

Spatial distribution of *Brucella* antibodies with reference to indigenous cattle populations among contrasting agro-ecological zones of Uganda



Fredrick Kabi^{a,b,*}, Vincent Muwanika^a, Charles Masembe^c

^a Department of Environmental Management, Molecular Genetics Laboratory, College of Agricultural and Environmental Sciences, Makerere University, P. O. Box 7062/7298, Kampala, Uganda

^b National Livestock Resources Research Institute (NaLIRRI), P.O. Box 96, Tororo, Uganda

^c Department of Bio-Sciences, College of Natural Sciences, Makerere University, Box 7062, Kampala, Uganda

ARTICLE INFO

Article history:

Received 11 December 2014

Received in revised form 2 June 2015

Accepted 8 June 2015

Keywords:

Agro-ecological zones

Brucella antibodies

Indigenous cattle populations

Spatial distribution

Uganda

ABSTRACT

Indigenous cattle populations exhibit various degrees of agro-ecological fitness and provide desirable opportunities for investments to improve sustainable production for better rural small-scale farmers' incomes globally. However, they could be a source of infection to their attendants and other susceptible livestock if their brucellosis status remains unknown. This study investigated the spatial distribution of *Brucella* antibodies among indigenous cattle populations in Uganda. Sera from a total of 925 indigenous cattle (410 Ankole *Bos taurus indicus*, 50 Nganda and 465 East African Shorthorn Zebu (EASZ) – *B. indicus*) obtained randomly from 209 herds spread throughout Uganda were sequentially analysed for *Brucella* antibodies using the indirect (I) and competitive (C) enzyme linked Immuno-sorbent assays (ELISA). Recent incidences of abortion within the previous 12 months and routine hygienic practices during parturition were explored for public health risks. *Brucella* antibodies occurred in approximately 8.64% (80/925) and 28.70% (95% CI: 22.52, 34.89) of the sampled individual cattle and herds, respectively. Findings have shown that Ankole and EASZ cattle had similar seroprevalences. Indigenous cattle from the different study agro-ecological zones (AEZs) exhibited varying seroprevalences ranging from approximately 1.78% (95% CI: 0, 5.29) to 19.67% (95% CI: 8.99, 30.35) in the Lake Victoria Crescent (LVC) and North Eastern Drylands (NED) respectively. Significantly higher odds for *Brucella* antibodies occurred in the NED (OR: 3.40, 95% CI: 1.34, 8.57, $p=0.01$) inhabited by EASZ cattle compared to the KP (reference category) AEZ. Recent incidences of abortions within the previous 12 months were significantly ($p<0.001$) associated with seropositive herds. These findings add critical evidence to existing information on the widespread occurrence of brucellosis among indigenous cattle populations in Uganda and could guide allocation of meagre resources for awareness creation. And deployment of control strategies including culling of older cattle and those which have aborted during advanced gestation, enforcement of hygiene practices and mass vaccination.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Brucellosis is a commonly encountered zoonotic disease in sub Saharan Africa (SSA) (Plumb et al., 2013; McDermott et al., 2013; Racloz et al., 2013). Its economic burden among pastoral and smallholder livestock farmers results into reduced success of poverty reduction initiatives through loss of productivity and income (WHO, 2006; MAAIF, 2010). Cattle brucellosis is primarily caused by *Brucella abortus* organisms (Radostits et al., 2000), which

occur in high concentration in placental membranes and fluids at parturition, aborted fetuses, unpasteurized dairy products consequently acting as sources of infection to other susceptible livestock and humans (Nabukenya et al., 2013; Racloz et al., 2013; Ducrotoy et al., 2014). Reproductive failure, lost milk yields and restrictions to lucrative markets (Mangen et al., 2002) comprise the main losses incurred due to cattle brucellosis. Undiagnosed *Brucella* infected cattle may provide sources of infection to farmers' households, animal health workers, butchers and consumers of unpasteurized dairy products (Mangen et al., 2002; Plumb et al., 2013). The consequences are widespread incidences of human brucellosis (Faye et al., 2005; Swai and Schoonman, 2009; Kunda et al., 2010). Recent reports by Nabukenya et al. (2013) indicate a seroprevalence of 7–10% among abattoir workers in Kampala and Mbarara.

* Corresponding author at: National Livestock Resources Research Institute (NaLIRRI), P. O. Box 96, Tororo, Uganda. Tel.: +256 776212292.
E-mail address: freddykabi@gmail.com (F. Kabi).

Indigenous cattle in Uganda constitute approximately 93.3% of the national herd and have adjusted to local agro-ecological conditions under the stewardship of rural smallholder farmers (MAAIF/UBOS, 2009; Balikowa, 2011). Their usefulness has been well emphasised by Kugonza et al. (2012), in addition to being able to thrive under marginal resources not conducive to other types of primary agricultural investments (FAO, 2009). Indigenous cattle, though popular, constitute the most brucellosis infected livestock species in Uganda (Mwebe et al., 2011). Several factors including traditional husbandry practices, poor sanitation during parturition, consumption of unpasteurized milk and their products, occurrence of latent phases of infection increases the risks of brucellosis dissemination (McDermott and Arimi, 2002; Kunda et al., 2007, 2010; Megersa et al., 2011; Nabukenya et al., 2013). For instance, approximately 70% of the milk consumed in Uganda is obtained from indigenous cattle (MAAIF/UBOS, 2009; Balikowa, 2011) and is sold unpasteurized through vendors. Indigenous cattle too, share pastures with wildlife increasing the risk of contamination with disease (Ocaido et al., 2009; Magona et al., 2009). Routine brucellosis surveillances to empower novel control strategies (Robinson 2003; Mekonnen et al., 2010) have therefore become a necessity. Although brucellosis location specific studies have been recently summarized (Mwebe et al., 2011), this survey provides additional information on nationwide spatial distribution with comparisons between contrasting agro-ecological zones (AEZs), indigenous cattle breeds and related risk factors to further equip strategic planning of disease control.

