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# Molecular Characterization of *Anaplasma* and *Ehrlichia* Species in Different Cattle Breeds and Age Groups in Mbarara District (Western Uganda)

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**Abstract:** *Anaplasma* and *Ehrlichia* sp. (AEs) cause significant economic losses to the livestock sector in Uganda. The aim of this study was to determine the prevalence of AEs in cattle from Kashaari county-Mbarara district (Uganda) so as to compare the prevalence of AEs in different cattle breeds, age groups, sub county of origin and management systems and predictor(s) of infection with AEs. Such information is deemed necessary to direct future tick-borne disease control programs. A single pair of primers was used to amplify a 492-498bp fragment of the 16SRNA gene spanning the V1 region conserved for both AEs. PCR products were transferred onto the Reverse Line Blot (RLB) membrane and AEs amplicons in the PCR products allowed to hybridize with AE species-specific oligonucleotides. The prevalence of *Ehrlichia* sp. was 5.1% (CI = 95%, 2.9-7.3%) whereas that of *Anaplasma* species was 5.3 % (CI = 95%, 3-7.6%). Individual AEs detected include; *A. bovis* (5.1%, CI = 95%, 2.9-7.3%), *E. ruminantium* (4.5%, CI = 95%, 2.4-6.6%), *A. marginale* (3.7%, CI = 95%, 1.8-5.6%), *A.* (*E.*) *phagocytophilum* (2.7%, CI = 95%, 1.1-4.3%), *E. ovina/canis* (2.7%, CI = 95%; 1.1-4.3%), *E.* sp. (*omatjenne*)(1.9%, CI = 95 %, 0.5-3.3%). Cattle breed was found to be the best predictor of infection. To further understand bovine tick-borne parasites in Uganda, we recommend that studies covering a wider area and over longer periods, investigation of breed as a predictor of infection, molecular genetic characterization, transmission and pathogenicity studies on the different strains of AEs be carried out.

**Key words:** *Anaplasma* and *Ehrlichia* sp. (AEs), cattle age, cattle breed, predictors of infection, prevalence, Reverse Line Blot hybridization (RLB)

## INTRODUCTION

Anaplasmosis is a Rickettsial disease of cattle, goats and sheep (Theiler, 1910; Theiler, 1911; Lestoquard, 1924; Ristic, 1968) mainly characterized by pyrexia, progressive anemia and icterus (Kuttler, 1984; Magona *et al*., 2008). The disease is widely distributed in the tropics affecting both wild and domestic ungulates (Minjauw and McLeod, 2003). Anaplasmosis, a disease of highly susceptible exotic cattle breeds also affects malnourished and stressed local breeds with infected animals becoming carriers for life; prone to show clinical signs of disease following periods of stress. *Anaplasma* and *Babesia* species are both vectored by *Boophilus microplus* (McCosker, 1979; Ristic, 1981) resulting in common mixed infections of both species. The effect of anaplasmosis is exacerbated in babesiosis endemic regions (Jonsson *et al*., 2008).

*Anaplasma marginale* is the causative agent of bovine anaplasmosis worldwide (Kocan *et al*., 2004; DelaFuente *et al*., 2007). *A. centrale* causes a less severe disease than that caused by *A. marginale* in which severe anemia and mortality of up to 50% are recorded (Minjauw and McLeod, 2003). *Boophilus* tick species are responsible for biological transmission of *Anaplasma* species (McCosker, 1979; Minjauw and McLeod, 2003), however, mechanical transmission of anaplasmosis by *Diptera* flies and sharp objects (for example; needles) occurs (Kocan *et al*., 2003; Minjauw and McLeod, 2003; DelaFuente *et al*., 2007).

*Ehrlichia* sp., the most important species being *E. ruminantium* (heart water), are transmitted by *Amblyomma* ticks (McCosker, 1979). Heart water is limited to Sub-Saharan Africa and some Caribbean islands (Uilenberg, 1983; Bekker *et al*., 2002; Minjauw and McLeod, 2003). The disease is severe in small ruminants, exotic and malnourished or stressed local breeds of cattle. High losses are also observed in naïve local cattle that have been moved to an area in which the disease is endemic (Minjauw and McLeod, 2003). Anaplasmosis and ehrlichiosis cause significant economic losses to the livestock industry in the Sub-tropical and Caribbean regions of the world (Uilenberg, 1983; Norval *et al*., 1995; Uilenberg, 1995; Deem *et al*., 1995;

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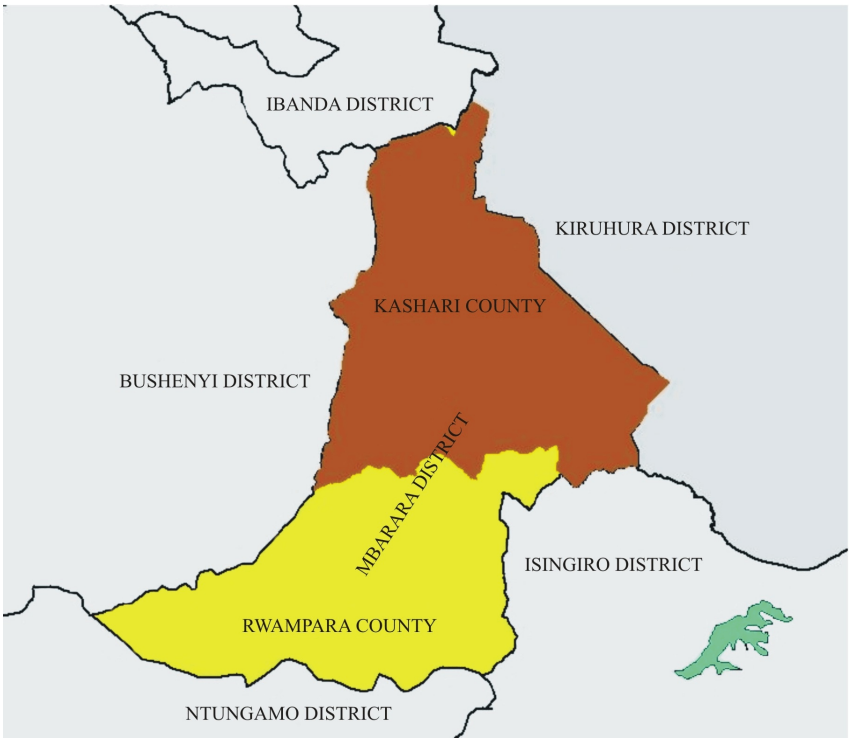


