Trop Anim Health Prod (2016) 48:603–611
DOI 10.1007/s11250-016-1005-x

REGULAR ARTICLES

Infections and risk factors for livestock with species of *Anaplasma*, *Babesia* and *Brucella* under semi-nomadic rearing in Karamoja Region, Uganda

Chiara Lolli[1] · Maria Luisa Marenzoni[2] · Paolo Strona[3] · Pier Giorgio Lappo[4] · Patrick Etiang[5] · Silvana Diverio[2]

Received: 3 September 2015 / Accepted: 9 February 2016 / Published online: 18 February 2016

Abstract A survey was conducted to estimate the prevalence of *Anaplasma*, *Babesia* and *Brucella* spp. infections in cattle, goats and sheep in the Karamoja Region of Uganda and to identify possible risk factors existing in this semi-nomadic and pastoral area. Low cost laboratory tests were used to diagnose infections (Rose Bengal test for *Brucella* spp. antibodies and direct microscopic examination for *Anaplasma* and *Babesia* spp.). Multivariable logistic regression models were applied to identify possible risk factors linked to gender, animal species, age (only for cattle) and districts. A total of 3935 cattle, 729 goats and 306 sheep of five districts of the Karamoja Region were tested. Seroprevalence for Brucella was 9.2 % (CI, 95 %: 8.4–10), for *Anaplasma* 19.5 % (CI 95 %: 18.4–20.6) and for *Babesia* 16 % (CI 95 %: 15–17.1). Significant differences in infections prevalence were observed against risk factors associated with districts and species. Cattle were the species with higher risk of the infections. Female gender was identified as at risk only for *Brucella* spp. infection. Cattle more than one year old had greater likelihood to be *Brucella* seropositive. Co-infections of *Anaplasma* and *Babesia* spp. were statistically associated, especially in goats and sheep. Further studies to identify risk factors related to host species and geographical districts are needed. The influence on the semi-nomadic agro-pastoral system in Karamoja of animal raids and animal mixing should be further investigated. Findings were important to sensitize Karamojong undertaking measures on infection control, especially on cattle, which are their main source of food.

Keywords *Anaplasma* · *Babesia* · *Brucella* · Semi-nomadic rearing · Risk factors · Uganda

✉ Silvana Diverio
silvana.diverio@unipg.it

Chiara Lolli
chiaralolli82@gmail.com

Maria Luisa Marenzoni
marialuisa.marenzoni@unipg.it

Paolo Strona
paolostrona@yahoo.it

Pier Giorgio Lappo
coopdev.lappo@hotmail.it

Patrick Etiang
etiang.patrick@gmail.com

1 Via Bontempi 3, 06089, Torgiano Perugia, Italy

2 Department of Veterinary Medicine, Via San Costanzo 4, 06126 Perugia, Italy

3 Africa Mission–Cooperazione e Sviluppo, Via Cesare Martelli 6, 29122 Piacenza, Italy

4 International Cooperation and Development NGO (C&D), Lugugo Bypass, Plot 5, P.O. Box 7205, Kampala, Uganda

5 Karamoja Veterinary Laboratory, Institute for International Cooperation and Development (C&D), Moroto, Uganda

Introduction

The Karamoja is the northeastern region of Uganda and its communities have among the lowest welfare indices in the country. The region is characterized by a semi-arid climate and the majority of the population subsists through agro-pastoral or purely pastoral livelihoods. Livestock are their main protein sources: blood, milk and meat, and animal products are often traditionally consumed raw, exposing people to pathogens (Gradé et al. 2009). Livestock is also the centre of relational, social and religious life of the population of the Karamoja (Lolli and Diverio 2010). During the dry season, adults and young members of the village practice transhumance

(Institute for International Cooperation and Development (C&D) 2010). In the past years, the Karamoja Region has been severely affected by a succession of shocks including severe drought (2006), prolonged dry spell (2007–2009) and subsequent flooding (in 2007), leading to crop failures, restricting access to adequate water, thereby creating high pressure on watering areas both for humans and livestock (Institute for International Cooperation and Development (C&D) 2010). The lack of readily accessible water makes hygiene difficult during the dry season (Institute for International Cooperation and Development (C&D) 2010). Human and livestock health are limited in Karamoja by poor coverage of public veterinary services and restricted access to allopathic veterinary drugs (Gradé et al. 2009). Furthermore, there is the lack of any systematic recording of veterinary cures because ethnoveterinary knowledge is traditionally passed on by word of mouth (Gradé et al. 2009). Long lasting civil insecurity caused by livestock riding conflicts among different tribes and villages heavily impairs food security for people enhancing famine and health hazards (Institute for International Cooperation and Development (C&D) 2010).

People in the Karamoja pastoral system live closely with livestock population, which can be affected by a range of infectious diseases. Routine annual migration of livestock from different districts, although preserves the environment from overgrazing, can favour disease transmission among mixing herds. The spreading of infectious pathogens is facilitated by overnight collection of the animals into the village's corrals for protecting them from inter-tribal raids and by livestock congregation at few water pumps during the drought. This causes overgrazing of the limited pasturage around watering points and heavy contamination of water sources with animal wastes. The presence of such organic matter in muddy or stagnant water sources can favour the survival of parasites and the transmission of infectious diseases (Oloya et al. 2006).