2. Materials and methods

2.1. Study area

The study area has been described in Kabi et al. (2014). Briefly, Uganda's total size is approximately 241,550.7 square kilometres (sq km), lies across the equator in Eastern Africa between longitudes 29.5° East and 35° East and between latitudes 4.5° North and 0.5° South. The country's mean altitude is 1100 m above sea level, ranging from 620 m (Albert Nile) to 5111 m (Mt. Rwenzori peak). Numerous water bodies occur, drained by rivers (Aswa, Kagera and the Nile) which influence the agro-ecological climatic features (UBOS, 2013). The 10 AEZs in this study have been delineated basing on a fairly similar socio-economic background and ecological conditions, farming systems and practices (MAAIF/MFPED, 2004).

2.2. Sample and data collection plan

The sample collection plan followed a landscape sampling strategy covering the 10 AEZs (Kabi et al., 2014). Samples and data for this study were collected from January 2011 to April 2012.

The sample size (n) was determined using the arithmetic formula,

$$n = \frac{z^2 p(1-p)}{d^2}$$

where n is the sample size, z is 1.96 at 95% confidence interval, p is the expected prevalence chosen to be 10% (Mwebe et al., 2011) and d is the margin of error (5%) (Thrusfield, 2003).

This sampling plan could enable the estimation of an individual cattle seroprevalence of 10% with a 95% confident interval (CI) and an error margin of 5%. Given the above formula, 138 head of cattle per breed (Ankole and EASZ) was considered adequate. Additionally, this study used a landscape sampling strategy defined by 50 grid cells (approximately 50 × 50 km). Within each grid cell, 4–6 indigenous cattle herds were randomly selected and similarly at the herd level, 4–5 head of cattle were randomly selected for sample collection.

This sampling strategy enabled a fairly uniform and widespread data collection across the different agro-climatic zones as designed under the NextGen Project (NextGen, 2010) aimed at establishing differences among indigenous cattle populations in dissimilar AEZs. The 10 AEZs have been defined on the basis of a fairly uniform socio-economic background and ecological conditions, farming systems and practices (MAAIF/MFPED, 2004). They include:– North Eastern Savannah Grasslands (NESG), North Eastern Drylands (NED), Kyoga Plains (KP), North Western Savannah Grasslands (NWSG), Para-Savannah Grasslands (PSG), Western Savannah Grasslands (WSG), Lake Victoria Crescent (LVC), Pastoral Rangelands (PR), South Western Farmlands (SWF) and Western Highland Ranges (WHR). These AEZs, grid cells and sampling sites are displayed in Fig. 1.

Latitude and longitude of each sampled site were obtained by a global positioning system (GPS) of an Etrex®, Garmin (Southampton, UK) handset. Cattle herd owners, their representatives such as cattle herdsman, or knowledgeable family members were interviewed to facilitate recording of administrative locations, recent incidences of abortions (within the previous 12 months), retained placenta and the associated hygiene practices. The sampled cattle breed, age (months) and gender were recorded on customized data sheets. The local animal health workers assisted with language translation and interpretation of the questions to cattle herd owners or herdsmen who represented the herd owner. The language translation and interpretation were validated in a meeting by the district veterinary officer and local village leaders.

2.3. Blood sample collection

About 5 ml of blood from well restrained cattle was collected from the jugular vein using sterile needles into plain vacutainers (Becton-Dickinson, Vacutainer System, UK). These were stored at ambient temperatures overnight and separation of sera was performed on the following day. The sera were kept in a cool box under ice and transported to the Molecular Genetics Laboratory at the Department of Environmental Sciences, Makerere University for storage at –20 °C.

2.4. Laboratory technics for serological testing using the I-ELISA and C-ELISA Svanovir® kits

The I-ELISA Svanovir® kit was used as a screening test to detect *Brucella* antibodies (with sensitivity of 0.95 and specificity of 0.97). The positive samples were confirmed by the Svanovir® Brucella-Ab C-ELISA kits (Svanova Biotech AB Uppsala, Sweden). The kit has sensitivity (Se) of 0.98 and specificity (Sp) of 0.99 and is able to detect IgM, IgG1, IgG2 and IgA (Nielsen, 2002; Gall and Nielsen, 2004; Rogan and Gladen, 1978). Samples were declared positive if they tested positive to both Indirect Antibody Enzyme Linked Immunosorbent Assay (I-ELISA) and Competitive Antibody Enzyme Linked Immunosorbent Assay (C-ELISA) in accordance with recommendations from the World Organisation for Animal Health (OIE, 2009). The serial interpretation was necessitated in order to eliminate any undeclared *Brucella* strain 19 vaccinated cattle and cross-reactions from any other gram negative bacteria.