Fig. 1: Map of Mbarara district showing the geographical location of Kashaari county


Fig. 2: Map of Kashaari County

Okello-Onen *et al*., 2003). In Mbarara district (Fig. 1) and Kashaari county (Fig. 2) in particular, attempts to improve the local breeds through importation of high yielding dairy breeds so as to increase milk production has often led to high mortalities due to Tick-Borne Diseases (TBDs) (Loria *et al*., 1999; Georges *et al*., 2001; Oura *et al*., 2004a). Consequently, cross breeding programs have not yielded benefits as expected, in part, due to TBDs like anaplasmosis and cowdriosis.

Mbarara district (Fig. 1) with her estimated cattle population of 715,000 heads in 26,266 herds (NBOS, 2001) is one of the leading districts in livestock production in Uganda. The district supplies surrounding areas, commercial centers and Kampala city with livestock products including meat, milk, and hides for export. This district, therefore, provides a significant supply of livestock products to the urban and rural population in Uganda. In order to improve milk and meat production so as to boost farmers' incomes and food security, there has been massive importation of exotic dairy breeds like Holstein Frisians, into Uganda and particularly Mbarara district. This has resulted into mixed breed cattle management systems being popular than ever before. Exotic cattle breeds are unfortunately highly susceptible to TBDs (Ajayi *et al*., 1982; Jacobsen, 1983; Paling *et al*., 1991) requiring high levels of tick control and/or boosting of the immunity of these animals through safe use of vaccines. Scaling up use of aracacides to control ticks and therefore TBDs is not only environmentally damaging but could interfere with establishment of endemic stability (Norval *et al*., 1992; Figueroa *et al*., 1998; Coleman *et al*., 2001) that has long been known to occur for anaplasmosis and ehrlichiosis and other TBDs. Further more, stepping up acaracide use to suppress tick population makes it impossible for use of the mixed breed management system for it disrupts endemic stability in local breeds. Endemic stability has been reported in indigenous breeds of Africa and not in exotic breeds (Norval *et al*., 1992; Perry and Young, 1995). Use of attenuated vaccines in control of TBDs is also met with several shortcomings (Morzaria *et al*., 2000; DelaFuente *et al*., 2002; Oura *et al*., 2003). Therefore, future control of Ticks and tick-borne diseases by vaccination requires development and use of safe multivalent subunit vaccines against all Tick-Borne Pathogens (TBPs) that infect cattle in the tropics (Morzaria *et al*., 2000; Oura *et al*., 2003). Successful use of multivalent subunit TBD vaccines requires that the seroprevalence of different TBPs in the tropics first be well understood (Oura *et al*., 2004a). Knowledge of prevalence of the different AEs is prerequisite information in formulation of multivalent subunit TBD vaccines. Moreover, such prevalence data is used in assessment of the impact of anaplasmosis and ehrlichiosis and other TBDs on livestock production (Minjauw and McLeod, 2003). Despite the fore mentioned apparent need for AEs prevalence data in control of anaplasmosis and ehrlichiosis, this information is very scarce and in most cases unavailable. This study was therefore carried out to determine the prevalence of AEs in cattle from Kashaari county (Fig. 2)-Mbarara district (Uganda) using the molecular diagnostic method of Reverse Line Blot hybridization (RLB). We further compared the prevalence of AEs in different cattle breeds, age, Sub County of origin and different management systems. Such prevalence data is deemed to help in development of intergraded TBD control methods including the use of; multivalent vaccines, pasture management programs, resistant cattle breeds and effective use of acaracides.

Grazing (management) system, age, agro-ecological zones (altitude above sea level) and breed of animals (Ajayi *et al*., 1982; Jacobsen, 1983; Paling *et al*., 1991) have been reported before to influence the probability of infection with Aes (Gitau *et al*., 1997; Maloo *et al*., 2001; Rubaire-Akiiki *et al*., 2004; Rubaire-Akiiki *et al*., 2006). This in turn influences the spatial and temporal distribution of anaplasmosis and ehrlichiosis. In view of the above, this research went ahead to explore the best predictor of infection with AEs with regard to management (grazing) system, age, breed and place of origin. Determination of predictor factors for infection with both AEs is intended to create a knowledge base on how factors like management system; breed and age of the animal can be integrated in sustainable and cost-effective TBD control.

## MATERIALS AND METHODS

**Study area and study design:** A cross sectional study was carried out between February and March, 2008. February is the end of a dry season and March is the beginning of a rainy season that stretches to the end of May. The average minimum and maximum annual temperatures are 14.6 and 26.3ºC, respectively. The annual rainfall is 822 mm occurring in 114 rainy days in the year shared in two rainy seasons (March-May and September–December) (Faye *et al*., 2005) The study area was recently described by Muhanguzi *et al*. (2010.) Briefly, the study was carried out in Mbarara district (Fig. 1) in Kashaari county (Fig. 2) which is located in south-western Uganda. Kashaari county being the county with the highest number of cattle estimated at 102,143 heads in about 3,752 herds was chosen for this study (NBOS, 2001). Most cross breeding programs have concentrated in this county as well. The main cattle breeds were (i) the Ankole, which is a hybrid of zebu (*Bos indicus*) and long-horned cattle (*Bos taurus*) that is well adapted to local conditions and thought to be resistant to TBDs (ii) the exotic breeds imported from Europe or the United States of America to improve the

