exported from the forest to the community by wildlife during crop and animal raids and livestock grazing activities (Weny et al., 2017). Although the lack of sequencing leaves Anaplasma ovis identification without rigorous confirmation, we consider that our positive PCR results for A. ovis MSP4 gene makes A. ovis identification the most likely interpretation. Accordingly, the risk of A. ovis infections in this study was found to be the same for age and sex, and this runs contrary to findings from Sudan in which risk was highest in juveniles and males (Lee et al., 2018). Infections of A. ovis were also found to be similar in males and females in this study, contrary to findings in Brazil in which males were found to be at higher risk (Da Silva et al., 2018). This may be due to poor animal welfare practices whereby male animals are not prioritized in disease control strategies.

This was the first study reporting the molecular epidemiology of Anaplasmataceae in small ruminants of Uganda, however, further studies using the 16S rRNA and other gene targets related to Anaplasmataceae (Ali et al., 2017; Ringo et al., 2019; Seo et al., 2018; Shi et al., 2020; Yang et al., 2016), would pave way for the characterization of Anaplasma infections in both small and large ruminants in Uganda to understand evolutionary changes and sources of variation for improved animal health.

There was an association between human settlement in the mid-lands and agricultural activities at the forest edge were associated and prevalence of Anaplasmataceae. In China, Anaplasma infections were associated with farming communities (Yang et al., 2016). Since ticks at the forest edge carry Anaplasma parasites (Punsantsogvoo et al., 2014), human-livestock activities at the forest edge increase infection rates. Increased community encroachment on the forest, such as in endemic communities of Rwempi, Nyabeya and Kapeka 3 create an environmental interface where small ruminants are continuously exposed to disease vectors at the forest edge. Infections were found to be higher in communities closer (<0.3 km from forest edge) than those farther inland. This increase in the infection risk in the surrounding villages leads to a greater disease burden in the communities at the forest edge. The presence of zoonotic species of A. phagocytophilum and A. capra raises public health importance of Anaplasmataceae (Shi et al., 2020). Policy failures to prioritize animal health and devise robust policies to limit small ruminant (and human) exposure to disease vectors will continue to lead to increased livestock losses in the animal industry and impact on human health and wellbeing.

5. Conclusion

It is clear from the high prevalence of Anaplasmataceae reported in this study that anaplasmosis in small ruminants in Uganda has in general been under-reported. Anaplasma ovis prevalence was higher in ovines than caprines that reflects the limited community treatments on ovines during routine animal disease control activities. There is a need to include regular surveys to provide data to guide policy makers and to prioritize small ruminant health in rural communities of East Africa.

Findings in the study support that more awareness is needed to promote ecosystem health in disease control strategies to eliminate reservoirs of infections, including those that are zoonotic. Agricultural activities at the forest edge increase human-domestic-wildlife interactions (Kasozi et al., 2019), leading to increased prevalence of infection. Global health disease control strategies which promote ecosystem health and limit community encroachments on the forest edge would support one health practice. In this study, we placed emphasis on A. ovis due to the acquisition of suitable control DNA, however, the large number of positives for Anaplasmataceae suggests other species are present and this demonstrates the need to conduct additional studies in the region including targeting A. phagocytophilum, and DNA sequencing which were impossible to conduct in this study due to budgetary restrictions. Furthermore, differences in sample size in caprines (n = 666) and ovines (n = 46), since in this community, there were just more caprines than ovines.

Declarations

Author contribution statement

K. Kasozi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

S. Welburn and E. MacLeod: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

D. Nalumunya, M. Namayanja, K. Matama, K. Zalwango, W. Matovu, G. Zirintunda, J. Ekou, S. Kembabazi, C. Mugasera, A. Kitibwa, D. Tayebwa, S. Musinguzi, M. Mahero, I. Ssengendo, A. Nanteza and E. Matovu: Contributed reagents, materials, analysis tools or data.

G. E.-S. Batiba and N. Marraiki: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data associated with this study can be accessed at https://figshare.com/s/2d7b22c5a014991fae4d.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Akejo, B.S., Otto, O., 2017. Ruminants, resilience and poverty: assessing the socioeconomic role of small ruminants in the pastoral areas of northeastern Uganda. In: Swedish University of Agricultural Sciences.

