Acta Tropica 197 (2019) 105042

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

# Brucellosis in dairy herds: A public health concern in the milk supply chains of West and Central Africa

Imadidden Musallam[a,*], Andrée Prisca Ndour[b], Damitoti Yempabou[b], Cha-Ah Crystella Ngong[c], Müller Fotsac Dzousse[c], Mohamed-Moctar Mouiche-Mouliom[c], Jean Marc Kameni Feussom[d,e], Jean Bosco Ntirandekura[f], Désiré Ntakirutimana[g], Adama Fane[h], Elisabeth Dembele[h], Adama Doumbia[h], Amah Akpemdo Paul-Henri Siméon Ayih-Akakpo[b], Pindemnewe Pato[i], Magnoudewa Pali[i], Arnaud S.R. Tapsoba[j], Germaine Minougou Compaore[j], Haladou Gagara[k], Aïda Issaka Garba[k], Bhagyalakshmi Chengat Prakashbabu[a], Laura Craighead[a], Emi Takahashi[a], John McGiven[l], Patrick Nguipdop-Djomo[m], Punam Mangtani[m], Rrianatou Alambédji-Bada[b], Ayayi Justin Akakpo[b], Javier Guitian[a]

[a] Veterinary Epidemiology, Economics and Public Health Group, Department of Pathobiology and Population Sciences, The Royal Veterinary College, Hawkshead Lane, Herts, UK
[b] Ecole Inter Etats des Sciences et Médecine Vétérinaires de Dakar (EISMV), Dakar, Senegal
[c] Ecole des Sciences et de Médecine Vétérinaire de Ngaounderé, Ngaounderé, Cameroon
[d] Cameroon Epidemiological Network for Animal Diseases (RESCAM), Ministry of Livestock, Fisheries and Animal Industries, Yaoundé, Cameroon
[e] Epidemiology, Public Health Veterinary Association (ESPV), Yaoundé, Cameroon
[f] Université du Burundi, Faculté d'Agronomie et de Bio-Ingénierie, Département de Productions Animales, Bujumbura, Burundi
[g] Laboratoire National Vétérinaire, Bujumbura, Burundi
[h] Laboratoire Central Vétérinaire, Bamako, Mali
[i] Ministère de l'Agriculture de la Production Animale et Halieutique, Direction de l'Élevage, Lomé, Togo
[j] Institut de l'Environnement et de la Recherche Agricole (INERA), Ouagadougou, Burkina Faso
[k] Laboratoire Central de l'Elevage, Niamey, Niger
[l] Department of Bacteriology, Animal and Plant Health Agency, OIE Brucellosis Reference Laboratory, FAO/WHO Collaborating Centre for Brucellosis, UK
[m] London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health, Department of Infectious Disease Epidemiology, London, UK

ARTICLE INFO

*Keywords:*
Brucellosis
Dairy herds
Milk
Cattle
Peri-Urban
Africa
Zoonosis

ABSTRACT

Ten herd-level cross-sectional studies were conducted in peri-urban dairy production areas of seven West and Central African countries (Burkina Faso, Burundi, Cameroon, Mali, Niger, Senegal and Togo). The objectives were to estimate herd level *Brucella* spp. seroprevalence and identify risk factors for seropositivity.

In each of the ten study areas, herds (between 52 and 142 per area, total = 965) were selected probabilistically and a structured questionnaire was administered to gather information on their structure and management. A bulk milk sample from each herd was tested by indirect ELISA for *Brucella* spp. For each area, herd seroprevalence estimates were obtained after adjusting for the assumed performance of the diagnostic test. Herd level risk factors for *Brucella* spp. seropositivity were identified by means of stratified logistic regression, with each peri-urban zone as a stratum. Area-specific models were also explored.