Human brucellosis is considered by the World Health Organization a neglected zoonotic disease, relevant in Uganda. It has been estimated to have an incidence rate of 0.7/1000 person-years. The disease becomes very serious in humans infected by immunodeficiency virus (Faye et al. 2005). Major recognized risk factors enhancing the incidence of human brucellosis include contact with infected animal materials or products (Nasinyama et al. 2014), poor milk hygiene (Dairy Development Authority (DDA) 2004), traditional lifestyle and habits, such as eating raw meat during the ceremonies (Oloffs et al. 1998; Lolli and Diverio 2010), keeping livestock, living far away from health centres and lack of awareness about the mode of transmission of brucellosis (Kunda et al. 2007). A previous study found a moderate knowledge of people on human and animal brucellosis, particularly concerning its transmission (Kansiime et al. 2014). Farmers, abattoir and animal health workers are at high risk of occupational exposure to the infection (Nasinyama et al. 2014).

Babesia and *Anaplasma* spp. infections are endemic in some areas of Uganda and represent a major challenge to livestock production (Rubaire-Akiiki et al. 2004). Ticks strictly transmit *Babesia*, as well as *Anaplasma*, which can be also transmitted iatrogenically or by biting flies. *Babesia* may cause zoonotic disease but in unusual situations (OIE 2015). Previous studies indicated the presence of these infections in some parts of the country (Kabagambe et al. 2001; Magona et al. 2008; Rubaire-Akiiki et al. 2004, 2006). Tick-borne diseases cause losses through mortality and impair improvement of livestock for reduced weight gains and milk production (Muhanguzi et al. 2010). Public health surveillance through collection of local epidemiological information and identification of related risk factors is crucial for implementing control strategies. These should be shared and integrated with local community of Karimojong, in particular with livestock farmers (Rubaire-Akiiki et al. 2004; Lolli and Diverio 2010).

So far, very limited data concerning the prevalence of livestock infections in the Karamoja Region are available (Newton et al. 1974; Mwebe et al. 2011). The aim of the study was to estimate the prevalence of the three most important microbial pathogens of livestock, namely *Brucella*, *Anaplasma* and *Babesia* species, in a population of cattle, sheep and goats reared in the Karamoja Region. The epidemiological study was carried out using low cost laboratory tests, to allow their future routine application in this poor rural area. Possible risk factors were also investigated. This study is a collaborative work among the Institute for International Cooperation and Development (C&D) and its lab team, the local District Veterinary Officers (DVOs), the Community Animal Health Workers (CAHWs) of Moroto, (Uganda) and the staff of the Laboratory of Ethology and Animal Welfare (LEBA) of the Department of Veterinary Medicine of Perugia University (Italy), aimed to gathering data to implement an Early Warning System (EWS) set for the Karamoja Region. This EWS is a sector of a project funded by European Commission Humanitarian Aid Department (ECHO) consisting of the detection, rapid verification and response to epidemic-prone and emerging disease threats, in order to minimize the impact of these threats on the health and economy of the population. For the success of the EWS the active participation of the local District Veterinary Services, other extension staff, Community Animal Health Workers, farmers and other organizations operating on the ground is very important.

Materials and methods

Area description

The study was conducted in the five districts (Abim, Kaabong, Kotido, Moroto and Nakapiripirit) of the Karamoja Region of Uganda. Topographically, the altitude ranges from 1400 to

3000 m. Karamoja has bi-modal variable rainfall patterns, occurring from September to October and March to April. Average annual temperature of 15–32.5 °C, and an average rainfall of 500–600 ml per year are recorded. The region is a semi-arid to arid savannah covered with seasonal grasses, thorny plants, and occasional small trees (Gradé et al. 2009).

Study design and herd selection

The sampling of animals (cattle, goats and sheep) was performed from October 2009 to March 2010 to obtain a whole coverage of the Karamoja territory and the estimated distribution of the animals, based on existing data (OCHA 2009). The villages to sample were randomly selected. All animals of these selected villages were tested to limit problems due to restricted accessibility of herds, absence of animal census and presence of continuous raids of animals among villages. Logistic and strategy procedures for organizing and randomly collecting the samples were carried out as "community mobilization" with the help of the local villages' CAHWs (Institute for International Cooperation and Development (C&D) 2010). This procedure was aimed to integrate and establish a constructive relationship between the veterinary team involved in this study and the communities. To that effect, the C&D Lab Team identified the stakeholders to be contacted who, for each village, included the DVOs, the CAHWs, the Elders, and the kraal leaders of the villages involved in the study. All experimental procedures followed in the present study were in agreement with the Ethical Committee of the Perugia University and were conducted in accordance with law dispositions of the Italian and Uganda Ministry of Health.

Data and sample collection

C&D Lab Team and LEBA staff carried out the collection of samples and the analysis at the C&D Moroto laboratory. Animals sampled were grazed on bushy communal pastures with different herds mixing freely, alongside wild ruminants. A clinical examination was performed on each animal before blood sampling. Body conditions were broadly assessed to detect any sign of emaciation, parotid and pre-scapular lymph nodes were palpated. Animals were examined to detect presence of ticks, diarrhoea, ocular or nasal discharges. Few clinical data were collected because usually not all the farmers, the only ones being able to supply anamnestic information of the animals, were present at sampling collection. Most often one farmer had the task of bringing his and the other farmers' herds of the same village to the sample collection point (Dr. Patrick Entiang, personal communication).