2.5. Data management and analysis

The data of *Brucella* seropositivity among the different age classes, sexes, breeds and AEZs were entered into Microsoft Excel® 2010, exported to Stata® ver. 12 (2012) package (Stata Corporation Texas, USA), cleaned and coded for statistical computation. The sampled cattle were categorised into 7–24, 25–36, 37–72, 73–192 months old age groups. Means (obtained using Wald statistics taking into account the clustering of animals by herds) of brucellosis seroprevalences among the different AEZs, breeds, sexes, age

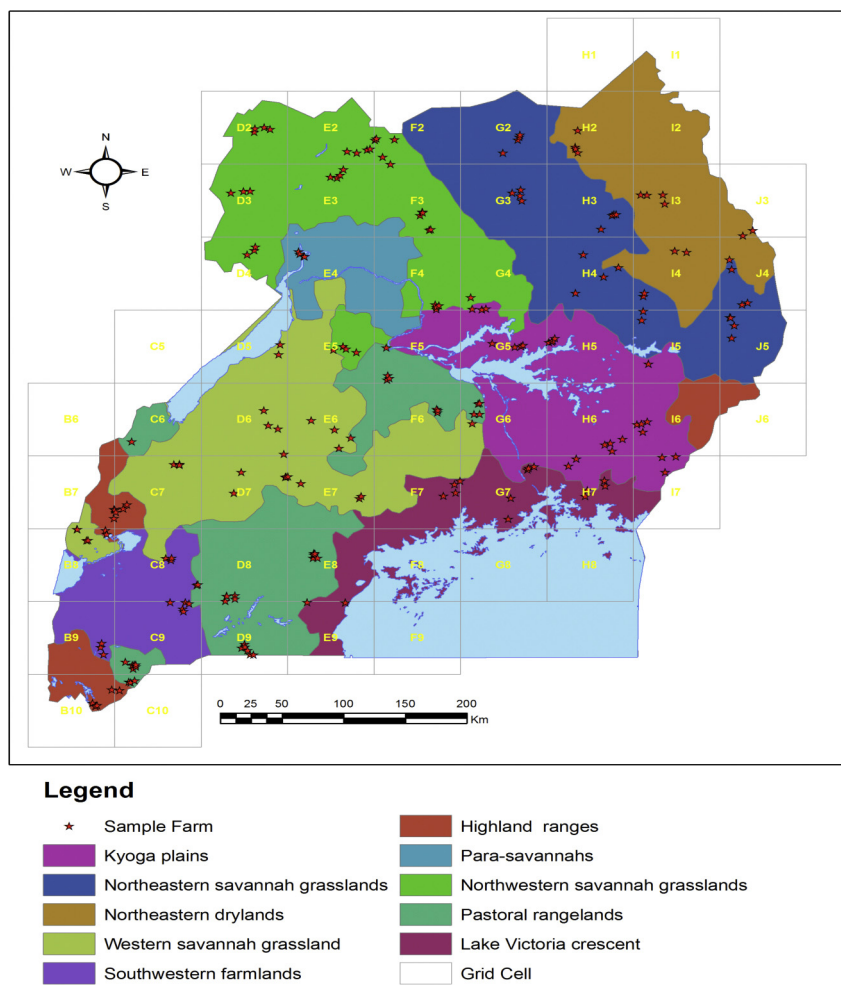


Fig. 1. The nationwide locations of indigenous cattle sample farms/ herds ($n = 209$) in the 10 AEZs of Uganda.

The locations of the 209 farms/ herds where 925 indigenous cattle blood samples were obtained to determine the spatial distribution of *Brucella* antibodies. Sample collection was guided by the 50 grid cells (approximately 50×50 km) in which 4–6 farm herds were selected and within each herd, 4–5 indigenous cattle were randomly selected. (Adopted from Kabi et al., 2014)

groups were computed at 95% confidence interval (CI). A generalized estimating equation (GEE) population-averaged univariable and multivariable logistic regression models with herd as a group variable and an exchangeable working correlation structure was engaged to estimate the odds ratios (OR) with robust intervals (95% CI) and corresponding p -values for variations within AEZs, breeds, gender and age groups. The 'xtlogit' command of Stata® ver. 12 (2012) package was used.

2.6. Spatial distribution of *Brucella* antibodies in Uganda

The inverse distance weighted interpolation (IDWI) of ArcMap® technology was used to determine the spatial distribution of *Brucella* antibodies in Uganda. IDWI employs the hypothesis that measurements that are close to one another are more alike than those that are farther apart. To forecast a value for any unmeasured location, IDWI will use the measured values surrounding the prediction location. Those known values closest to the prediction location will have more influence on the predicted value than those farther away. Thus, IDWI assumes that each measured point has a local influence that diminishes with distance.

Using the GIS data of the 209 cattle herds and the *Brucella* antibody seropositivity per herd, a continuous *Brucella* antibody spatial distribution map was created to display the spatial distribution

of *Brucella* antibodies among indigenous cattle populations across the entire landscape of Uganda. The spatial distribution of *Brucella* antibodies obtained from apparently healthy indigenous cattle populations in Uganda (January 2011–April 2012) was interpolated using 209 study herd seroprevalence values to create a nation-wide spatial effect. The IDWI on the spatial analyst extension of ArcMap version 10 was used to generate the continuous *Brucella* antibodies distribution map on a red–yellow color for higher and light green for lower antibody occurrence. Parameters were set so that for each pixel in the continuous raster an average seroprevalence was calculated based on *Brucella* seropositivity at herd level. Being a weighted average, the weights were higher for herds near the pixel (red) and lower for more distant herds (green). An appropriate exponent value of 20 km was chosen to generate a continuous *Brucella* antibodies prevalence map over the 209 individual herd seroprevalence values. Fig. 2 shows the results of the spatial distribution of *Brucella* antibodies in Uganda.