Estimated herd seroprevalences were: Lomé (Togo) 62.0% (95% CI:55.0–69.0), Bamako (Mali) 32.5% (95% CI:28.0–37.0), Bujumbura (Burundi) 14.7% (95%CI:9.4–20.8), Bamenda (Cameroon) 12.6% (95% CI:7.6–21.9), Ouagadougou (Burkina Faso) 3.0% (95% CI:1.0–9.1), Ngaoundere (Cameroon) 2.3% (95% CI:1.0–7.0), Thies (Senegal) 1.3% (95% CI:0.1, 5.3), Niamey (Niger) 1.2% (95% CI:0.08-5.3), Dakar (Senegal) 0.2% (95% CI:0.01–1.7) and Niakhar (Senegal) < 0.04%. Logistic regression modelling revealed transhumant herds to be at lower risk of infection (adjusted OR: 0.25, 95% CI: 0.13 - 0.5) and in one of the areas (Bamenda), regular purchase of new animals was found to be strongly associated with *Brucella* spp. seropositivity (adjusted OR = 5.3, 95% CI: 1.4–25.9). Our findings confirm that *Brucella* spp. circulates among dairy cattle supplying milk to urban consumers in West and Central Africa, posing a serious public health concern. Control programs

* Corresponding author.
*E-mail address:* IMusallam@rvc.ac.uk (I. Musallam).

https://doi.org/10.1016/j.actatropica.2019.105042
Received 14 March 2019; Received in revised form 27 May 2019; Accepted 28 May 2019
Available online 29 May 2019

are urgently needed in areas such as Lomé or Bamako, where more than 30% of the herds show evidence of infection.

## 1. Introduction

Globally, brucellosis is assumed to represent one of the highest economic and public health burdens of any zoonosis, with *Brucella melitensis, B. abortus, B. suis* and *B. canis* all producing disease in human and animal populations (Corbel, 2006; Whatmore et al., 2014; Al Dahouk et al., 2017). Ruminants are the primary hosts for *B. melitensis* and *B. abortus,* with humans becoming infected following consumption of raw milk and dairy products, by direct contact with aborted foetuses, afterbirth and parturition fluids and during slaughter practices (FAO, 2010; WHO, 2005; Doganay and Aygen, 2003).

In humans, the acute form of the disease is debilitating with general malaise, fever, arthralgia and backache reducing a patient's ability to work. An acute brucellosis episode has an estimated disability weight (DW) of 0.210, highlighting the high impact of brucellosis on individuals (Dean et al., 2012; WHO, 2015). The World Health Organisation (WHO) estimated that in 2010 there were 0.83 million cases of human brucellosis globally (47% of these were identified as foodborne in origin) although the actual figure is likely to be much higher than this, due to widespread under-reporting and misdiagnosis (WHO, 2015; Kirk et al., 2015; Jennings et al., 2007).

In 2011, the World Bank ranked brucellosis among the top ten diseases globally for all the main domestic ruminant species in terms of Livestock Units Lost (LUL; World Bank, 2011). Losses are the result of abortions and associated reduction in milk yield (McDermott et al., 2013; Oseguera Montiel et al., 2015). In cattle, the disease is mainly associated with the species *B. abortus*, which has been successfully eliminated from Australia, New Zealand, Canada, Japan and several European countries through the vaccination of susceptible animals, followed by a test-and-slaughter policy (CFSPH, 2009).