Table 1: Oligonucleotide probe sequences which were hybridized onto the reverse line blot membrane

| Oligonucleotide probe | Sequence | References |
|---|---|---|
| *Ehrlichia/Anaplasma* catch-all | GGG GGA AAG ATT TAT CGC TA | (Bekker *et al*., 2002; Kamst-van and Zwart, 2002; Oura *et al*., 2004a) |
| *A. centrale* | TCG AAC GGA CCA TAC GC | (Bekker *et al*., 2002; Kamst-van and Zwart, 2002; Oura *et al*., 2004a) |
| *A. marginale* | GAC CGT ATA CGC AGC TTG | (Bekker *et al*., 2002; Georges *et al*., 2001; Kamst-van and Zwart, 2002; Oura *et al*., 2004a) |
| *A. phagocytophilum* | TTG CTA TAA AGA ATA ATT AGT GG | (Bekker *et al*., 2002; Kamst-van and Zwart, 2002) |
| *A. phagocytophilum* | TTG CTA TGA AGA ATA ATT AGT GG | |
| *A. phagocytophilum* | TTG CTA TAA AGA ATA GTT AGT GG | |
| *A. phagocytophilum* | TTG CTA TAG AGA ATA GTT AGT GG | |
| *E. ruminantium* | AGT ATC TGT TAG TGG CAG | |
| *A. bovis* | GTA GCT TGC TATGRG AAC A | (Bekker *et al*., 2002; Kamst-van and Zwart, 2002; Oura *et al*., 2004a) |
| *E. chaffeensis* | ACC TTT TGG TTA TAA ATA ATT GTT | |
| *E.* sp. *omatjenne* | CGG ATT TTT ATC ATA GCT TGC | |
| *E. ovina/canis* | TCT GGC TAT AGG AAA TTG TTA | |

dairy production (iii) cross breeds of those two main breeds, and (iv) other minor breeds such as boran zebu or East-African shorthorn zebu (Faye *et al*., 2005).

**Sampling and sample size determination:** Six of the 9 sub counties of Kashaari county were selected using computer generated random numbers. The selected sub counties were Biharwe, Bubaare, Kakiika, Kashare, Rubaya and Rwanyamahembe. The used expected prevalence of AEs as taken from literature (Ssenyonga *et al*., 1992) was 42%, N (population size) = 102,143 heads of cattle. Using Win Episcope 2.0 Soft ware, accepted error of 5%, at 95% level of confidence, the sample size was calculated as thus, sampling fraction = 0.366, sample size n = 374, adjusted sample size n(a) = 373, Used value of n = 375 heads of cattle. The sampled animals were then stratified according to age, breed, management system and sub county of origin and prevalence of AEs analyzed against these variables. Each of the sampled farmers was requested to complete a questionnaire so as to get information about the farmers' biodata, livestock kept, management systems used by different farmers, problems that farmers face in livestock production and assessment of livestock productivity.

**Clinical samples:** 1 mL of jugular blood was collected into EDTA coated vaccutainer tubes from each of the sampled animals. Blood samples were transported on ice to the laboratory for further storage and processing. In the laboratory, blood samples were stored at -20ºC until required for PCR.

**DNA extraction and PCR:** DNA extraction was done as earlier described by D'Olveira *et al*. (1995) and DNA Stored at -20ºC until needed for PCR. PCR was completed according to (Bekker *et al*., 2002). A single pair of primers, Ehr-F (GGA ATT CAG AGT TGG ATC MTG GYT CAG) as previously described by Schouls *et al*. (1999) and the reverse primer Ehr-R (5'-Biotin-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3') as modified by Bekker *et al*. (2002) was used to amplify a 492-498bp fragment of the 16SRNA gene spanning the V1 region. This region is conserved for both AEs. Primers were obtained from Isogen (Maarssen, the Netherlands). The reverse primer was biotinylated at the 5' end to facilitate visibility of PCR products upon hybridization with oligonucleotide probes (Table 1). The specific oligonucleotides which were hybridized on the membrane contained an N-terminal N-(trifluoracetamidohexyl-cyanoethyl, N, N-Diisopropyl phosphoramidite [TFA])-C6 amino linker (Isogen) to help link the oligonucleotides (Table 1) onto the Biodyne C membrane (Pall Biosupport, Ann Arbor, Mich.).

**Reverse line blot hybridisation:** Before PCR products were applied onto a biodyne C blotting membrane, a volume of 15 µL of each of the PCR product was diluted to an end volume of 150 mL of 2x SSPE/0.1% SDS, heated for 10 min at 100°C, and cooled on ice immediately. A 10 sec short centrifugation run at 12000 rev. /min was carried out before denatured PCR samples were applied into miniblotter (Pall Biosupport, Ann Arbor, Mich.) slots and incubated for 60 min at 42ºC. RLB was completed as described previously by Gubbels *et al*. (1999).

## RESULTS

To determine the prevalence of AEs in Kashaari county, 375 blood samples were collected. The animals sampled were characterized according to age, breed, management system and sub county of origin. Polymerase Chain Reaction (PCR) and Reverse Line Blot hybridization (RLB) assays were done to find out AEs with which animals were infected. The probable effect of age, breed, management system and sub county of origin on the prevalences of these piroplasms were tested by back ward logistic regression.