2.7. Ethical clearance

This study obtained ethical clearance from Makerere University Institute of Environment and Natural Resources (MUIENR) and approval from the higher degree committee of Makerere University. The permission to conduct this study was offered by the

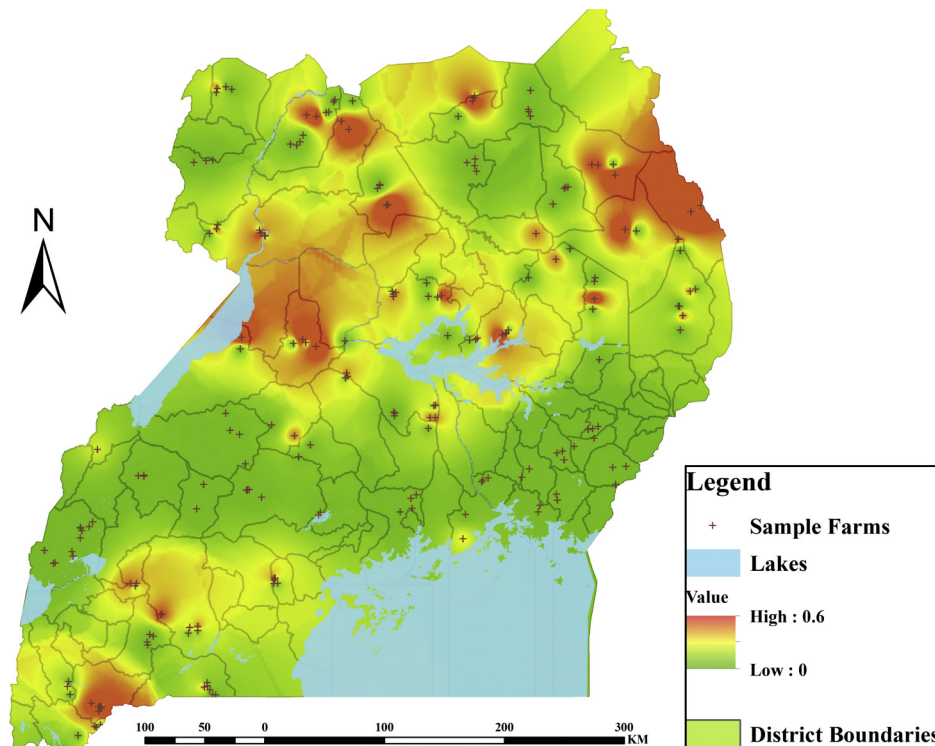


Fig. 2. The spatial distribution of *Brucella* antibodies among indigenous cattle populations in Uganda (January 2011–April 2012). The spatial distribution of *Brucella* antibodies (January 2011–April 2012) was interpolated using 209 herd seroprevalences to create a nation-wide spatial effect. An inverse distance weighted interpolation (IDWI) on the spatial analyst extension of ArcMap 10 was used to generate the continuous antibody distribution map on a red–yellow color for higher and green for lower *Brucella* antibody seroprevalence. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Uganda National Council for Science and Technology (UNCST) reference number NS 325. The district veterinary personnel and farmers granted oral agreement to use their cattle for this study.

3. Results

3.1. Trend of occurrence of *Brucella* antibodies in the study cattle

Blood samples were obtained from 925 cattle comprising 50 Nganda (*B. indicus*, 46 females and 4 males), 410 Ankole (*B. taurus indicus*, 379 females and 31 males), and 465 EASZ (*B. indicus*, 387 females and 78 males) from which sera were prepared. The small sample size of males kept for breeding purposes unfortunately encourages their communal use which may increase the risks of brucellosis transmission between herds. In this study, a sample was considered seropositive if it tested positive to the two tests (I-ELISA and C-ELISA), while a herd was considered positive if at least one individual within the herd tested positive to both I-ELISA and C-ELISA. Two samples from the NESG failed the C-ELISA test and therefore regarded as false positives to the I-ELISA test. The individual seroprevalence of brucellosis among the study cattle was estimated to be 8.64% (80/925). The herd antibody prevalence of brucellosis was estimated to be 28.70% (95% CI: 22.52, 34.89). The occurrence of *Brucella* antibodies increased along the age gradient among the study cattle, while the Ankole and EASZ exhibited similar seroprevalence value, although the Nganda had lower seroprevalence. Female cattle exhibited higher seroprevalence compared to the males. All the study AEZs were harboring at least one seropositive head of cattle with seroprevalence ranging from a low 1.78% (95% CI: 0, 4.94) to a high 19.67% (95% CI: 8.99, 30.35) in the LVC and NED, respectively, as shown in Table 1.

3.2. The risk of *Brucella* antibodies' occurrence by age, sex, breed and AEZs

The different risk factors for *Brucella* antibodies in the study cattle under the different categories was analysed using univariable and multivariable logistic regression models. During the analyses of both the univariate and multivariable logistic regressions, the first level of each independent variable was used as a reference category. The OR of being seropositive was 2.86 times higher among the study cattle of 73–192 months of age compared to that of 7–24 months of age but this was not statistically significant. However, when the lower age categories were combined (7–72 months) and compared in a logistic regression, with 73–192 months, a significantly higher OR (1.90: 1.12, 3.21; $p=0.01$) was observed. Within the sex categories, the females exhibited a higher non-significant seroprevalence and a significantly ($p=0.05$) higher OR of 2.45 (95% CI: 0, 6.20) times compared to their male counterparts. Indigenous cattle sampled from the NED exhibited a significantly higher OR of *Brucella* antibodies (3.40: 95% CI: 1.34, 8.87; $p=0.01$) than those resident in the reference category of KP AEZ. The results are shown in Table 1. The multivariable logistic regression model showed that study cattle resident in the NED (OR 6.25, p -value = 0.007) were significantly associated with *Brucella* antibodies compared to those in the reference category of KP AEZ as shown in Table 2.

