SEROPREVALENCE SURVEY OF BRUCELLOSIS AMONG CATTLE IN SELECTED DISTRICTS OF SOUTH KIVU PROVINCE, EASTERN OF DR CONGO

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ABSTRACT

Brucella is one of the major zoonotic pathogens worldwide, and it is responsible for enormous economic losses as well as considerable human morbidity in endemic areas. Across-sectional study was carried out on different farms in four territories of the South-Kivu province, in Eastern of DR Congo to determine the prevalence of Brucella among cattle and the potential major risk factors. A total of 835 serum samples from randomly selected unvaccinated cattle over a period of 3 years were collected from 100 herds of cattle and examined for antibodies to *Brucella abortus* using the competitive ELISA technique. Data associated with risk factors of brucellosis were analysed by CDC Epi-info™ version 7 using Fisher's exact test. An overall seroprevalence of 27.3% (228/835) was obtained where Uvira territory showed a highest individual sero-prevalence rate (46.0%) followed Kalehe, Mwenga and Kabare territories with respectively 27.6%, 21.3%; and 16.1%. However, logistic regression analysis revealed that age of cattle and grazing system ($p<0.0001$) were statistically significant for seropositivity to *Brucella* spp where by cattle of 1-3 years old showed higher seroprevalence compared to those > 6 years with 46% and 7.1% respectively. Similarly, cows kept in communal grazing system were highly seropositive than the ones kept in Cowshed system with 28.1% and 8.1% respectively. In conclusion, brucellosis was endemic at considerable prevalence in cattle from South Kivu province. We advocate testing animals before movement, the implementation of stamping out policy as well as coordinated surveillance for the disease among diverse cattle populations in South Kivu province to significantly reduce the public health risks associated with Brucella infections in cattle.

Keywords: Diagnosis, Public health, Risk factors, Zoonosis infection

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Introduction

Bovine brucellosis is usually caused by Brucella abortus and occasionally by Brucella melitensis where cattle are kept together with infected sheep or goats OIE (2015). Brucellosis is amongst the neglected zoonosis and causes significant economic loss in cattle production in many regions of the world WHO (2009). Largely due to the lack of public awareness, it is one of the most important zoonotic infections, especially in pastoral and mixed crop-livestock farming systems in Africa Mcdermott and Arimi (2002). Brucellosis is an infectious bacterial disease that primarily infect livestock but also humans Pappas *et al*., (2005). The disease is endemic in most Sub-Saharan African countries Faye *et al* (2005; Karimuribo *et a.l.,* (2007); Omer *et a*l., (2000). In the Democratic Republic of Congo (DRC), and especially the Eastern part of the country, South Kivu region where this study was conducted is sharing borders and uncontrolled animal trans-border movement with many Sub Saharan countries where Brucellosis were confirmed such as Tanzania, Rwanda, Kenya, Uganda and Burundi is suspected to be infected. Any confirmation study has been done in South Kivu region to confirm suspected cases while the disease has been already reported in the area based on clinical diagnosis by the Ministry of livestock and agriculture IPAPEL (2012).

In D.R.Congo, the livelihood of smallholder farmers is heavily dependent on cattle, which apart for milk production, they are used for drought power, meat, income, transport and manure, and other social or cultural activities. However, cattle productivity in smallholder farms is primarily affected by diseases, in addition to lack of adequate grazing, poor husbandry practices and lack of adequate veterinary services. In animals, the disease is manifested by reproductive disorders such as abortions, infertility, and retention of placenta, stillbirth, reduction of milk production and loss of animals Gwida *et al.,* (2010). In addition, full-term calves may die soon after birth (Karimuribo *et al.,* (2007). The organisms are excreted in urine, reproductive discharges and in milk. The variation in the prevalence of the disease may be influenced by the characteristics of animal populations, management factors and other biological features such as herd immunity, persistence of infection in calves and vaccination status that largely determine the epidemiology of brucellosis (Faye *et al.,* (2005); Muma *et al*., (2006) . The establishment of the smallholder dairies, and most recently, the introduction of the agrarian reform programme in the year 2000 brought about increased movement of cattle between the commercial and smallholder sectors. This has created a unique cattle management system with the potential of changing the epidemiology of brucellosis and other infectious diseases. While brucellosis continues to be closely monitored in the commercial farming sector, there is lack of information on its seroprevalence and the risk factors

associated with the disease in smallholder cattle.