PCR/RLB results: Before PCR products were applied on the RLB membrane, 5-10 PCR products including positive and negative PCR controls were randomly selected and 5 µL of the product in a loading dye electrophroretically (120 V/20 min) separated in 2% agarose gel so as to confirm that the right gene fragment size (base pairs-bp) was amplified. The gel was stained in ethidium bromide for 20-30 min to enhance visibility

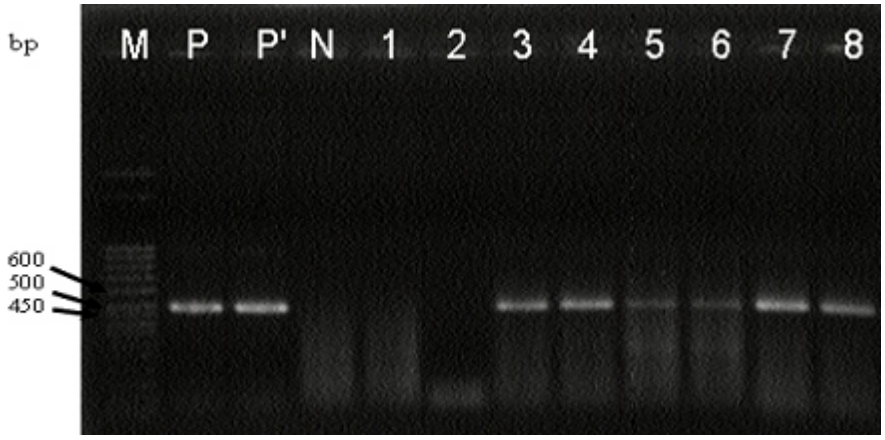


Fig. 3: 2% agarose gel representation of 11 PCR products
M: Molecular marker, P: positive control (*A. marginale*), P': positive control (*E. ruminatium*), N, negative control, 1-8 Test sample PCR products. 490bp was the expected fragment size and can be seen in the two positive controls P and P' as well as Test samples 3, 4, 7 and 8 which were strongly positive for *Anaplasma* /or *Ehrlichia species.* Test samples 5 and 6 were weakly positive while 1 and 2 and the negative control, N, were negative

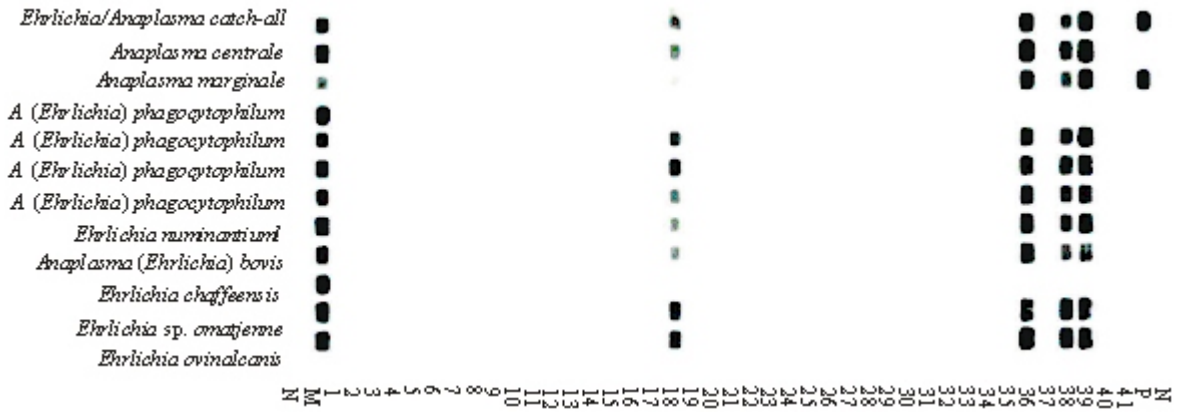


Fig. 4: Reverse line blot representation of 41 test sample PCR products
PCR: products were applied in vertical lanes and Species-specific oligonucleotides in horizontal rows. N; RLB negative control, M; reverse line blot membrane positive marker, 1-41; PCR products from test DNA samples, P; PCR positive control (*A. marginale*), N; PCR negative control. Samples 1-17, 19-35, 37, 40 and 41 were negative; sample 18, 36, 38 and 39 had dual infection with *E. ovina/canis*, *E.* sp. *omatjenne*, *A.* (*Ehrlichia*) *bovis*, *E. ruminantium*, *A.* (*E.*) *phagocytophilum*, *A. marginale* and *A. centrale*

of the bands under ultraviolet light. The expected fragment size was 490bp. Polymerase Chain Reaction (PCR) would therefore be taken as having worked if a 490bp fragment is separated in the PCR positive control lane and not in the PCR negative control lane. Otherwise, PCR would be repeated before progressing to RLB. A representative 2% agarose gel of 11 PCR products is shown in Fig. 3.

To determine which AEs were present in any of the blood samples collected, the PCR products from each of the samples were allowed to hybridize with *Anaplasma*/*Ehrlichia* species- specific oligonucleotides already appended onto the RLB membrane. PCR products were applied in vertical lanes and *Anaplasma*/*Ehrlichia* species-specific oligonucleotides in horizontal rows. Black rectangular blots on the Hyper ECL film indicate a positive signal and their absence indicate negative signal. A signal was taken as a true signal when such a blot showed both on the *Anaplasma/Ehrlichia* catch-all and on the section corresponding to the species-specific oligonucleotide. Representative results of the exposed (with RLB membrane/ECL detection reagents) and developed hyper ECL film are as in Fig. 4.