3.3. *Brucella* herd seropositivity and its association with recent abortion within the herds

Results obtained from the interview of the 209 households indicated that 52 herds recorded an abortion in the past 12 months. While *Brucella* (Indirect and C-ELISA) test results indicated that *Brucella* antibody prevalence among herds which had a recent (in the previous 12 months) incidence of abortion and those with no his-

Table 1
Summary results of univariable logistic regression (LR) analysis of *Brucella* seroprevalence among the different indigenous cattle categories in Uganda.

Risk factors	Category level	n = 925	Estimated seroprevalence (%)	95% CI	Univariable LR analysis		
					OR	95% CI of OR	p-Value of OR
Age	7–24	70	4.28	0, 8.99	1	–	Reference
	25–36	83	2.40	0, 5.71	0.55	0.08, 3.39	0.52
	37–72	332	7.53	4.33, 10.72	1.81	0.53, 6.19	0.33
	73–192	440	11.36	7.93, 14.79	2.86	0.86, 9.44	0.08
				Overall p-value = 0.03			
Breed	Ankole	410	8.29	5.35, 11.23	1	–	Reference
	Nganda	50	4.0	0, 9.22	0.46	0.10, 1.97	0.29
	EASZ	465	9.46	6.46, 12.45	1.15	0.72, 1.84	0.54
				Overall p-value = 0.44			
Sex	Male	124	4.03	0.64, 7.41	1	–	Reference
	Female	801	9.36	7.08, 11.64	2.45	0.97, 6.20	0.05*
AEZ	Sampled districts						
KP	Iganga, northern Bugiri, Tororo, Kaberamaido	134	6.71	1.26, 12.16	1	–	Reference
LVC	Southern Masaka, Bukomansimbi, Buikwe, Mpigi, Jinja, Mayuge	56	1.78	0, 4.94	0.25	0.03, 2.04	0.19
NED	Northeastern Kotido, eastern Kitgum, northern Nakapiripiriti	61	19.67	8.99, 30.35	3.40	1.34, 8.57	0.01*
NESG	Pader, Kitgum, Katakwi, Abim	127	7.08	2.13, 12.04	1.05	0.40, 2.75	0.90
NWSG	Adjumani, western Nebbi, Arua, Yumbe, northern Gulu, northern Apac	155	11.61	5.50, 17.71	1.82	0.79, 4.21	0.15
PSG	Eastern Nebbi, southwestern Gulu, western Masindi	16	18.75	0, 39.23	3.20	0.77, 13.34	0.10
PR	Masindi, Nakasongola, southern Mubende, eastern Mbarara, southern Ntungamo	166	12.04	7.67, 16.42	1.90	0.83, 4.32	0.12
SWF	western Mbarara, northern Ntungamo, Rukungiri	36	8.33	1.48, 15.18	1.26	0.32, 4.92	0.73
WHR	Kabale, Kasese, western Kyenjonjo	61	3.27	0, 7.54	0.47	0.09, 2.24	0.34
WSG	Hoima, Kibaale, eastern Kyenjonjo,	133	2.65	0, 6.39	0.37	0.10, 2.04	0.43
				Overall p-value = 0.01			

AEZs – Agro-Ecological Zones, NESG – North Eastern Savannah Grasslands, NED – North Eastern Drylands, KP – Kyoga Plains, NWSG – North Western Savannah Grasslands, PSG – Para-Savannah Grasslands, WSG – Western Savannah Grasslands, LVC – Lake Victoria Crescent, PR – Pastoral Rangelands, SWF – South Western Farmlands, WHR – Western Highland Ranges, OR – odds ratio.

* Significantly (Ref. $p \leq 0.05$).

tory of a recent abortion was 86.7% and 13.3%, respectively. Study cattle with a recent (in the previous 12 months) abortion were very significantly ($p \leq 0.0001$) associated with *Brucella* seropositivity (Table 3). All households reported lack of protective gear and yet assisted cattle during parturition and disposed aborted fetuses, placental membranes into nearby bushes which could later be devoured by dogs.

4. Discussion

Indigenous cattle have been patronised by rural smallholder farmers in Uganda (MAAIF/UBOS, 2009), however their brucellosis status is largely unknown, which could be an impediment to initiatives for increased productivity and poverty reduction. This countrywide study established the individual *Brucella* antibody prevalence of approximately 8.64% with reference to the different indigenous cattle breeds sampled from the 10 AEZs. This is comparable to 10% seroprevalence recently reported by Mwebe et al. (2011). The Ankole and EASZ cattle had similar seroprevalence levels, although the Nganda exhibited a lower seroprevalence of 4%. The individual seroprevalence among Nganda cattle is comparable to 5% previously described by Makita et al. (2011) in the areas surrounding Kampala. Nganda cattle are reared in central Uganda in smaller herds in a crop-livestock farming system characterized by restricted movements as compared to the other two indigenous breeds reared in either western or eastern Uganda. The herd sero-

prevalence of approximately 28.70% established in this study was much lower compared to 100% previously reported by Magona et al. (2009). However, our herd seroprevalence is similar to 20% reported in Tanzania by Kunda et al. (2010) amidst the indigenous Shorthorn Zebu and 26.1% reported by Megersa et al. (2011) among pastoral cattle in Ethiopia. Normally, higher herd seroprevalence such as that reported by Magona et al. (2009), are due to larger cattle herds involved in long distance pastoral system in search for pasture and water, which may be shared with wildlife such as in Nakasongola district of Uganda. These phenomena provide suitable circumstances for *Brucella* organism transmission.