Therefore, this study was conducted to estimate the seroprevalence of brucellosis and associated risk factors in cattle farms from smallholder dairy farms in South Kivu region where there is no screening policy for both animals designated for slaughter, animal herds and human. In South Kivu region, Brucella seems to be a threat for both food security and human health even if data are not available while the disease is spreading from one region to another.

Materials and methods

Description of the study areas

The study was conducted in smallholder dairy cattle farms of Uvira, Mwenga, Kabare and Kalehe territories of South Kivu, in Eastern of the Democratic Republic of Congo from May 2014 to October 2015 (Fig 1). These selected areas represented the different agro-ecological regions of South Kivu and smallholder dairy farms and where there is no use of Brucellosis vaccine and are areas where Brucellosis outbreaks have been suspected and reported IPAPEL (2007). The production systems are found in table 1.

South Kivu province where this study was conducted is located in Eastern part of DRC and borders the provinces of North Kivu to the North, Kivu lake North East, Maniema to the West, and Katanga to the South (Figure1). It shares its borders with the countries of Burundi, Rwanda and Tanzania in the East. The area is about 65 070 km^2, with a total population size of 4 614 768 (71 persons per km^2). Koppen-Geiger Climate classification systems classify its climate as tropical wet and dry (Aw1) and the altitude is 1531 m above the sea level with an average rainfall of about 1 500 mm with more than 50 % of the total land used for grazing FAO (2012).

Study design and sampling of individual animals

A cross sectional study was carried out using a stratified sampling procedure to select herds and individual cattle per herd. The details of the study design, sampling of herds and individual animals have been described previously by Matope *et al*., ((2011). In each study area, the approximate number of farms was listed with the assistance of local veterinary/ agricultural office. Herds that were co-grazed were grouped together and considered as one and only herds with a minimum of 8 cattle ≥ 1 year were included in the study. The sample sizes of herds in each area was predetermined as described by Dohoo *et al.*, (2003), by assuming that brucellosisexisted at 25% inter-herd and 15% intra-herd seroprevalence.

The sample sizes of individual animals were estimated using the diagnostic sensitivity (Se) and specificity (Sp) of Rose Bengal test (RBT) of 90% and 75%, respectively and for the competitive enzyme-linked immunosorbent assay (cELISA) 98% and 99% respectively based on previous validation studies (Mcgiven et al 2003). For bleeding, cattle were selected by systematic random sampling and where it was not possible; at least six animals were selected from those present in the herd and blood samples taken.

Epidemiological data collection

Sample data sheets were used to record separately the information on individual animal variables (sex, age, grazing system and animal origin). Herd level data that included: herd structure, size, history of purchases of animals and farm management practices were collected by interviewer-administered questionnaire. This herd data was envisaged for further use in studying the herd-level risk factors for brucellosis.

Laboratory tests

The clotted blood samples were centrifuged at 3000 x g for 15 minutes and 2 ml of serum were collected into cryo-tubes and stored at -20°C until laboratory tests were performed. The RBT, conducted as previously described was used to screen sera for anti-Brucella antibodies OIE (2008). The buffered B. abortus antigens and control sera (positive and negative) used were obtained from VLA, Weybridge (UK). Since a serial testing was used (to increase on test specificity), then only the RBT positive (agglutinations visible by the unaided eye) were tested using the Svanovir ™ Brucella-Ab c-ELISA test kits (Svanova Biotech, Uppsala, Sweden) for confirmation. The c-ELISA was done according to the manufacturer's instructions and essentially as described elsewhere by Matope *et al*., (2011) and Muma *et al.,* (2006). Only animals positive on both RBT and c-ELISA were classified Brucella seropositive.