Ali, Y., Rahbari, S., Shayan, P., Sadeghi-dehkordi, Z., Bahonar, A., 2017. Molecular detection of Anaplasma marginale and Anaplasma ovis in sheep and goat in west highland pasture of Iran. Asian Pacific J. Tropical Biomed. 7 (5), 455-459.

Balinandi, S., Chitimia-Dobler, L., Grandi, G., Nakayiki, T., Kabasa, W., Bbira, J., Lutwama, J.J., Bakkes, D.K., Malmberg, M., Mugisha, L., 2020. Morphological and molecular identification of ticks of tick species (Acarina: Ixodidae) infesting cattle in Uganda. Parasitol. Res. 119 (8), 2411-2420.

Bardosh, K., Waiswa, C., Welburn, S.C., 2013. Conflict of interest: use of pyrethroids and amides against tsetse and ticks in zoonotic sleeping sickness endemic areas of Uganda. Parasites Vectors 6 (1), 204.

Bekker, C.P.J., Vos, S. De, Taou, A., Spargano, O.A.E., Jongejan, F., 2002. Simultaneous application of msp1 and msp2 gene sequences to detect previously unknown anaplasma genotype in cattle from Uganda. Int. J.Animal Veterinary Adv. 7, 580-587.

Bilgic, H.B., Baler, S., Kose, O., Unlu, A.H., Hacilarboglu, S., Eren, H., Weitz, T., Karagec, T., 2017. Prevalence of tick-borne haemoparasites in small ruminants in Turkey and diagnostic sensitivity of single-PCR and RLB. Parasites Vectors 10 (1), 211.

Da Silva, N.B., Taus, N.S., Johnson, W.C., Mira, A., Schnitter, L., Valente, J.D.M., Vidotto, O., Masterson, H.E., Vieira, T.S.W.I., Ueti, M.W., Vieira, R.F.C., 2018. First report of Anaplasma marginale infection in goats, Brazil. PloS One 13 (8), 1-6.

Dahmani, M., Davout, B., Sambou, M., Bassene, H., Scandola, F., Ameer, T., Raout, D., Fenollar, F., Medimannov, O., 2019. Molecular investigation and phylogeny of species of the Anaplasmataceae infecting animals and ticks in Senegal. Parasites Vectors 12 (1), 405.

Devendra, C., 1994. Small Ruminants: potential value and contribution to sustainable livestock farming as a means of poverty alleviation in rural southern India. Trop. Anim. Health Prod. 26 (2), 303-308.

El Imam, A.H., Hassan, S.M., Gameel, A.A., El Hussein, A.M., Taha, K.M., 2005. Goats - a pathway out of poverty. Small Rumin. Res. 60 (1-2 SPEC. ISS.), 238-239.

Fenollar, F., Mediannikov, O., 2019. Molecular investigation and phylogeny of Theileria, Anaplasma and Ehrlichia species among apparently healthy sheep in central and western Kenya. Onderstepoort J. Vet. Res. 86 (1), 1-8.

Herrero, M., Grace, D., Njuki, J., Johnson, N., Enahoro, D., Silvestri, S., Rutagarama, I., Kasozi, K.I., Namayanja, M., Gaithuma, A.K., Mahero, M., Matovu, E., Yamagishi, J., Sugimoto, C., MacLeod, E., 2019. Prevalence of hemoprotozoan parasites in small ruminants in Kenya. Ticks and Tick-Borne Diseases 9 (3), 598-604.

Irons, M.G., Odu, C.H., Proctor, T., Odu, C.K., Bester, B., 2006. Effects of housing density and management practices on the prevalence of ticks and tick-borne diseases in sheep. Ticks and Tick-Borne Diseases 7, 1331.

Irons, M.G., Odu, C.H., Proctor, T., Odu, C.K., Bester, B., 2006. Effects of housing density and management practices on the prevalence of ticks and tick-borne diseases in sheep. Ticks and Tick-Borne Diseases 7, 1331.

Kasozi, K.I., Namayanja, M., Gaithuma, A.K., Mahero, M., Matovu, E., Yamagishi, J., Sugimoto, C., MacLeod, E., 2019. Prevalence of hemoprotozoan parasites in small ruminants in Kenya. Ticks and Tick-Borne Diseases 9 (3), 598-604.

Lalli, J.C., Sivashanmugam, N., Menon, U., 2013. The role of livestock in developing countries. Animal 7 (SUPPL.1), 3-18.