West and Central Africa are rapidly-urbanising regions, with an increased demand for dairy products being met by a burgeoning dairy sector, largely located in the peri-urban areas that surround major cities (Ducrotoy et al., 2017; Guneralp et al., 2017). Brucellosis is suspected to be endemic throughout the region, although the lack of prevalence estimates, together with various practical and political factors at both local and regional levels, have left brucellosis in livestock largely uncontrolled (Akakpo et al., 2009; Alonso et al., 2016; Craighead et al., 2017). Although the available evidence is scarce and should be interpreted with caution, previous studies have shown high levels of infection among dairy herds located in dairy production areas in West and Central Africa. In Mali for example it was estimated in 2003 that up to 30% of milk and dairy products at selling points in Bamako were contaminated with *Brucella* (Bonfoh et al., 2003). More recently, in 2014, it was estimated that 25.6% of dairy herds in the Adamawa and North Regions of Cameroon were seropositive against *Brucella* spp. (Awah-Ndukum et al., 2018). Lack of brucellosis control programs is particularly worrying in expanding dairy systems, in which husbandry practices are known to favour disease spread (Ogugua et al., 2018). Furthermore, the absence of milk hygiene controls and sociocultural habits of unpasteurized dairy product consumption, common in Sub-Saharan Africa, amplify the potential public health impact of *Brucella* infection in these settings. An initial step towards the formulation of locally-appropriate brucellosis control programmes is the characterization of the infection status of dairy farms. Thus, the objectives of this study were to provide herd-level estimates of *Brucella spp*. seroprevalence among bovine dairy herds in ten of the major peri-urban dairy zones across West and Central Africa and identify herd-level risk factors for seropositive status against *Brucella* spp.

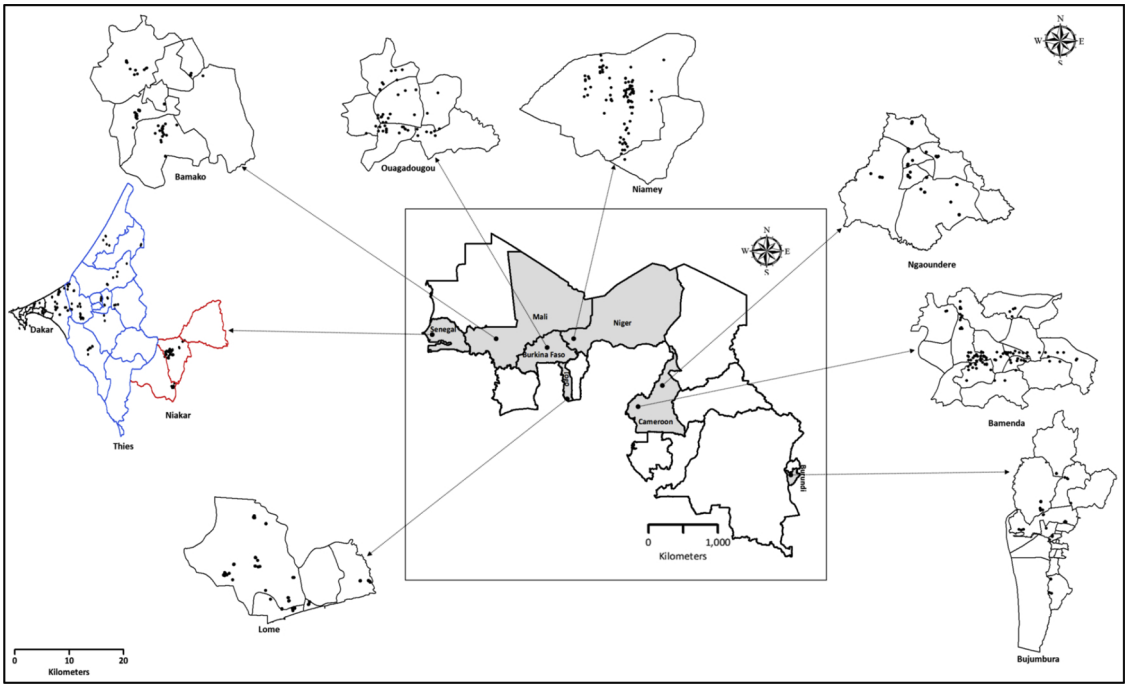


**Fig. 1.** Map of the ten study areas in seven West and Central African countries (Burkina Faso, Burundi, Cameroon, Mali, Niger, Senegal and Togo) showing point locations of cattle herds included in the cross-sectional studies of bovine brucellosis in peri-urban dairy herds, February 2017 - January 2018. One dot on the map may present more than one herd.