Prevalence of different Anaplasma and Ehrlichia sp. in Kashaari County: Of the 375 cattle sampled, 21 (5.6%, 95% confidence interval - CI, 3.3-7.9%) of them were

Table 2: Prevalence of Anaplasma and Ehrlichia species in cattle from Kashaari county (Uganda)

| Variable | No. of examined | Positive | Percentage positive (95% CI) |
|---|---|---|---|
| **Individual prevalence** | | | |
| Total positive (AEs) cases | 375 | 21 | 5.6 (3.3-7.9) |
| *Anaplasma* sp. | 375 | 20 | 5.3 (3.0 -7.6) |
| *Ehrlichia* sp. | 375 | 19 | 5.1 (2.9-7.3) |
| *A.* (*E.*) *bovis* | 375 | 19 | 5.1 (2.9-7.3) |
| *A. centrale* | 375 | 17 | 4.5 (2.4-6.6) |
| *E. ruminantium* | 375 | 17 | 4.5 (2.4-6.6) |
| *A. marginale* | 375 | 14 | 3.7 (1.8-5.6) |
| *A.* (*E.*) *phagocytophilum* | 375 | 10 | 2.7 (1.1-4.3) |
| *E. ovina/canis* | 375 | 10 | 2.7 (1.1-4.3) |
| *E.* sp. (*omatjenne*) | 375 | 7 | 1.9 (0.5-3.3) |
| **Sub county** | | | |
| Kakiika | 54 | 5 | 9.3 (6.4-12.2) |
| Rubaya | 44 | 4 | 9.1 (6.2-12.0) |
| Kashare | 82 | 6 | 7.3 (4.7-9.9) |
| Rwanyamahembe | 68 | 4 | 5.9 (3.5-8.3) |
| Biharwe | 49 | 1 | 2.0 (0.6-3.4) |
| Bubaare | 78 | 1 | 1.3 (0.1-2.5) |
| **Age group** | | | |
| Calves 1 (0-5.9 months) | 47 | 3 | 6.4 (3.9-8.9) |
| Calves 2 (6-8.9 months) | 50 | 3 | 6.0 (3.6-8.4) |
| Adult cattle (Above 2 years ) | 212 | 12 | 5.7 (3.3-8.1) |
| Young cattle (9- 24 months) | 67 | 3 | 4.5 (2.4-6.6) |
| **Animal breed** | | | |
| Exotic (Pure Friesians) | 13 | 3 | 23.1 (18.8-27.4) |
| Cross breed (Ankole long horned cattle and other zebu breeds x Friesians) | 152 | 11 | 5.2 (2.9-7.5) |
| Local (Ankole long-horned cattle) | 210 | 7 | 4.6 (2.5-6.7) |
| **Management system** | | | |
| Extensive | 349 | 21 | 6.0 (3.6-8.4) |
| Semi-intensive | 22 | 0 | 0 (0) |
| Intensive 4 | 0 | 0 (0) | |

Table 3: Summary of statistical variables that were analyzed as predictors of infection with *Anaplasma* and *Ehrlichia* sp.

| Variable | | No. (n) positive (95 % CI) | Percentage prevalence | $X^2$ | p-value |
|---|---|---|---|---|---|
| Breed | Exotic | 3 | 23.1 (18.8-27.4) | *7.85 | 0.02 |
| | Cross breed | 11 | 5.2 (2.9-7.5) | | |
| | Local | 7 | 4.6 (2.5-6.7) | | |
| Age | Calves 1 | 3 | 6.4 (3.9-8.9) | 0.21 | 0.967 |
| | Calves 2 | 3 | 6.0 (3.6-8.4) | | |
| | Adult cattle | 12 | 5.7 (3.3-8.1) | | |
| | Young cattle | 3 | 4.5 (2.4-6.6) | | |
| Management system | Extensive | 21 | 6.0 (3.6-8.4) | 1.386 | 0.271 |
| | Semi-intensive | 0 | 0 (0) | | |
| | Intensive | 0 | 0 (0) | | |
| Sub county of origin | Kakiika | 5 | 9.3 (6.4-12.2) | 6.775 | 0.258 |
| | Rubaya | 4 | 9.1 (6.2-12.0) | | |
| | Kashare | 6 | 7.3 (4.7-9.9) | | |
| | Rwanyamahembe | 4 | 5.9 (3.5-8.3) | | |
| | Biharwe | 1 | 2.0 (0.6-3.4) | | |
| | Bubaare | 1 | 1.3 (0.1-2.5) | | |

*: Only breed of animal was statistically significant ($p<0.05$) predictor at bi-variate analysis. However all the variables (breed, management system, age and sub county of origin) were modeled (Multi-variate analysis) to find out the best predictor of infection of cattle with AEs.

positive with AEs. The prevalence of *Ehrlichia* sp. was 5.1% (95% CI, 2.9-7.3%) whereas that of *Anaplasma* species was 5.3% (95% CI, 3.0-7.6%). The most and least prevalent *Anaplasma* sp. detected were *A. bovis* (5.1%, 95% CI, 2.9-7.3%) and *Anaplasma* (*Ehrlichia*) *phagocytophilum* (*A.* (*E.*) *phagocytophilum*) (2.7%, 95% CI, 1.1-4.3%) respectively. *E. ruminantium* (4.5%, 95% CI, 2.4-6.6%) and *E.* sp. (*omatjenne*) (1.9%, 95% CI, 0.5-3.3%) were the most and least detected *Ehrlichia* sp. respectively. Forty eight percent (95% CI, 42.9-53.1 %) of the positive samples were positive for the human infective *A.* (*E.*) *phagocytophilum*. Different AEs detected are summarized in Table 2. AEs occurred together as dual infections in 90.5% (95% CI, 87.5-93.5%) of all the positive cases.

The sub counties of Kakiika and Bubaare recorded the highest and lowest prevalence of AEs at 9.3% (95% CI, 6.4-12.2%) and 1.3% (CI = 95%, 0.1-2.5%)

respectively. There is no statistically significant difference ($X^2$ = 6.775, p = 0.258; 5df) between prevalence of AEs across the sub-counties of Kashaari County.

Calves-1 (0 - 5.9 months of age) and calves-2 (6- 8.9 months of age) had the highest prevalence of AEs at 6.4% (95% CI, 3.9-8.9%) and 6.0% (95% CI, 3.6-8.4%) respectively. Cattle between 9-24 months of age (young cattle) had the lowest prevalence of AEs of 4.5% (CI = 95%, 2.4-6.6%). There is no statistically significant difference ($X^2$ = 0.21, p = 0.967; 3df) in the prevalence of AEs among cattle of different age groups.