Blood samples (10 ml) were collected from each animal in non-heparinized and heparinized vacutainers (Becton-Dickinson, Vacutainer System, UK) by jugular venepuncture. After blood sampling, species, gender and district of each animal were recorded. Age of cattle was assigned to three age categories as follows: 0–6 months, 7–12 months, older than 12 months. Cattle breed categories included East African Short-Horned Zebu cattle, Ankole and Boran. Heparinized blood samples were used to make thin and thick blood smears, stained by Giemsa method, to find hemoparasites consistent with *Anaplasma* spp. and *Babesia* spp. (Office International Epizooties (OIE) 2015). Non-heparinized blood samples were stored at 4 °C for a maximum of 24 h and centrifuged at 2500 rpm for 10 min to collect the sera. Thirty μl of each serum were used to detect antibodies against *Brucella* spp. by screening them with Rose Bengal test, in accordance with published protocols (Office International Epizooties (OIE) 2015).

Statistical analysis

The estimation of the prevalence, defined as the proportion of positive animals out of the total number of animals tested for each investigated infectious diseases, was obtained using the software Episheet (http://krothman.hostbyet2.com/episheet.xls). Each independent variable (species, gender, district, presence of co-infections) was examined separately for association with the outcomes (positivity to *Brucella* spp., *Anaplasma* spp. or *Babesia* spp. infection, and co-infections, defined as presence of at least two of the investigated etiological agents) to assess the relationship between possible risk factors and the infection. The effect of age was analysed only for cattle, grouping animal age into two classes: cattle younger or equal than 12 months and cattle older than 12 months. Variables scoring $p \leq 0.20$ in univariate model or considered to be biologically relevant (i.e. gender) were included in the multivariable model. Odds ratios (OR) and corresponding 95 % confidence intervals (95 % CI) were obtained by means of logistic regression. Data were analysed by commercial software R, version 2.8.1 (R, Development Core Team 2007). A value of $p < 0.05$ was considered significant for the analysis.

Results

In the five districts, a total of 4970 livestock were sampled, comprising of 3935 (79.2 %) cattle, 729 (14.7 %) goats and 306 (6.2 %) sheep. Females were the 72 % of the animals and males 27.9 %. Out of 3935 cattle, 1027 were calves (aged from 0 to 6 months), 151 heifers or steers (7–12 months) and 2658 adults (older than 12 months). Age was not recorded in 99 animals. Overall, 73.7 % of cattle were older than 12 months. In 76 cases out of 4970, blood samples collected were not suitable for laboratory tests or their results were doubt. Therefore, these samples were not included in the overall prevalence analysis. Species, gender and age (only for cattle) distribution in the sampled livestock roughly represented the composition of livestock population in Karamoja.

As commonly referred during the dry season in Uganda, general health condition of the majority of the animals was poor, with evident emaciated state. Heavy tick infestations were observed in cattle, which presented heavier tick burdens compared to small ruminants. Some cases of coughing, corneal opacity, skin infections in cattle and few cases of limping animals and abortions in goats were reported. Skin lesions were common, as a result of local methods of branding. Suspected cases of lumpy skin disease, especially in Loputuk, Nakiloro and Iriiri districts were only occasionally recorded (Dr. Patrick Entiang, personal communication).

The prevalence of *Brucella* spp., *Anaplasma* spp., and *Babesia* spp. infections and co-infections in the sampled livestock for district is reported in Fig. 1. The estimated prevalence of these infections and co-infections in the Karamoja Region, subdivided for animal species, is reported in Tables 1 and 2. The effects of gender, species and district on the prevalence risk of infections and co-infections are reported in Tables 3, 4, and 5. Final multivariable model for co-infections (presence of at least two of the investigated infections) in the sampled livestock of the Karamoja Region is reported in Table 6. Cattle older than 12 months were more associated with seropositivity against *Brucella* spp. compared with younger ones (OR = 2.2, 95 % CI: 1.6–2.9). However, being older than one year was a protective factor for *Anaplasma* spp. (OR = 0.7, 95 % CI: 0.6–0.97) and *Babesia* spp. infections (OR = 0.8, 95 % CI: 0.7–1.0).

The likelihood of an animal to be simultaneously infected with *Babesia* spp. and *Anaplasma* spp. was statistically significant (OR = 3.2, 95 % CI: 2.7–3.8) in all species. On the contrary, *Brucella* spp. infection was not associated with vector-borne infections. Considering single species, cattle had a risk 2.5 times higher to be infected with *Anaplasma* spp. if they were *Babesia* spp. positive (OR = 2.49, 95 % CI: 2.1–2.95), while goats and sheep respectively 12.2 (95 % CI: 4.16–35.55) and 45.8 times (95 % CI: 3.88–541.35), even if the prevalence of these infections in small ruminants was lower than in cattle (Table 2).