## 2. Methods

### 2.1. Study design and study population

Cross-sectional studies were conducted in ten peri-urban dairy production zones across seven West and Central Africa countries between February 2017 and January 2018, in Burkina Faso (Ouagadougou), Burundi (Bujumbura), Cameroon (Ngaoundere and Bamenda), Mali (Bamako), Niger (Niamey), Senegal (Dakar, Thies and Niakhar) and Togo (Lomé) (Fig. 1).

The target population was defined as 'all bovine dairy herds present in the predefined peri-urban zone'. The study unit was defined as 'any herd where lactating cows are managed together as a unit regardless of herd size'. For this purpose, all lactating cows kept together, owned by the same person/household and usually kept in the same location were included. The most commonly practised livestock production systems in the region are sedentary, unrestricted grazing and transhumant livestock production and very few herds are of the nomadic type (SWAC-OECD/ECOWAS, 2008). For purpose of this study, peri-urban dairy production zones were broadly defined as areas of concentration of dairy farms in the proximity of urban areas to which they supply milk/dairy products. The geographical boundaries for each 'peri-urban dairy production zone' were defined through discussion with personnel from the veterinary and livestock production services, dairy farm associations and private veterinarians in each zone by delimiting on a map the area where 'most' farms concentrate. The size of the areas ranged from 333 km $^2$ (Lomé) to 7069 km$^2$ (Ngaoundere).

Ethical approval was granted by the Ethics and Welfare Committee of the Royal Veterinary College (RVC) and the Ethics Committee at the Interstate School of Veterinary Science and Medicine of Dakar (EISMV). Informed consent for questionnaire administration and collection of biological samples was obtained verbally from herd owners before sampling and interviewing. Each study was conducted using the same protocol and any departures from protocol were recorded.

### 2.2. Sampling frame, sampling strategy and sample size calculations

Where available, a comprehensive list of all known dairy herds within each zone was used as the sampling frame. Where such lists were unavailable, a snowballing approach was used among local milk collectors and sellers to identify dairy herds in the zone. Sampling began once no new herds could be identified.

Using an expected herd–level prevalence of 15%, based on previously published data (Akakpo et al., 2009; Boukary et al., 2013), it was calculated that sampling between 60 and 90 herds per zone would result in an absolute precision of 5% with 95% confidence in populations of between 125 and 250 herds.

$$ni = \left(\frac{1.96}{d}\right)^2 * P * (1 - P)$$

$$n = \frac{ni * N}{ni + N}$$

Where, $\boldsymbol{n_i}$ is the sample size for the infinite population, ***1.96*** is the Z-value corresponding to a 95% confidence interval of the standard normal distribution, $\boldsymbol{d}$ is the expected absolute error (5%), $\boldsymbol{P}$ is the expected prevalence at a herd level, $\boldsymbol{n}$ is the number of herds to be sampled after correction for the finite population and $\boldsymbol{N}$ is the population size. In this study we are assuming that within a zone herds are independent (i.e. not clustered). Therefore, a target sample size of between 60 and 90 herds per zone was used.

Official lists of dairy herds were used in seven zones (Bamako, Bamenda, Bujumbura, Dakar, Ngaoundere, Niakhar and Thies) and lists derived from a snowballing approach were used in three zones (Niamey, Ouagadougou and Lomé). The size and source of the sample frames is presented in Table 1. Herds were randomly selected from the lists and owners of the selected herds were informed of the study objectives and methods. If any owners declined to participate or no lactating cows were present in the herd at the time of the visit, the subsequent herd from the list was approached. In three zones where herd numbers were low (Ouagadougou, Thies, Niamey) all farms were selected.

### 2.3. Collection and testing of milk samples

Milk samples were collected in 50 ml sterile screw cap polyethylene tubes with 5 ml of 5% formalin added immediately. Where available, a bulk milk sample was collected, otherwise a small volume of milk from each lactating cow was collected into a single container and 50 ml of milk taken from this mix. Milk samples were kept chilled in a cool box with ice before arrival at the diagnostic laboratory, where they were centrifuged, aliquoted into 1 ml units and frozen at −20 °C for storage.