Data analysis

The epidemiological and animal bio-data were stored in a computer data base and statistical analysis was performed using CDC Epi-info™ version 7. In order to improve the estimation of brucellosis seroprevalence, individual animal level- data were weighted according to the inverse of the sampling fraction Dohoo *et al*., (2003). A sampling weight was obtained as a product of the proportion of herds sampled against the total number of herds in each study area and the proportion of cows sampled in a herd. A logistic regression was used to identify the risk factors associated with prevalence of ASF based on the cELISA results.

Results

Distribution of herds and animals sampled

A total of 835 cattle from 100 herds from the four territories of the study area were sampled and tested for presence of antibodies to Brucella spp. The majority of animals were female 699 (83.8%) from which less than half 298 (35.7%) had an age between 1 and 3 years while the minority of them 155 (18.6%) were above 6 years old.Over half 798 (95.5%) were kept in communal grazing system and only 37 (4.5%) used cowshed system. A total of 205 cattle of both sex were samples in Kabare territory, 211 in Mwenga, 209 in Uvira and 210 animals in Kalehe. In addition, 750 (89.8%) of catlle were originated from locally raised while only 85 (10.2%) were purchased.

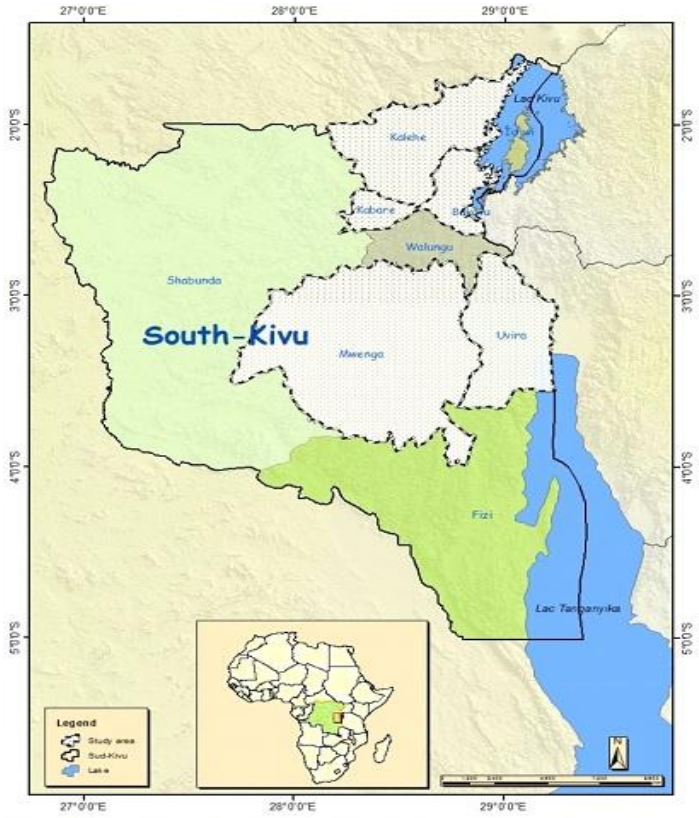
Brucela sero-positivity according to the geographical location

During the investigation, 100 boivine helds were visited from which 835 sera were sampled. Of the 835 cattle tested for Brucella antibodies, 228 were positive by c-ELISA giving an overall sero-prevalence of 27.3%. The highest sero-prevalence (45.5%) was observed in Uvira territory followed by Kalehe (27.6%) and the lowest (16.1%) was Kabare (Table 3).

Brucellosis seroprevalence in cattle according to sex, age, grazing system, origin and geographical region in South Kivu province, DR Congo

The results obtained from the logistic analysis revealed that the age and grazing system (p = <0.0001) were the main factors associated significantely with seropositivity of cattle for antibodies to *Brucella* spp. Indeed, brucellosis seroprevalence was observed to decrease with increasing age of cattle. There were significantly higher numbers of seropositive cattle in the 1-3 years age group (p<0.0001, OR = 11.3; 95%CI: 1.6 – 3.5) compared to those over 6 years. Similarly, Cattle kept in communal grazing system were more Brucella positive than those kept in cowshed system (p=0.00018, OR=4.4; 95% CI: 1.9 – 10.4) (Table 4).