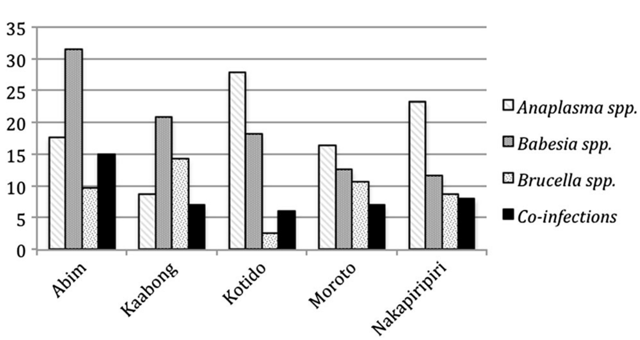

Fig. 1 Prevalence of *Anaplasma* spp., *Babesia* spp., and *Brucella* spp. infections and co-infections in the five districts of the Karamoja Region

Table 1 Estimated prevalence (% and 95 % confidence interval, CI) for *Brucella* spp., *Anaplasma* spp., and *Babesia* spp. infections in the sampled livestock of the Karamoja Region

Pathogen	Species	Prevalence (%)	95 % confidence interval
Brucella spp.	Cattle	384/3935 (9.8)	8.9–10.7
	Goats	64/729 (8.8)	6.9–11.1
	Sheep	8/306 (2.6)	1.2–5.3
	Total	456/4970 (9.2)	8.4–10.0
Anaplasma spp.	Cattle	912/3935 (23.2)	21.9–24.5
	Goats	40/729 (5.5)	4.0–7.5
	Sheep	15/306 (4.9)	2.9–8.1
	Total	967/4970 (19.5)	18.4–20.6
Babesia spp.	Cattle	776/3935 (19.7)	18.5–21.0
	Goats	16/729 (2.2)	1.3–3.6
	Sheep	3/306 (1.0)	0.2–3.1
	Total	795/4970 (16.0)	15.0–17.1

Discussion

This is the first detailed report about *Brucella* spp., *Anaplasma* spp. and *Babesia* spp. infections in the Karamoja Region, carried out on three different animal species (cattle, goats and sheep). Several differences in the distribution of these infections were found in relation to different district areas of the Karamoja or specific livestock species. In our study we recorded different prevalence of infections compared with those previously reported (Kabagambe et al. 2001; Magona et al. 2009, 2011; Rubaire-Akiiki et al. 2004, 2006). This could be due to different distribution of risk factors.

Prevalence data of *Brucella* infections in livestock found in our study confirmed those previously registered in Uganda (seroprevalence from 3 to 17 %; Newton et al. 1974; Oloffs et al. 1998; Faye et al. 2005; Kashiwazaki et al. 2012; Nizeyimana et al. 2013; Miller et al. 2015; Mugizi et al. 2015), but differed from that of a survey carried out in the Nakapiripirit district (prevalence 0 %; Mwebe et al. 2011). However, prevalence comparison among different surveys is difficult because the prevalence of infections depends on many risk factors, which widely vary according to year, geographic location, breeding and dairy system, livestock population and species tested, as well as on type, sensitivity and specificity of laboratory tests utilized.

The main risk factors associated with a high seroprevalence of brucellosis in livestock are poor milk hygiene, poor awareness and traditional lifestyle (Oloffs et al. 1998). In Uganda, the two major sources of milk are the zero-grazing and pastoral dairy systems (Dairy Development Authority (DDA) 2004). The pastoral system holds almost all herds in the Karamoja Region. In all the country 30 % of the produced milk is consumed at farm-level, while 70 % is available for marketing. On the opposite, in the Karamoja most of the milk

Table 2 Prevalence of co-infections of *Brucella* spp., *Anaplasma* spp., and *Babesia* spp. in the sampled livestock of the Karamoja Region

Prevalence and (%) co-infections

Species	*Brucella* spp. and *Anaplasma* spp.	*Brucella* spp. and *Babesia* spp.	*Anaplasma* spp. and *Babesia* spp.	*Brucella* spp., *Anaplasma* spp., and *Babesia* spp.
Cattle	81/3935 (2.1)	60/3935 (1.5)*	293/3935 (7.4)***	19/3935 (0.5)
Goats	5/729 (0.7)	1/729 (0.1)	6/729 (0.8)***	1/729 (0.1)
Sheep	0/306 (0)	0/306 (0)	2/306 (0.7)**	0/306 (0)
Total	86/4970 (1.7)	61/4970 (1.2)	301/4970 (6.1)***	20/4970 (0.4)

* statistically significant result with $p < 0.05$

** statistically significant result with $p < 0.01$

*** statistically significant result with $p < 0.001$

is consumed at farm-level or in the nearby villages. Of the marketed milk, 80 % is sold unpasteurized through informal channels (Dairy Development Authority (DDA) 2004). In Uganda, it is of public health concern that people in the pastoral system, living closely with a livestock population, are more prone to contract brucellosis (Magona et al. 2009). The main risk factors associated with a high seroprevalence of brucellosis in livestock are poor milk hygiene, poor awareness and traditional lifestyle (Oloffs et al. 1998). An association between free-grazing or pastoral systems and higher seroprevalence of brucellosis has been already reported in other areas of Uganda (Kabagambe et al. 2001; Faye et al. 2005; Magona et al. 2009; Makita et al. 2011; Mwebe et al. 2011). An additional risk factor for the transmission of *Brucella* infections in the Karamoja population could be the habit of eating raw meat during the ceremonies, as well as by skin abrasions and inhalation of airborne animal manure particle (OIE 2015). Another potential risk factor might be the consumption of raw blood, a common practice in some Karamojong tribes, which represents a subsistence factor extremely relevant especially during food shortages (Lolli and Diverio 2010).