Once all sample collection in a zone had been completed, samples were brought to room temperature and tested for *Brucella* spp. antibodies using Brucelisa160 M indirect ELISA (iELISA; assays provided by the OIE brucellosis reference centre Animal and Plant Health Agency (APHA), Addlestone, Surrey, UK). The cut-off optical density (OD) was calculated as 50% of the mean value of the eight intermediate control wells. All samples with an OD equal to or above the cut-off value were considered positive. Testing of the milk samples was carried out in six different diagnostic laboratories across the study countries. For the purpose of quality control, testing procedure was standardised across all laboratories with positive and negative controls provided. Moreover, the raw OD data and photos of all ELISA plates where inspected by the first author, who decided acceptance of the results of each ELISA plate.

### 2.4. Data collection

A structured questionnaire was administered at the time of sampling using Open Data Kit (ODK) on Android tablets in a choice of English or French. Herd name, location, composition (number of lactating cows,

**Table 1**
Number and data source of known dairy herds (sampling frame) within each of the 10 peri-urban zones in seven West and Central African countries included in a cross-sectional study of brucellosis, February 2017 - January 2018.

| Country | Zone | Number of dairy herds in the list (Sampling frame) | Data source |
|---|---|---|---|
| *Burkina Faso* | Ouagadougou | 129 | Snowballing |
| *Burundi* | Bujumbura | 157 | Veterinary services |
| *Cameroon* | Ngaoundere | 301 | Veterinary services |
| | Bamenda | 304 | Veterinary services |
| *Mali* | Bamako | 160 | Veterinary services |
| *Senegal* | Dakar | 150 | Veterinary services |
| | Thies | 147 | Veterinary services |
| | Niakhar | 170 | Veterinary services |
| *Togo* | Lome | 176 | Snowballing |
| *Niger* | Niamey | 135 | Snowballing |

**Table 2**
Herd composition and husbandry and management practices for 10 peri-urban dairy production zones in seven West and Central African countries included in a cross-sectional study on brucellosis, February 2017–January 2018.

| | Peri-urban zone | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Bamako | Bamenda | Bujumbura | Dakar | Lomé | Ngaoundere | Niakhar | Niamey | Ouagadougou | Thies |
| *Herd composition* | Heifers | Minimum | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | Median | 8 | 5 | 7 | 4 | 12 | 3 | 4 | 4 | 7 | 5 |
| | | Maximum | 38 | 22 | 27 | 30 | 36 | 30 | 30 | 2 | 25 | 30 |
| | Lactating cows | Minimum | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 2 |
| | | Median | 19 | 10 | 11 | 9 | 25 | 10 | 6 | 10 | 9 | 12 |
| | | Maximum | 66 | 55 | 73 | 50 | 66 | 80 | 40 | 38 | 70 | 40 |
| | Bulls | Minimum | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| | | Median | 6 | 2 | 1 | 1 | 12 | 1 | 2 | 2 | 1 | 1 |
| | | Maximum | 25 | 28 | 20 | 3 | 41 | 15 | 20 | 20 | 15 | 4 |
| | Adult herd size (median) | | 21 | 36 | 12 | 37 | 49 | 16 | 13 | 18 | 14 | 27 |
| | | | Bamako No (%) | Bamenda No (%) | Bujumbura No (%) | Dakar No (%) | Lomé No (%) | Ngaoundere No (%) | Niakhar No (%) | Niamey No (%) | Ouagadougou No (%) | Thies No (%) |
| Husbandry and management practices | Transhumance (Yes) | | 17 (25.8) | 70 (73) | 56 (64.4) | 80 (89.9) | 75 (97.4) | 58 (59.2) | 103 (86.6) | 45 (91.8) | 41 (78.8) | 72 (94.7) |
| | Regular mixing with other flocks for water or grazing (Yes) | | 23 (34.8) | 60 (62.5) | 26 (29.9) | 71 (79.8) | 61 (79) | 61 (62.2) | 112 (94.1) | 42 (85.7) | 8 (15.4) | 59 (77.6) |
| | Natural insemination | | 60 (90.9) | 96 (100) | 81 (93.1) | 79 (88.8) | 77 (100) | 90 (91.8) | 115 (96.6) | 49 (100) | 37 (71.2) | 68 (89.5) |
| | Regular borrowing of bulls for service (Yes) | | 16 (24.2) | 14 (14.6) | 52 (59.8) | 7 (7.9) | 5 (6.5) | 8 (8.2) | 19 (16) | 11 (22.4) | 5 (9.6) | 7 (9.2) |
| | Regular purchasing of new animals (Yes) | | 38 (57.6) | 41 (42.7) | 49 (56.3) | 37 (41.6) | 41 (53.2) | 34 (34.7) | 30 (25.2) | 13 (26.5) | 30 (57.7) | 32 (42.1) |