All the different study AEZs harbored cattle with *Brucella* antibodies varying from about 1.78% to 19.67%. The lower antibody prevalences of cattle sampled from LVC, WSG, WHR and KP (1.78–6.71%) are comparable to a seroprevalence of 5% observed among cattle in peri-urban areas of Kampala (Makita et al., 2011). Similarly, Magona et al. (2009) estimated the individual and herd brucellosis seroprevalence of zero-grazed crossbred Friesian cattle from the eastern districts of Busia, Bugiri, Mayuge, Tororo, Manafwa, Budaka, Iganga (part of LVC and KP AEZs) to be 3.3% and 5.5%, respectively. This low seroprevalence strengthens the fact that smaller cattle herds whose movements in search for pasture is limited are more likely to harbor lower brucellosis frequencies compared to pastoral cattle in the NED AEZ of Uganda. The seroprevalence ranges of 7.08–18.75% observed in this study were evident among cattle resident in the NESG, SWF, PR and

Table 2
Summary results of multivariable logistic regression (LR) analysis of *Brucella* seroprevalence among the different indigenous cattle categories in Uganda.

Risk factor	Category level	n = 925	Multivariable LR analysis		
			OR	95% CI of OR	p-Value of OR
Age (months)	7–24	70	1	–	Reference
	25–36	83	0.67	0.09, 4.53	0.68
	37–72	332	1.86	0.48, 7.16	0.36
	73–192	440	2.98	0.82, 10.84	0.09
Breed	Ankole	410	1	–	Reference
	Nganda	50	0.27	0.05, 1.46	0.13
	EASZ	465	0.39	0.14, 1.10	0.07
Sex	Male	124	1	–	Reference
	Female	801	1.96	0.79, 4.88	0.14
AEZ	KP	134	1	–	Reference
	LVC	56	0.22	0.03, 1.54	0.12
	NED	61	6.25	1.63, 23.94	0.007**
	NESG	127	1.94	0.47, 7.98	0.35
	NWSG	155	2.61	0.70, 9.73	0.15
	PSG	16	4.27	0.65, 27.74	0.12
	PR	166	1.53	0.60, 3.92	0.37
	SWF	36	0.72	0.21, 2.47	0.60
	WHR	61	0.30	0.06, 1.47	0.13
	WSG	133	0.28	0.05, 1.44	0.13

AEZs – Agro-Ecological Zones, NESG – North Eastern Savannah Grasslands, NED – North Eastern Drylands, KP – Kyoga Plains, NWSG – North Western Savannah Grasslands, PSG – Para-Savannah Grasslands, WSG – Western Savannah Grasslands, LVC – Lake Victoria Crescent, PR – Pastoral Rangelands, SWF – South Western Farmlands, WHR – Western Highland Ranges, OR – odds ratio.

** Very significantly (Ref. $p \leq 0.001$), overall p -value for the model = 0.000.

Table 3
Brucella herd seroprevalence and its association with recent abortion recorded within the herds in the previous 12 months.

Brucella herd seropositivity (I-ELISA and C-ELISA tests)	Abortions reported			LR results of <i>Brucella</i> sero-status herds and its association with abortion incidence	
	None (%)	Recorded (%)	Total (%)	OR (95% CI)	p-Value
Seropositive herds	8 (13.3)	52 (86.7)	60 (100)	Abortion	131.85: 45.54, 381.71
Seronegative herds	142 (95.3)	7 (4.7)	149 (100)		
Total number of herds	150	59	209		0.001***

I – Indirect enzyme linked immunosorbent assay, C-ELISA – competitive enzyme linked immunosorbent assay, OR – odds ratio, % – percentage, LR – logistic regression.

*** Very highly significant.

PSG AEZs. These are comparable to 15.8% and 12.8% previously reported among the pastoral and agro-pastoral cattle respectively in Mbarara district (Faye et al., 2005). Indigenous cattle in these AEZs are kept in larger herds, graze and obtain water communally, and sometimes share pastures with wild animals in conservation areas such as Queen Elizabeth, Lake Mburo and Murchison falls National Parks. The highest antibody prevalence of about 19.67% was observed in the NED AEZ, a semi-arid area characterized by extensive cattle movement, practiced to obtain adequate pasture feed and water. Extensive cattle movements in search of pastures result into widespread acquisition and dissemination of *Brucella* organisms, commonly observed on communal pastures and water (for instance in swampy and wetlands areas). A similar scenario was been observed in Kenya by Kadohira et al. (1997) who demonstrated that free communal grazing and abortion in cattle were significant risk factors for higher brucellosis seroprevalence.

The current study has shown that brucellosis seropositivity among the older cattle (>72 months) was higher than the younger age categories (7–72 months) combined. This agrees with previous studies (McDermott and Arimi, 2002; Faye et al., 2005; Berhe et al., 2007; Magona et al., 2009; Mai et al., 2012) which revealed that the occurrence of brucellosis seropositivity increases with advancing age. This is attributed to the fact that pastoralists keep cattle for

long periods sometimes beyond their economic value. This practice results into repeated exposure to *Brucella* organisms and consequently higher disease seropositivity.

Female cattle exhibited higher odds of brucellosis seroprevalence than their male counterparts in the univariate logistic regression analysis, which strengthen the fact that female cattle may be infected compared to their male counterparts. More importantly however, females usually outnumber males within herds and will discharge *Brucella* organisms during abortions, in the retained fetal membranes and associated vaginal fluids (Makita et al., 2011; Megersa et al., 2011). Such phenomena critically influence deployment of control strategies including vaccinations and hygiene practices being more emphasized in the females than the males.