Differences in prevalence of *Brucella* infections reported in literature might also be due to the use of different diagnostic tests of infection (direct or indirect, with various sensitivity and specificity). No single test is appropriate in all epidemiological situations and all have limitations, causing false negative or positive results. As recommended in contexts similar to that of our study (Eisler et al. 2012), we decided to adopt the criterion of using test with low cost and easy equipment. This is because the diagnosis is the key to control and prevent livestock diseases and these low cost diagnostic tests can be easily implied in local laboratories. Rose Bengal test is considered highly sensitive (risk of false positive results), and direct microscopic examination highly specific (risk of false negative results) (Office International Epizooties (OIE) 2015). Nizeyimana et al. (2013) did not found significant differences

Table 3 Effect of gender, species and district on risk prevalence of *Brucella* spp. infection in the sampled livestock of the Karamoja Region

Factor	Level	*N*	Prevalence (%)	OR*	95 % confidence interval		*p* value
					Lower limit	Upper limit	
Gender	Male[1]	1387	106/1387 (7.6)	1	–	–	–
	Female	3579	350/3579 (9.8)	1.35	1.07	1.7	0.012
Species	Cattle[a]	3931	384/3931 (9.8)	1	–	–	–
	Goats	729	64/729 (8.8)	0.72	0.54	0.96	0.026
	Sheep	306	8/306 (2.6)	0.23	0.11	0.46	0.000
District	Kotido[a]	846	22/846 (2.6)	1	–	–	–
	Kaabong	913	130/913 (14.2)	6.68	4.19	10.63	0.000
	Moroto	1069	114/1069 (10.7)	4.91	3.07	7.84	0.000
	Nakapiripirit	1743	152/1743 (8.7)	3.78	2.4	5.96	0.000
	Abim	395	38/395 (9.6)	4.07	2.36	6.99	0.000

*Odds ratio

[1] Reference category

N = number of observations

Table 4 Effect of gender, species and district on risk prevalence of *Babesia* spp. infection in the sampled livestock of the Karamoja Region

Factor	Level	*N*	Prevalence (%)	OR*	95 % confidence interval		*p* value
					Lower limit	Upper limit	
Gender	Male[a]	1366	224/1366 (16.4)	1	–	–	–
	Female	3532	571/3532 (16.2)	1.13	0.95	1.35	0.179
Species	Cattle[a]	3875	776/3875 (20)	1	–	–	–
	Goats	725	16/725 (2.2)	0.09	0.05	0.14	0.000
	Sheep	298	3/298 (1)	0.05	0.02	0.15	0.000
District	Kotido[a]	845	154/845 (18.2)	1	–	–	–
	Kaabong	913	191/913 (20.9)	1.54	1.21	1.97	0.000

*Odds ratio

[a] Reference category

N = number of observations

between Rose Bengal test and an indirect ELISA in detecting antibodies against *Brucella* spp., concluding that Rose Bengal test could be used as a cheap screening of infection in animals.

In our study, goats and sheep had a lower probability to be *Brucella* seropositive compared with cattle, with sheep showing the lowest prevalence. This is an important finding because no previous prevalence data in sheep in Uganda were available. Miller et al. (2015) found that goat and cattle *Brucella* seropositivity were strictly correlated, hypothesizing that goats were an active component in the transmission cycle of brucellosis within farms. Different ranges of seroprevalence (2-100 %) of brucellosis in goats in Uganda have been previously reported (Kabagambe et al. 2001; Mwebe et al. 2011; Miller et al. 2015). The reason of this species variability is not evident; it might be related to different susceptibility to infection. In our study, no identification of *Brucella* at species level was performed, but it is possible that different geographical areas are characterized by different risk factors. We discovered that cattle were the most likely to be seropositive for *Brucella*, while goats and sheep had low positivity. This is an important finding considering that cattle are the most important source of food in the Karamoja Region. An additional possible untested risk factor could be that cattle are the main goal of the raids among tribes, unlike goats and sheep. Cattle movement from one village to another during raids could increase spreading of *Brucella* infection. Species-associated factors should be investigated in future studies for better understanding the reason of different species exposure,.

Age might be another risk factor, since cattle live longer than small ruminants, therefore having more probability to be affected by infections. Age of cattle (older than 12 month) was found to be a risk factor for *Brucella* seropositivity, in

Table 5 Effect of gender, species and district on risk prevalence of *Anaplasma* spp. infection in the sampled livestock of the Karamoja Region

Factor	Level	*N*	Prevalence (%)	OR*	95 % confidence interval		*p* value
					Lower limit	Upper limit	
Gender	Male[a]	1388	279/1388 (20.1)	1	–	–	–
	Female	3582	688/3582 (19.2)	0.91	0.77	1.07	0.24
Species	Cattle[a]	3935	912/3935 (23.2)	1	–	–	–
	Goats	729	40/729 (5.5)	0.23	0.16	0.3	0.000
	Sheep	306	15/306 (4.9)	0.16	0.1	0.27	0.000
District	Kotido[a]	236	236/846 (27.9)	1	–	–	–
	Kaabong	913	79/913 (8.7)	0.29	0.22	0.38	0.000
	Moroto	1071	176/1071 (16.4)	0.62	0.49	0.77	0.000
	Nakapiripirit	1744	406/1744 (23.3)	0.86	0.71	1.04	0.111
	Abim	396	70/396 (17.7)	0.48	0.35	0.65	0.000

*Odds Ratio

[a] Reference Category

N = number of observations

Table 6 Final multivariable model for co-infections (presence of at least two of the investigated infections) in the sampled livestock of the Karamoja Region