Exotic cattle (Pure Friesians) had the highest level of infection at 23.1 % (CI = 0.95, 18.8-27.4%). Despite the small number of pure exotic cattle (Friesians) sampled, the prevalence of AEs was significantly ($X^2$ = 7.85, p = 0.02) higher among this group; 23.1% ( 95% CI, 18.8-27.4%) compared to other breeds. Local (Ankole long-horned cattle /other zebu breeds) had the lowest level of infection of 4.6% (95% CI, 2.5-6.7%) compared to the rest of the cattle breeds.

Extensively managed animals had the highest prevalence of infection with AEs of 6% (95% CI, 3.6-8.4%). All the semi-intensively and intensively managed cattle were negative for both AEs. There was no statistically significant difference in the prevalence of AEs among differently managed cattle was not statistically significant ($X^2$ = 1.386, p = 0.271, 2df) Extensively managed animals refer to those animals which were left to graze in none or poorly fenced off farms during the day and kept in night bomas at night. Such animals were watered on communal watering places and were given no commercial concentrates at all. Semi-intensively managed animals (n = 25) here means those animals which were either housed in permanent/semi permanent structures or kept in well fenced off and paddocked farms with watering places. Such animals were given some commercial concentrates especially at the time of milking addition to feeding on natural pastures. These herds mixed with no neighboring herds. Intensively managed animals (n = 4) are those, which were kept indoors and pastures harvested for them. They were also supplemented with commercial concentrates.

The best model that describes the predictor of infection with AEs was obtained from; Logit (EAs) = $\beta_0$ + $\beta_1$ + $\beta_2$ + e; where $\beta_0$ = background /constant risk, 1...n = variables like age groups, management system, breed and sub county of origin; and *e* = error value. The final model output was;

Logit P (A/E) = -1.2 - 1.827crossbreed
- 1.691 exotic cattle + 0.658.

Where Logit (E/A) is the logarithm to base 10 of probability of infection with AEs, $\beta_0$ = constant risk of infection = -1.2 and *e* = error value = 0.658. Predictors of infection with AEs and their associated statistical significance, which were entered into the binary logistic model, are summarized in Table 3.

## DISCUSSION

**Prevalence of *Anaplasma* and *Ehrlichia* sp. in Kashaari County:** Of the 375 cattle sampled, 5.6% (95% CI, 3.3-7.9%) were positive with AEs. The prevalence of *Ehrlichia* sp. was 5.1% (95%CI, 2.9-7.3%) whereas that of *Anaplasma* sp. was 5.3% (95% CI, 3.0-7.6%). The most and least prevalent *Anaplasma* sp. detected were *A. bovis* and *A.* (*E*). *phagocytophilum* at 5.1% ( 95% CI, 2.9-7.3%) and 2.7%, (95% CI, 1.1-4.3%) respectively. *E. ruminantium* and *E. sp. omatjenne* were the most and least detected *Ehrlichia* sp. at 4.5 % (95% CI, 2.4-6.6%) and 1.9% (95% CI, 0.5-3.3%) respectively. These prevalences of AEs are much lower than that previously reported by Ssenyonga *et al.* (1992). The big difference in the reported prevalence then and in this study can be explained by the lapse of time and the likely changes in either tick control regimes or weather/climate. The most plausible explanation, however, is the differences in the specificity of the methods used in the Ssenyonga *et al.* (1992) study and RLB used in the current study. Where as serological tests detect exposure to infection (and not necessarily current infection), molecular diagnostic techniques, in this case RLB, detect current/active infection (Bekker *et al.*, 2002; Nagore *et al.*, 2004a; Nagore *et al.*, 2004b; García-Sanmartín *et al.*, 2006). Recent molecular studies (using RLB) of Tick-Borne Disease Pathogens (TBDPs) have reported higher (Oura *et al.*, 2004b) AEs prevalences than reported here. Differences in the study areas, time of the study and sampling methods explain the differences in prevalences recorded in these studies and this study. In this study, just 1/21 infection was a single infection. This implies that vectors for both AEs -*Boophilus* and *Amblyomma* sp. (McCosker, 1979; Minjauw and McLeod, 2003) are present in Kashaari. This could as well be explained by mechanical transmission in Aes.

**Novel *Anaplasma* and *Ehrlichia* sp. detected in Kashaari county-Uganda:** Ten (10) out of the twenty one (21) positive animals showed a strong signal for *A.* (*E.*) *phagocytophilum* the causative agent of tick-borne fever in sheep and pasture fever in cattle and now named the cause of human granulocytic ehrlichiosis-HGE (human Anaplasmosis) (MacLeod and Gordon, 1933; Chen *et al.*, 1994; Engvall *et al.*, 1996; Stuen, 2007). *A.* (*E.*) *phagocytophilum* an *Ixodes ricinus* transmitted (MacLeod, 1932; MacLeod and Gordon, 1933; Stuen, 2007) human infective parasite has been known now for over 200 years to be widely distributed TBP in Europe (Brodie *et al.*, 1986; Stuen, 2007) as well as many

parts of The United States of America (Chen *et al*., 1994; Belongia *et al*., 1997; Walls *et al*., 1997; Stanka *et al*., 1998). Cases of *A*. (*E*.) *phagocytophilum* or Human Granulocytic Ehrlichiosis (HGE) have not been reported in Uganda or other sub-Saharan countries. A possible case of HGE was initially reported in 1999 in the Free State Province of South Africa (Pretorius *et al*., 1999) and recently an Anaplasma species closely related to *A*. (*E*.) *phagocytophilum* was isolated from canine blood from South Africa (Inokuma *et al*., 2005). The strong RLB signals observed with *A*. (*E*.) *phagocytophilum* specific-oligonucleotide sequences signify the presence of *A*. (*E*.) *phagocytophilum* or a very closely related *Anaplasma* (*Ehrlichia*) sp. in cattle in Kashaari county. The possible occurrence, or indeed the occurrence, of *A*. (*E*.) *phagocytophilum* or *A*. (*E*.) *phagocytophilum*- like organisms in this new area can be explained by the parasites' unique epidemiological features that have been recently reviewed by Stuen (2007). These include the parasites' ability to be transmitted by a multiplicity of tick and mite species; to virtually infect all mammals and rodents and possible spread by migratory birds. Where as we cannot authoritatively mention the likely ways in which this parasite came to be in this new area, it is very likely that the parasite has been present in Kashaari county and other parts of the country but has never been studied before. Importation of animals from South Africa into Uganda and Kashaari in particular especially the recent importation of thousands of Boer goats for breeding purposes are suggested ways this parasite might have been introduced if at all it is a new parasite. This, however, needs to be explored further by molecular genetic characterization, transmission, pathogenicity and phylogenetic studies.