Lalljee, S.V., Soundararajan, C., Singh, Y.D., Sargison, N.D., 2019. The potential of small ruminants in alleviating poverty in the pastoral areas of northeastern Uganda. Socioeconomic role of small ruminants in the pastoral areas of northeastern Uganda. Trop. Anim. Health Prod. 51 (2), 303-311.

Lee, S.H., Mosaaid, E., Ibrahim, A.M., Ismail, A.A., Adjou Moumouni, P.F., Liu, M., Ringo, A.E., Gao, Y., Guo, H., Zheng, W., Elstratiou, A., Galon, E.M., Li, J., Therkose, I., Inoue, N., Suzuki, H., Xuan, X., 2019. Molecular detection and genetic characterization of anaplasma species in small ruminants reveals the presence of novel strains closely related to A. phagocytophilum in Turkey. Vector Borne Zoonotic Dis. 15 (10), 480-506.

Lee, S.H., Mossaad, E., Ibrahim, A.M., Adjou Moumouni, P.F., Liu, M., Ringo, A.E., Aboge, G.O., Adjou Moumouni, P.F., Lee, S.H., Jirapattharasate, C., Liu, M., Gao, Y., Guo, H., Zheng, W., Elstratiou, A., Galon, E.M., Li, J., Therkose, I., Inoue, N., Suzuki, H., Xuan, X., 2019. Molecular detection and genetic characterization of anaplasma species in small ruminants reveals the presence of novel strains closely related to A. phagocytophilum in Turkey. Vector Borne Zoonotic Dis. 15 (10), 480-506.

Lee, S.H., Mossaad, E., Ibrahim, A.M., Adjou Moumouni, P.F., Liu, M., Ringo, A.E., Aboge, G.O., Adjou Moumouni, P.F., Lee, S.H., Jirapattharasate, C., Liu, M., Gao, Y., Guo, H., Zheng, W., Elstratiou, A., Galon, E.M., Li, J., Therkose, I., Inoue, N., Suzuki, H., Xuan, X., 2019. Molecular detection and genetic characterization of anaplasma species in small ruminants reveals the presence of novel strains closely related to A. phagocytophilum in Turkey. Vector Borne Zoonotic Dis. 15 (10), 480-506.

Said, M. Ben, Belkahia, H., Alberti, A., Zobba, R., Bousrih, M., Yahiaoui, M., Daaloul- Balinandi, S., Chitimia-Dobler, L., Grandi, G., Nakayiki, T., Kabasa, W., Bbira, J., Lutwama, J.J., Bakkes, D.K., Malmberg, M., Mugisha, L., 2020. Morphological and molecular characterization of the presence of Rhipicephalus microplus in southeast Africa. Ticks and Tick-Borne Diseases 11 (7), 1331-1337.

Vudrilo, P., Okwee-Acii, J., Tayebwa, D.S., Byaruhanga, J., Kakoosa, S., Wampande, E., Omara, R., Mahindo, J.B., Tweyongyere, R., Owiny, D.O., Hatta, T., Tsugi, N., Umemiya-Shirafuji, R., Xuan, X., Kanemaeda, M., Fujisaki, K., Suzuki, H., 2016. Emergence of multi-ancestral resistant Rhipicephalus ticks and its implication on chemical tick control in Uganda. Parasites Vectors 9 (1), 4.

Weny, O., Okwee-Acii, J., Okeek, S.G., Tumwine, G., Nyandoro, S., Monay, S., Sizibere, H., Goldberg, T.L., 2017. Prevalence and risk factors associated with hemoparasites in cattle and goats at the edge of Kilube national park, western Uganda. J. Parasitol. 103 (1), 69-74.

Yang, J., Li, Y., Liu, Z., Liu, J., Niu, Q., Ren, Q., Chen, Z., Guan, G., Luo, J., Yin, H., 2015. Molecular detection and characterization of Anaplasma spp. in sheep and cattle from Xijiang, northwest China. Parasites Vectors 8 (1), 108.

Yang, J., Liu, Z., Niu, Q., Liu, J., Han, R., Liu, G., Shi, Y., Liu, J., Yin, H., 2016. Molecular survey and characterization of a novel Anaplasma species closely related to Anaplasma capra in ticks, northwestern China. Parasites Vectors 9 (1), 603.

Lalli, J.C., Sivashanmugam, N., Menon, U., 2013. The role of livestock in developing countries. Animal 7 (SUPPL.1), 3-18.