Factor	Level	*N*	Prevalence (%)	OR*	95 % confidence interval		*p* value
					Lower limit	Upper limit	
Gender	Male[a]	1388	123/1388 (8.9)	1	–	–	–
	Female	3582	281/3582 (7.8)	0.97	0.77	1.21	0.759
Species	Cattle[a]	393	392/3935 (10.0)	1	–	–	–
	Goats	729	12/729 (1.6)	0.13	0.07	0.25	0.000
	Sheep	306	2/306 (0.01)	0.07	0.02	0.26	0.000
District	Kotido[a]	845	56/845 (6.6)	1	–	–	–
	Abim	376	63/376 (16.8)	1.92	1.43	2.59	0.000

*Odds ratio

[a] Reference category

N = number of observations

agreement with previous studies (Magona et al. 2009; Faye et al. 2005; Makita et al. 2011; Mugizi et al. 2015). Continuous exposure to the etiological agent during animal lifetime can possible lead to enhanced stimulation of antibody production along increasing age.

Our findings showed the female gender has a major risk of having antibodies against *Brucella* compared to males. This is in contrast to Magona et al. (2009), who did not find gender related risk factors for *Brucella* seropositivity in cattle in Uganda. Gender difference might be due to the activation of different transmission routes and pathogenesis of infection, which in cattle are strongly linked to the achievement of sexual maturity and to the tropism of the bacterium for the pregnant uterus. Accordingly, females could be more frequently exposed to *Brucella* infection being more prone to get in contact with aborted and infected materials, thereby becoming the main bacterium reservoir. In our study, no information about the management of referred abortions, contact with wildlife, or practice of sharing water with other flocks or wildlife was collected. All these possible risk factors should be further investigated, as already suggested by other authors (Miller et al. 2015).

Geographical location also influenced the prevalence of the infection, since four districts had a significant higher probability to have *Brucella* seropositivity compared to the district of Kotido. Seroprevalences detected at Abim, Kaabong, Moroto, and Nakapiripirit were similar with the overall one of the Karamoja Region, while positivity registered in Kotido district was significantly lower. Differences in prevalence based on district or geographical location have been already reported (Kashiwazaki et al. 2012; Mugizi et al. 2015). The knowledge of district-associated factors, such as livestock management or presence of characteristic hygienic conditions, is essential to undertake specific control measures accordingly to geographical or social distribution of infection.

Another interesting aspect concerning *Brucella* infection, not developed in this survey, is the variation of seroprevalence during years detected by other authors (Kashiwazaki et al. 2012). It was noticed that prevalence of infections changes for different causes during the time, so more information on risk factors could be obtained if a constant monitoring would be undertaken. Accordingly, longitudinal studies, considering all these variables, should be carried out to understand the reasons.

Livestock prevalence of *Anaplasma* and *Babesia* infections was respectively 19.5 and 16 %. However, direct comparison of prevalence reported in other studies was not easy because they generally investigated single species, like *Anaplasma marginale* and *Babesia bigemina* (Oura et al. 2011; Rubaire-Akiiki et al. 2004, 2006; Magona et al. 2011) or detected antibodies instead of haemoparasites (Rubaire-Akiiki et al. 2004, 2006; Magona et al. 2011). In our study, goats and sheep were found less at risk of *Anaplasma* and *Babesia* infections than cattle. This may be due to common vector-associated factors (i.e. heavy tick infestations were recorded only in cattle during clinical examination), or to the tropism of specific strains or variants of *Anaplasma* and *Babesia* spp. having different susceptibility for animal species. In this study, like for *Brucella* infection, the etiological agents have been identified at genus level. However, it should be also investigated if a different management among sheep, goats and cattle existed. Moreover, animal age, already identified as risk factor in cattle for *Brucella* seropositivity, was not investigated in sheep and goats. Their smaller dimensions compared with cattle could make less body area of sheep and goats available to questing ticks than in cattle. However, the detection of these two vector-borne infections permitted to analyse the frequency of co-infections and to study the relationship between tick-borne and non tick-borne infections. We observed that an animal had three times more probability to have co-infections with *Babesia* spp. and *Anaplasma* spp., particularly in goat and sheep, while *Brucella* infection was not linked with the other infections. This could be explained because *Anaplasma* and *Babesia* infections are transmitted by common carriers, whereas a different route of transmission is used by *Brucella* bacterium. As regards age, being older than 12 months resulted to be a protective factor for *Anaplasma* and *Babesia* infections. As already hypothesized by Magona et al. (2008), this might be related to the laboratory tests used to detect these two pathogens, which directly identify the agents and not the serological response of the host. It is possible that animals negative to direct microscopic examination, with low or absent haemoparasitic burden and subclinical infections, were positive to the test for antibodies against the same agents. Livestock gender was not found to be a risk factor in *Anaplasma* and *Babesia* infections, since these were equally distributed in all animals probably because there is not a gender predisposition to vector-attacks.