However, no significant difference was observed in seroprevalence between males and females (p=0.451, OR= 0.9; 95%CI: 0.6 - 1.5) as well as locally raised and purchased cattle (p=0.464, OR= 0.9; 95%CI: 0.5 – 1.5). In addition, cattle sampled in Uvira (p<0.0001, OR= 4.1 ; 95%CI : 10.1-0.5) and Kalehe (p=0.006 ; OR=2.1 ; 95%CI : 0.2 – 0.6) territories were more likely to be seropositive for antibodies to *Brucella* spp. when compared to those sampled in Kabare. Whilst the female cattle showed similar likelihood of being seropositive with the male (p=0.451, OR= 0.9 ; 95%CI : 0.6 – 1.5) (Table 4).



Figurel. Map of South Kivu province showing the different study territories

Source: Arc-GIS coordinates

Table 1. Cattle production system and common practices in south Kivu province, Eastern DRCongo

Territories	Known practices	Production system
Uvira	Farmers share facilities for grazing and prevalence common grazing system.	Communal grazing system
Kalehe	Farmers share facilities for grazing and prevalence common grazing system.	Communal grazing system
Mwenga	Self-contend units	Zero-grazing
Kabare	Self-contend units	Zero-grazing

Table 2: Characteristics of cattle tested for Brucellosis in South Kivu province

Variable	Characteristics	Number of animal tested	Percentage
Sex	female	699	83.8
	male	136	16.2
Age (Year)	1 to 3	298	35.7
	3 to 5	194	23.2
	5 to 6	188	22.5
	>6	155	18.6
Grazing system	Comm. grazing	798	95.5
	cowshed	37	4.5
Territories	Kabare	205	24.6
	Kalehe	210	25.1
	Mwenga	211	25.3
	Uvira	209	25
Origin of animal	Locally raised	750	89.8
	Purchased	85	10.2

Table 3 : Brucela sero-positivity according to geographical locations

Territories	**No herds sampled**	**No animal tested**	**Positive cattle**	**Seroprevalence(%)**
Kabare	25	205	33	16.1
Kalehe	24	211	58	27.6
Mwenga	26	209	45	21.3
Uvira	25	210	92	45.5
Total	**100**	**835**	**228**	**27.3**

Table4: Logistic regression analysis of factors associated with Brucellosis seroprevalence in cattle from South Kivu province, DR Congo using the Chi-square test.

Variables	Characteristics	Sero-positive based on X^2 test		OR	95%CI	p-value
		Positives n(%)	Negatives n(%)			
Sex	female	192(27.5)	507(72.5)	1	-	-
	male	36(26.5)	100(73.5)	0.9	0.6 - 1.5	0.451
Age (year)	>6	11(7)	144(93)	1		
	1 to 3	140(46)	158(58)	11.3	1.6- 3.5	<0.0001
	3 to 5	52(26.8)	142(73.2)	4.7	0.1 - 0.4	<0.0001
	5 to 6	25(13.3)	163(86.7)	1.9	0.2 - 1.03	0.24
Grazing system	Com. grazing	225(28.1)	573(71.9)	1		
	cowshed	3(8.1)	34(91.9)	4.4	1.9 - 10.4	0.00018
Territories	Kabare	33(16)	172(84)	1	-	-
	Mwenga	45(21.3)	166(78.7)	1.4	0.4 - 1.1	0.107
	Uvira	92(44)	117(56)	4.1	0.1 - 0.5	<0.0001
	Kalehe	58(27.6)	152(72.4)	2.1	0.2 - 0.6	0.006
Orig. of animal	Locally raised	204(27.2)	546(72.8)	1	-	-
	Purchased	24(28.2)	61(71.8)	0.9	0.5 – 1.5	0.464