This study also demonstrates that the history of a recent abortion in the previous 12 months within cattle herds is strongly associated with higher likelihoods of *Brucella* infection, since a total of 86.7% seropositive herds reported a recent (within the past 12 months) history of abortion. This is in agreement with a study carried out in northern Tanzania by Kunda et al. (2010), who noted that the occurrence of abortion within cattle herds had strong association with brucellosis infection. Similar finding were reported by Kadohira et al. (1997) in Kenya and Magona et al.

(2009) in Nakasongola district of Uganda among indigenous cattle. Although Magona et al. (2009) established a seroprevalence of 3.3% among crossbred cattle, but no abortion was recorded, possibly due to the lower seroprevalences. All households reported having assisted cattle during parturition, disposed dead aborted fetuses and placental membranes into nearby bushes which could later be devoured by dogs or other wild carnivores. This is noted as a critical public health risk for brucellosis as previously reported (Magona et al., 2009; Megersa et al., 2011).

The traditional attachment of rural households to indigenous cattle such as assistance at delivery and rearing favorite cows within the herd for several years are likely predisposing factors for the occurrence of brucellosis within cattle keeping communities. Such findings have been reported by Kunda et al. (2010). Further studies are suggested to establish the prevalence of human brucellosis among households with a history of abortion among their cattle herds.

5. Conclusions

This study has shown that cattle resident in the NED AEZ, advancing age category (>72 months) and a recent history of abortion within the herd in the previous 12 months were important risk factors for brucellosis seropositivity. Apart from being a source of infection to cattle households and animal health service providers, indigenous cattle could also disseminate brucellosis to naïve exotic and crossbred cattle in their proximity, though not quantified in this study. The authors suggest urgent creation of public awareness on hygiene practices among the pastoralists and smallholder farmers. Culling of older cattle (>72 months) and those which have had an abortion especially in the third trimester as may be more associated with brucellosis. Routine testing and culling of positive cases and mass vaccination as immediate control measures to be considered for deployment.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors are grateful to all the farmers who participated in this study for their hospitality and kind support. The Next generation methods to preserve farm animal biodiversity (NextGen project) and Eastern Africa Agricultural Productivity Project (EAAPP) of the National Agricultural Research Organisation (NARO) Uganda provided the necessary logistics for this study for which we are very appreciative. We recognize the technical support provided by John Padde, Johnson Mayega and Moses Namutale of the Department of Environmental Management. We additionally acknowledge the technical assistance provided by Esau Martin, Milton Bahati from National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) Entebbe. We acknowledge all the DVOs and their staff in the study areas for their support. This work constitutes part of F Kabi's PhD thesis supervised by Associate Professors C. Masembe and V. Muwanika, whose relentless efforts are highly treasured.