A variable situation for vector-borne infections was present considering the district-factors. All the four districts were at lower risk of *Anaplasma* infection than Kotido (reference district). Kaabong and Abim had a greater risk of *Babesia* infection, while Nakapiripirit had lower risk and Moroto had prevalence comparable with Kotido. Considering co-infections, Abim resulted more at risk to have vector-borne infections than the other districts. The reason of this should be further investigated. Previous studies observed spatial variations in tick abundance (Magona et al. 2011). However, other factors than vector-associated variables take part in infections: geographical, seasonal and climatic characteristics could cause a different tick infestation load, as previously reported in other studies, that found highest tick challenge in the lowland zone compared with midland and uplands zones (Rubaire-Akiiki et al. 2004). Also villages with high density of anti-tick plants were found having lower risk of tick infestation because these plants determine an unfavourable tick survival (Magona et al. 2011). The identification of these factors could be very useful to control the infections in a natural way: Rubaire-Akiiki et al. (2006) suggested that when areas at lower risk are identified, the movement of animals in an appropriate area could reduce the risk of infestation. Differently from another study (Magona et al. 2011), no seasonal variation has been analysed in the present study because sample collection was performed over a limited period of time. Other socio-economical factors should be systematically included in further epidemiological studies, because they can be relevant for the spread of infections (Krecek et al. 1995; Okello et al. 2014). The practice of raids characterizing the Karamoja and not analysed in the current study, could be an additional factor differently expressed in the districts. Raids cause animal mixing and moving favouring the spread of infectious diseases, probably even more efficiently compared with animal marketing, already identified as a risk factor for animal brucellosis (Kabagambe et al. 2001). Raided animals are distributed amongst raiders, a tradition which enhances the risk of spreading or introducing infections to new areas and herds (Oloya et al. 2006).

A clear positive output of this study was the development of timely information sharing within the regional on-going EWS. However, disease surveillance should be further supported for firmly establishing it as a recognized tool to contain socio-economic losses from animal diseases and to better control livestock diseases in the Region of the Karamoja of Uganda and in the neighbouring countries.

Acknowledgments The authors wish to thank the C&D staff and organization for embracing and supporting this study, in particular Carlo Ruspantini, Cristiana Strozzi, Cristina Raisi, Federico Soranzo, Pierangela Cantini, and Antonio Petrella. We are enormously grateful to the staff of the C&D Laboratory of Moroto (Michael Omodo, Samuel Okiror, Mariko Iiko, and Fred Akena), for their invaluable help in collecting data, and to Costanza Azzari for her collaboration. We are extremely grateful to all the Karamojong farmers who allowed and helped us in sampling their animals. Without the precious collaboration of all these people this study could not be possible.

The study was supported by the project funded by the European Commission Humanitarian Aid Department (ECHO), with DanChurchAid (DCA) heading a consortium of international and local organizations with in-depth and long-term experience with pastoral communities that include Karamoja Agro-pastoral Development Programme (KADP), Agency for Technical Cooperation and Development (ACTED) and Institute for International Cooperation & Development (C&D) (Project ECHO/HF/BUD/2008/01010—Drought Preparedness, Karamoja and Pokot, implemented in Uganda and Kenya).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Dairy Development Authority (DDA), 2004. Annual report of the Dairy Development Authority in Uganda for 2003–2004. Available from: http://www.dda.or.u/.

Eisler, M.C., Magona, J.W., and Revie, C.W., 2012. Diagnosis of cattle diseases endemic to sub-Saharan Africa: evaluating a low cost decision support tool in use by veterinary personnel. PLoS One. 7(7), e40687.

Faye, B., Castel, V., Lesnoff, M., Rutabinda, D., and Dhalwa, J., 2005. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). Preventive Veterinary Medicine 67(4), 267-81.

Gradé, J.T., Tabuti, J.R., and Van Damme, P., 2009. Ethnoveterinary knowledge in pastoral Karamoja, Uganda. Ethnopharmacology 122(2), 273-93.

Institute for International Cooperation and Development (C&D), 2010. Livestock disease surveillance in Karamoja. The importance of early detection of diseases and epidemics for livestock dependent communities. Ed. Ruspantini C., Uganda.

Kabagambe, E.K., Elzer, P.H., Geaghan, J.P., Opuda-Asibo, J., Scholl, D.T., and Miller, J.E., 2001. Risk factors for *Brucella* seropositivity in goat herds in eastern and western Uganda. Preventive Veterinary Medicine 52(2), 91-108.

Kansiime, C., Mugisha, A., Makumbi, F., Mugisha, S., Rwego, I.B., Sempa, J., Kiwanuka, S.N., Asiimwe, B.B., and Rutebemberwa, E., 2014. Knowledge and perceptions of brucellosis in the pastoral communities adjacent to Lake Mburo National Park, Uganda. BMC Public Health 14, 242.

Kashiwazaki, Y., Ecewu, E., Imaligat, J.O., Mawejje, R., Kirunda, M., Kato, M., Musoke, G.M., and Ademun, R.A., 2012. Epidemiology of bovine brucellosis by a combination of rose bengal test and indirect ELISA in the five districts of Uganda. Journal of Veterinary Medical Science 74(11), 1417-22.

Krecek, R.C., Cornelius, S.T., and McCrindle, C.M., 1995. Socio-economic aspects of animal diseases in southern Africa: research priorities in veterinary science. Journal of South African Veterinary Association 66(3), 115-20.

Kunda, J., Fitzpatrick, J., Kazwala, R., French, N.P., Shirima, G., MacMillan, A., Kambarage, D., Bronsvoort, M., and Cleaveland, S., 2007. Health-seeking behaviour of human brucellosis cases in rural Tanzania. BMC Public Health 7, 315.

Lolli, C., and Diverio, S., 2010. The Disease Surveillance (Part III). In: Livestock Disease Surveillance in Karamoja. The importance of early detection of diseases and epidemics for livestock dependent communities. Institute for International Cooperations and Development (C&D), Ed. Ruspantini C., Uganda, 20-24.