Discussion

The results on brucellosis seroprevalence and the associated risk factors investigated in cattle from smallholder dairy farms selected from various agro-ecological regions of South Kivu showed that brucellosis is present in all study areas with mean seroprevalence of 27.3 %. The seropositive reactions were likely to be caused by field Brucella spp. because the c-ELISA which was used as a confirmatory test has a high specificity in individual animals which minimizes false positive reactions caused by cross-reacting antibodies produced against other Gram-negative bacteria such as Yersinia enterocolitica O:9, E. coli O:157 and some Salmonella spp Nielsen *et al* (2004). This individual seroprevalence that we obtained was extremely higher than the one that Matope *et al* (2011) found from cattle in Zimbabwe. Animal age class, grazing system and the ecological sampling area were factors that mostly influenced the seroprevalence of Brucellosis in South-Kivu region. The observed brucellosis seroprevalence results agree with those of previous studies in Zimbabwe by Madsen (1989) and Mohan *et al.,* (1996).

The research identified that the differences in seroprevalence is likely to be attributed to certain risk factors such as cattle management practices, population dynamics; and biological features, for instance herd immunity that largely influence the prevalence of Brucella spp Al-Majali *et al* (2009); McDermott and Armi (2002); Reviriego *et*

al (2000). The prevalence was high in Uvira territory and Kalehe compare to others. However, the observed results for Uvira may be contributed to a high proportion of farms that shared facilities for grazing and watering of cattle compared to the other study areas which kept their herds as self-contained units (data not shown). The practice of mixing of cattle, either through grazing or sharing of watering points is an important risk factor for brucellosis according to the reports of Al-Majali *et al.,* (2009), Madsen(1989), Muma *et al*., (2007), and Reviriego *et al*., (2000). The continual movement of cattle from commercial to smallholder farming areas could present a risk of introducing brucellosis in the latter since the disease has been previously noted to be more prevalent in commercial farms compared to communal areas in some countries. The movement of animals between herds has been established to be an important risk for Brucella spp. infection in other regions of the world Al-Majali (2009); Omer *et al.,* (2000).

It was clear from the study that the preponderance of seropositive in the 1-3 years age group was high in the study area. This may be related to the onset of sexual maturity, which is associated with increased risk of infection with Brucella spp., especially following abortions Muma *et al*., (2007). However, the age at which sexual maturity is attained varies with breeds of cattle and this is likely to influence the observed relationship between age and positive reactors in different sub-populations. Although the observations about age and brucellosis seroprevalence differ with somer reports Faye *et al*., (2005; Muma *et al.,* (2007), but present a certain similarity to those of previous findings by Matope *et al*., (2011), Omer *et al.,* (2000) and Pappas *et al*., (2005). It is likely that in endemic areas, the risk of Brucella infection is greater in younger naïve animals compared to older cows, some of which may not exhibit detectable antibody titters, possibly due to latency which is common in chronic brucellosis.

The study showed the lack of difference in seropositive reactors between males and females may indicate that the risk of infection with Brucella spp. is independent of sex of cattle. Similar findings have also been reported by Bayemi *et al.,* (2009) and Matope *et al.,* (2011). However, this relationship has been shown to vary with different cattle subpopulations Muma *et al*., (2006); Muma *et al*., (2007).

Conclusion

Our results testify that brucellosis is presence in South-Kivu province, in the Eastern part of DR Congo with a highest seroprevalence observed in Uvira territory where farms are sharing facilities for grazing and watering of cattle compared to the other study areas which kept their herds as self-contained units. Furthermore, our findings show that age group and sex grazing system of cattle play significant roles in the epidemiology of brucellosis in cattle in the study region. According to these findings more diverse epidemiological and molecular studies are recommended to be conducted through out the province in order to determine the possible entry of infected animals and establish risks for human

infection as well as investigate the presence of *Brucella* species in DR Congo for better implementation of appropriate control measures to prevent the pread of the disease.

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