*Ehrlichia* sp. (*omatjenne*) - an apparently apathogenic *Ehrlichia* sp. (Du Plessis, 1990, Allsopp *et al*., 1997) was detected in 1.9% (CI = 95%, 0.5-3.3%) of the sampled animals. *E.* sp. (*omatjenne*) has been detected in several ruminants in South Africa including Boer goats (Allsopp *et al*., 1997). Importation of several Boer goats into the country as explained for *A.* (*E*.) *phagocytophilum* may explain the presence of this none previously observed *Ehrlichia* species in Kashaari county. This should be verified by use of molecular genetic characterization and strain isolation/characterization studies. Where as this *Ehrlichia* species in general is not known as a pathogenic species in ruminants, a strain (*E*. sp. (*omatjenne*) 1) of this parasite has been used experimentally to produce disease indistinguishable from cowdriosis in sheep (Du Plessis, 1990) there by not ruling out its pathogenicity in natural conditions. Where it occurs, its main importance is attributed to its contribution to sero-positivity to *E. ruminantium* with which it greatly cross-reacts. This results in reports of heart water in areas where it actually does not occur or higher prevalences of the disease in areas where the disease does not occur in such alarming levels (Allsopp *et al*., 1997). In Uganda where TBD diagnosis is mostly by serology (personal observation), presence of *E*. sp. (*omatjenne*) will complicate the diagnosis and treatment of heart water.

*E. ovina /canis* (*E. canis*) was detected in 2.7% (CI = 95%, 1.1-4.3%) of the samples. *E. canis* is the causative agent of Canine Monocytic Ehrlichiosis (CME) worldwide (Neer *et al*., 2002; Siarkou *et al*., 2007). *E. canis* which is transmitted by *Rh. sanguineous* (Inokuma *et al*., 2006) has recently been reported to cause disease in humans with clinical signs compatible to those of Human Monocytic Ehrlichiosis (HME) (Perez *et al*., 2006). It is common in tropical and subtropical regions (Ristic and Holland, 1993) and has recently been reported in eighty one percent (68/78) of the dogs in southern Sudan (Inokuma *et al*., 2006). Despite the fact that canine ehrlichiosis has not been studied in Africa and particularly in Uganda, the single study done in eastern Sudan above indicates likely high burden of canine *Ehrlichial* species in sub-Saharan Africa. *E. canis* is here reported as an incidental infection in cattle most (6/10) of from Kashare sub county where dogs were used to lead cattle to communal watering places. This should have provided an avenue for ticks *(Rh. sanguineous)* to transmit infection from dogs to cattle. Establishment *E. canis* infection in cattle is likely to be a very important epidemiological feature in the spread of CME and the human form of the disease described by Perez *et al*. (2006).

**Prevalence of *Anaplasma* and *Ehrlichia* sp. in different sub counties and management systems in Kashaari County:** Extensively managed and communally watered cattle from the sub counties of Kakiika, Rubaya, Kashare and Rwanyamahembe recorded the highest prevalence of AEs at 9.3% (CI = 95%, 6.4-12.2%), 9.1% (CI = 95%, 6.2-12.0%), 7.3% (CI = 95%, 4.7-9.9%) and 5.9% (CI = 95%, 3.5-8.3%) respectively. Herd mixing facilitates tick attachment, feeding and introduction of TBPs. On the other hand, restrictedly grazed (paddocked) and on-farm watered cattle from the sub counties of Biharwe and Bubaare had the lowest levels of infection at 2.0% (CI = 95%, 0.6-3.4%) and 1.3% (CI = 95%, 0.1-2.5%). This is supported by the observation that all positive (n = 21) animals were extensively managed animals. Overall, There was no statistically significant difference between prevalence of AEs across the sub-counties ($X^2 = 6.775$, p = 0.258; 5df)) of Kashaari county and among differently managed cattle ($X^2 = 1.386$, p = 0.271, df = 2). This can partly be explained by the fact that most farmers in Kashaari County practice extensive management system. As a result, 346 of the 375 animals sampled were extensively managed and therefore the sample used was representative of the study area and other areas with

comparable cattle demographics. We would expect that the type of management system is a very important factor that predisposes cattle to infection with TBPs like AEs because, as explained above, different management systems offer different host (cattle) - vector (tick) contact times and therefore different transmission rates. Where as the current results clearly show that there is no statistically significant difference between the prevalence of AEs in cattle kept under different management systems, this could have been because cattle from different management systems were not equally represented. Unless proven otherwise by further studies with equal inclusion of animals from different management systems we take it that there was no statistically significant difference in prevalence of AEs among different management systems.