Magona, J.W., Walubengo, J., Olaho-Mukani, W., Jonsson, N.N., Welburn, S.C., and Eisler, M.C., 2008. Clinical features associated with seroconversion to *Anaplasma* marginale, *Babesia bigemina* and *Theileria parva* infections in African cattle under natural tick challenge. Veterinary Parasitology 155(3-4), 273-80.

Magona, J.W., Walubengo, J., Galiwango, T., and Etoori, A., 2009. Seroprevalence and potential risk of bovine brucellosis in zerograzing and pastoral dairy systems in Uganda. Tropical Animal Health Production 41(8), 1765-71.

Magona, J.W., Walubengo, J., Olaho-Mukani, W., Jonsson, N.N., Welburn, S.W., and Eisler, M.C., 2011. Spatial variation of tick abundance and seroconversion rates of indigenous cattle to *Anaplasma* marginale, *Babesia bigemina* and *Theileria parva* infections in Uganda. Experimental and Applied Acarology 55(2), 203-13.

Makita, K., Fèvre, E.M., Waiswa, C., Eisler, M.C., Thrusfield, M., and Welburn, S.C., 2011. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. BMC Veterinary Research 7, 60.

Miller, R., Nakavuma, J.L., Ssajjakambwe P., Vudriko, P., Musisi, N., and Kaneene, J.B., 2015. The Prevalence of Brucellosis in Cattle, Goats and Humans in Rural Uganda: A Comparative Study. Transboundary and Emerging Diseases doi: 10.1111/tbed.12332.

Mugizi, D.R., Boqvist, S., Nasinyama, G.W., Waiswa, C., Ikwap, K., Rock, K., Lindahl, E., Magnusson, U., and Erume, J., 2015.Prevalence of and factors associated with *Brucella* sero-positivity in cattle in urban and peri-urban Gulu and Soroti towns of Uganda. Journal of Veterinary Medical Science 77(5): 557-564.

Muhanguzi, D., Matovu, E., and Waiswa, C., 2010. Prevalence and characterization of Theileria and *Babesia* Species in cattle under different husbandry systems in Western Uganda. International Journal of Animal and Veterinary Advances 2(2), 51-58.

Mwebe, R., Nakavuma, J., and Moriyón, I., 2011. Brucellosis seroprevalence in livestock in Uganda from 1998 to 2008: a retrospective study. Tropical Animal Health Production (3), 603-8.

Nasinyama, G., Ssekawojwa, E., Opuda, J., Grimaud, P., Etter, E., and Bellinguez, A., 2014 *Brucella* sero-prevalence and modifiable risk factors among predisposed cattle keepers and consumers of un-pasteurized milk in Mbarara and Kampala districts, Uganda. African Health Science Dec. 14(4), 790-6.

Newton, F.J., Jones, E., Connor, R.J., Davidson, B.J., and McGovern, P.T., 1974. A survey of bovine brucellosis in four districts of Uganda. British Veterinary Journal 130: 249-254.

Nizeyimana, G., Mwiine, F.N., and Ayebazibwe, C., 2013. Comparative *Brucella abortus* antibody prevalence in cattle under contrasting husbandry practices in Uganda. Journal of South Africa Veterinary Association 84(1), E1-5.

Office for the Coordination of Humanitarian Affairs (OCHA), 2009. Available from: www.ugandacluster.ugs.

Office International Epizooties (OIE), 2015. Manual of diagnostic tests and vaccines for terrestrial animals 2015. Accessed on December 2015 from http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/.

Okello, A., Welburn, S., and Smith, J., 2014. Crossing institutional boundaries: mapping the policy process for improved control of endemic and neglected zoonoses in sub-Saharan Africa. Health Policy and Planning Jul 7, pii: czu059.

Oloffs, A., Baumann M.P.O., Afema, J., and Nakavuma, J., 1998. Experiences with a strategy to investigate bovine brucellosis in a rural area in Southwest Uganda, Revue d'Elevage et de Médicine Veterinaire des Pays Tropicaux, 51(2), 101–105.

Oloya, J., Muma, J.B., Opuda-Asibo, J., Djønne, B., Kazwala, R., and Skjerve, E., 2006. Risk factors for herd-level bovine-tuberculosis seropositivity in transhumant cattle in Uganda. Preventive Veterinary Medicine 80, 318– 329.

Oura, C.A., Tait, A., Asiimwe, B., Lubega, G.W., and Weir, W., 2011. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108(6), 1365-74.

R Development Core Team. R, 2007. A Language and Environment for Statistical Computing. Austria: R Foundation for Statistical Computing, Vienna, Austria.

Rubaire-Akiiki, C., Okello-Onen, J., Nasinyama, G.W., Vaarst, M., Kabagambe, E.K., Mwayi, W., Musunga, D., and Wandukwa, W., 2004. The prevalence of serum antibodies to tick-borne infections in Mbale District, Uganda: the effect of agro-ecological zone, grazing management and age of cattle. Journal of Insect Science 4:8

Rubaire-Akiiki, C.M., Okello-Onen, J., Musunga, D., Kabagambe, E.K., Vaarst, M., Okello, D., Opolot, C., Bisagaya, A., Okori, C., Bisagati, C., Ongyera, S., and Mwayi, M.T., 2006. Effect of agro-ecological zone and grazing system on incidence of East Coast Fever in calves in Mbale and Sironko Districts of Eastern Uganda. Preventive Veterinary Medicine 75(3-4), 251-66.