References

- Balikowa, D., 2011. Dairy Development in Uganda: A Review of Uganda's Dairy Industry. Dairy Development Authority (DDA), Uganda, Available at: <http://www.fao.org/3/a-aq292e.pdf> (accessed 10.07.14.).
- Berhe, G., Belihu, K., Asfaw, Y., 2007. Seroprevalence and epidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. *Int. J. Appl. Res. Vet. Med.* 5, 65–71.
- Ducrotoy, J., Bertu, J., Ocholi, A., Gusi, M., Bryssinckx, W., Welburn, S., Moriyón, I., 2014. Brucellosis as an emerging threat in developing economies: lessons from Nigeria. *PLoS Negl. Trop. Dis.* 8 (7), e3008.
- FAO, 2009. Livestock keepers—guardians of biodiversity. *Animal Production and Health: Paper No.167* 2009:59. ISBN: 978-92-5-106369-9. <http://www.fao.org/docrep/012/i1034e/i1034e00.htm> (accessed 21.08.14.).
- Faye, B., Castel, V., Lesnoff, M., Rutabinda, D., Dhalwal, J., 2005. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). *Prev. Vet. Med.* 67 (4), 267–281.
- Gall, D., Nielsen, K., 2004. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Rev. Sci. Tech. Off. Int. Epiz.* 23, 989–1002.
- Kabi, F., Masembe, C., Muwanika, V., Kirunda, H., Negrini, R., 2014. Geographic distribution of non-clinical *Theileria parva* infection among indigenous cattle populations in contrasting agro-ecological zones of Uganda: implications for control strategies. *Parasites Vectors* 7, 414.
- Kadohira, M., McDermott, J.J., Shoukri, M.M., Kyule, M.N., 1997. Variations in the prevalence of antibody to *Brucella* infection in cattle by farm, area and district in Kenya. *Epidemiol. Infect.* 118, 35–41.
- Kugonza, D.R., Nabasiyrye, M., Hanotte, O., Mpairwe, D., Okeyo, A.M., 2012. Pastoralists' indigenous selection criteria and other breeding practices of the long-horned Ankole cattle in Uganda. *Trop. Anim. Health Prod.* 44 (3), 557–565.
- Kunda, J., Fitzpatrick, J., Kazwala, R., French, N.P., Shirima, G., MacMillan, A., Kambarage, D., Bronsvort, M., Cleaveland, S., 2007. Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health* 7, 315.
- Kunda, J., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G.S., MacMillan, A., Cleaveland, S., 2010. Quantifying risk factors for human brucellosis in Rural Northern Tanzania. *PLoS One* 5 (4), e9968. <http://dx.doi.org/10.1371/journal.pone.0009968>
- MAAIF, 2010. Agriculture for food and income security, agriculture sector development strategy and investment plan: 2010/11–2014/15. <http://www.agriculture.go.ug/userfiles/AgriculturalSectorDevelopmentStrategyandInvestmentPlan29.pdf> (accessed 20.02.14.).
- MAAIF/MFPED, 2004. (Ministry of Agriculture, Animal Industry and Fisheries, and Ministry of Finance, Planning and Economic Development). Increasing incomes through exports: a plan for zonal agricultural production, agro-processing and marketing. Entebbe, Uganda; http://www.foodnet.cgiar.org/scrrip/docs/databases/ifpristudies_ug_nonscrip/pdfs/Government_of.Uganda/Plan_for_zonal_ag_production_processing_and_marketing.pdf (accessed 20.02.14.).
- MAAIF/UBOS, 2009. The national livestock census report 2008. Available: <http://www.agriculture.go.ug/userfiles/NationalLivestockCensusReport2009.pdf> (accessed 20.08.14.).
- Mai, H.M., Irons, P.C., Kabir, J., Thompson, P.N., 2012. A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. *BMC Vet. Res.* 8, 144, 1746–6148/8/144.
- Mangen, M.J., Otte, J., Pfeiffer, D., Chilonda, P., 2002. Bovine Brucellosis in Sub-Saharan Africa: Estimation of Sero-Seroprevalence and Impact on Meat and Milk off Take Potential. FAO, Rome.
- Magona, J., Walubengo, J., Galiwango, T., Etoori, A., 2009. Seroprevalence and potential risk of bovine brucellosis in zerograzing and pastoral dairy systems in Uganda. *Trop. Anim. Health Prod.* 41, 1765–1771. <http://dx.doi.org/10.1007/s11250-009-9375-y>
- Makita, K., Fèvre, E.M., Waiswa, C., Eisler, M.C., Thrusfield, M., 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 Vet. Res.* 7, 60.
- McDermott, J.J., Arimi, S.M., 2002. Brucellosis in Sub-Saharan African: epidemiology, control and impact. *Vet. Microbiol.* 90 (1–4).
- McDermott, J., Grace, D., Zinsstag, J., 2013. Economics of brucellosis impact and control in low-income countries. *Rev. Sci. Tech. Off. Int. Epiz.* 32, 249–261.
- Megersa, B., Biffa, D., Niguse, F., Rufael, T., Asmare, K., Skjerve, E., 2011. Cattle brucellosis in traditional livestock husbandry practice in Southern and Eastern Ethiopia, and its zoonotic implication. *Acta Vet. Scand.* 53, 24.
- Mekonnen, H., Kalayou, S., Kyule, M., 2010. Serological survey of bovine brucellosis in barka and arado breeds (*Bos indicus*) of Western Tigray, Ethiopia. *Prev. Vet. Med.* 94, 28–35.
- Mwebe, R., Nakavuma, J., Moriyón, I., 2011. Brucellosis seroprevalence in livestock in Uganda from 1998 to 2008: a retrospective study. *Trop. Anim. Health Prod.* 43, 603–608.
- Nabukenya, I., Kaddu-Mulindwa, D., Nasinyama, G., W, 2013. Survey of *Brucella* infection and malaria among Abattoir workers in Kampala and Mbarara Districts, Uganda. *BMC Public Health* 13, 901. <http://dx.doi.org/10.1186/1471-2458-13-901>
- NextGen Project, 2010. Next generation methods to preserve farm animal biodiversity by optimizing present and future breeding options. <http://www.nextgen.epfl.ch/page-64,067.html;jsessionid=D4367339C598E0F3A02E7C45F2D845E9> (accessed 08.08.14.).
- Nielsen, K., 2002. Diagnosis of brucellosis by serology. *Vet Microbiol* 90, 447–459.
- OIE, 2009. Bovine brucellosis, Terrestrial manual. http://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.04.03_BOVINE_BRUCCELL.pdf
- Ocaido, M., Muwazi, R., Opuda-Asibo, J., 2009. Disease incidence in ranch and pastoral livestock herds around Lake Mburo National Park, in South Western Uganda. *Trop. Anim. Health Prod.* 41, 1299–1308.
- Plumb, G.E., Olsen, S.C., Buttke, D., 2013. Brucellosis: 'One Health' challenges and opportunities. *Rev. Sci. Tech. Off. Int. Epiz.* 32 (1), 271–278.

- Racloz, V., Schelling, E., Chitnis, N., Roth, F., 2013. Persistence of brucellosis in pastoral systems. *Rev. Sci. Tech. Off. Int. Epiz.* 32, 61–70.
- Robinson, A., 2003. Guidelines for Coordinated Human and Animal Brucellosis Surveillance. Rome: FAO animal production and health paper. Available: <http://www.fao.org/docs/eims/upload/215249/y4723e00.pdf> (accessed 18.08.14.).
- Radostits, O.M., Gay, C.C., Inchcliff, K.W., 2000. *Veterinary Medicine. A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses*, 9th ed. W.B. Saunders Company Ltd., New York, pp. 867–882.
- Rogan, W.J., Gladen, B., 1978. Estimating prevalence from the results of a screening test. *Am. J. Epidemiol.* 107, 71–76.
- Swai, E.S., Schoonman, L., 2009. Human brucellosis: seroprevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. *Zoonoses Public Health* 56, 183–187, <http://dx.doi.org/10.1111/j.1863-2378.2008.01175.x>
- Thrusfield, M., 2003. *Veterinary Epidemiology*, 3rd ed. Blackwell Science, Oxford.
- UBOS, 2013. Statistical abstract 2013. Uganda Population and Housing Census. Available: <http://www.ubos.org> (accessed 18.07.14.).
- WHO, 2006. The control of neglected zoonotic diseases: a route to poverty alleviation. In: Report of a Joint WHO/DFID-AHP Meeting, 20–21 September 2005, WHO Headquarters, Geneva, with the participation of FAO and OIE. Available: <http://www.who.int/zoonoses> [accessed 20.10.14.).