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

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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.

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.

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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 herdsmen, 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