**Prevalence of *Anaplasma* and *Ehrlichia* sp. in different cattle age groups:** Calves-1 (0 -5.9 months of age) and calves-2 (6- 8.9 months of age) had the highest prevalence of AEs at 6.4% (CI = 95%, 3.9-8.9%) and 6% (CI = 95%, 3.6-8.4%) respectively while those between 9- 24 months of age (young cattle) had the lowest prevalence of 4.5% (CI = 95%, 2.4-6.6%) The differences in number of calves and adults infected and apparently healthy under the same tick control regime for both adults and calves can be explained by the difference in the dominant immune response, both acquired and innate, to AEs infection in calves between 0-9 months and those above 9 months of age (James *et al*., 1985; Potgieter and van Rensburg, 1987; Deem *et al*., 1995; Norval *et al*., 1995). This helps calves to maintain some level of parasitemia with apparently no disease. Acquired immunity plays a similar role in adult cattle that became infected and recovered from infection as calves (Krigel *et al*., 1992; Byroma *et al*., 2000, Kleef *et al*., 2002). Since AEs are intracellular, acquired cell mediated immunity to these organisms is more effective and more long lasting than hummoral /innate immunity (Du Plessis, 1970, Byroma *et al*., 2000). Low prevalence of AEs observed in adult cattle is probably due to the fact that a comparable number of adult cattle were infected and that these infections would be cleared by a strong acquired immunity. This is probably why despite numerical differences in infection with AEs across different ages groups, there is no statistically significant difference ($X^2$ = 0.21, p = 0.967; 3df) in the prevalence of AEs among cattle of different age groups implying an equilibrium of infection across ages.

Judging from the current AEs prevalences in Kashaari County, it is very unlikely that endemic stability against heart water and anaplasmosis occurs. This is because for endemic stability against tick-borne diseases to occur, infection rates ought to be high with low levels of clinical disease. In the current study the prevalences of *E. ruminantium* and *A. marginale (*the highly pathogenic bovine AEs) are very low at 4.5 % (CI = 95 %, 2.4-6.6%) and 3.7 % (CI = 95%, 1.8-5.6%) respectively. Low infection levels with both AEs are due to tick (vector) population suppression by overuse of acaracides or unfavorable conditions. Over use of acaracides is a likely cause because in my personal communication with farmers, I realized that most of the farmers use synthetic pyrethroid acaracides twice a week. This tick control pressure is sufficient to keep very small numbers of ticks on animals and therefore low levels of infection and as a result disrupt endemic stability. The season when sampling was done could have contributed to vector population suppression and hence low levels of infection because it was at the beginning of the wet season when tick growth had not recovered from a long December-February dry season. The prevalence of AEs in the very young calves (0-5.9 months of age) in it self was very low at 6.4% (CI = 95%, 3.9-8.9%). This was even lower in calves between 6-9 months of age at 6% (CI = 95%, 3.6-8.4%). This means that a large proportion of animals are getting to adult stages when they are not infected and therefore when they are naïve. This creates a large population of immunologically unchallenged cattle that is likely to suffer immensely due to TBDS in the event of any break in intensive acaracide use.

**Prevalence of *Anaplasma* and *Ehrlichia* species in different cattle breeds:** Exotic (Pure Friesians) cattle had the highest level of infection at 23.1% (CI = 95%, 18.8-27.4%). There are very few pure exotic cattle in Kashaari County kept purposely as breeding stock for crossbreeding programs, consequently, a small number of pure exotic cattle (Friesians) were sampled. Despite this small sample size of pure exotic cattle, the prevalence of AEs in this group was significantly higher compared to other breeds ($X^2$ = 7.85, p = 0.02, 2df). Local (Ankole long-horned cattle /other zebu) breeds had the lowest level of infection at 4.6% (CI = 95% 2.5-6.7%). Exotic breeds of cattle have been reported before as more susceptible to TBPs infection and TBD establishment than local breeds (Lohr *et al*., 1975; Wilson *et al*., 1975; Ajayi *et al*., 1982; Minjauw and McLeod, 2003). The high infection rate with AEs observed in this study is attributed to this difference in genetic resistance to ticks and tick-borne diseases earlier reported.

**Predictor (s) of infection with *Anaplasma* and *Ehrlichia* sp.:** Breed was found to be the best predictor if infection with AEs. The order of infection with AEs was; Local < cross< Exotic breeds. The final backward regression output which describes the predictor of infection with AEs was obtained as; Logit P (A/E) = -1.2 - 1.827crossbreed -1.691 exotic cattle +0.658. Where Logit P (A/E) is the logarithm to base 10 of probability of infection with *Anaplasma /Ehrlichia* species, $\beta_0$ is the background /constant risk of infection (-1.2) and e is the

error value (0.658). If the susceptibility indices for different breeds in terms of infection with AEs are established, direct substitution in the equation above will give a numerical value the antilogarithm to base 10 of which will be the value to which risk of infection will be increased with change in breed of animal. Where as the design of this study could not allow for determination of susceptibility indices for different breeds in terms of infection with AEs so as to come up with the numerical value to which risk of infection is increased with change in breed of animal, it is only sufficient to report here that breed of animal is a predictor of infection with AEs. Once studies have been designed, carried out and susceptibility indices for different breeds in terms of infection with AEs established, then the above model could be used to guide the choice of breed for purposes of TBD control.

In this study we demonstrated presence of a broad range of highly pathogenic AEs species like *A. marginale* and *E. ruminantium* in cattle from Kashaari County. Novel AEs like *A.* (*E.*) *phagocytophilum*, *E. sp. omatjenne* and *E. ovina/canis* were also detected. Under the conditions of the current study, breed of animal was found to be the best predictor of infection with AEs. The level of infection was strongly associated with breed of animal with local breeds being the least affected.

From these findings, we recommend that longitudinal studies covering a wider area and over a longer period of time be carried out so as to study the effect of weather changes and fluctuations in tick (vector) density on the prevalence of AEs. Further studies should be carried out to investigate cattle breed as predictor of infection with AEs and other tick-borne parasites. Molecular genetic characterization, transmission and pathogenicity studies should be carried out on the different strains of AEs in Kashaari County and particularly those that are being reported for the first time in this study. Lastly the sensitivity of RLB should be compared with serological techniques used in TBD diagnosis.

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