**Table 3**
Total number of dairy herds sampled, seropositive bulk milk samples and questionnaires completed across ten peri-urban zones in seven West and Central African countries, February 2017 - January 2018.

| Country | Zone | No. of herds sampled | No. (%) tested positive by milk iELISA | No. of questionnaires completed |
|---|---|---|---|---|
| Burkina Faso | Ouagadougou | 52 | 1 (2.8) | 52 |
| Burundi | Bujumbura | 87 | 14 (16.1) | 87 |
| Cameroon | Bamenda | 100 | 14 (14) | 96 |
| | Ngaoundere | 142 | 4 (2.8) | 98 |
| Mali | Bamako | 120 | 40 (33.3) | 66 |
| Niger | Niamey | 80 | 2 (2.5) | 49 |
| Senegal | Dakar | 89 | 1 (1.1) | 89 |
| | Niakhar | 119 | 0 (0) | 119 |
| | Thies | 76 | 2 (2.6) | 76 |
| Togo | Lomé | 100 | 62 (62) | 77 |
| TOTAL | | 965 | 140 (14.5) | 809 |

bulls and heifers), husbandry and management practices were all recorded. A pilot questionnaire was administered in ten herds in the study area of Dakar, then modified accordingly to form the final version (see Table 2 for variables recorded). Training and written guidelines were provided to all staff before commencing data collection and questions were posed by a bilingual administrator using the local language.

### 2.5. Data analysis

#### 2.5.1. Seroprevalence estimation

Apparent herd level seroprevalence (AP) was calculated for each zone by dividing the total number of seropositive herds by the total number of herds sampled. True seroprevalence (TP) for each zone was then calculated by adjusting for iELISA sensitivity (Se) and specificity (Sp) values as:

$$TP = (AP + Sp - 1)/(Se + Sp - 1)$$

According to the manufacturer the Se and Sp values for the iELISA are 0.98 and 0.99, respectively. Exact 95% confidence intervals (CI) for estimated true seroprevalences were obtained based on sampling from the hypergeometric distribution implemented in @Risk 7.5 for Excel (PalisadeCorporation Inc., Newfield, NY, USA).

#### 2.5.2. Univariable analysis

Univariable associations between potential risk factors and the herd serological status were assessed for each of the four highest seroprevalence zones (Lomé, Bamako, Bujumbura and Bamenda), each zone was considered separately. Associations were not assessed for the remaining six areas because of the low number of positive herds. Serological status was considered as a binary outcome, either positive or negative. Adult herd size was categorized as either ≤ median or > median. Associations between individual risk factors and the herd serological status were assessed using the Chi-squared test of association.

#### 2.5.3. Multivariable analysis

Significant explanatory variables in the univariate analysis ($p \leq 0.05$) were assessed for collinearity using Cramer's phi prime statistic (Ø) with variables considered collinear where Ø > 0.7, when a pair of variables was found to be collinear, only the more biologically plausible variable was kept for multivariable analysis.

Multivariable analyses were conducted, first, independently for each of the four zones with highest prevalence and secondly for all zones simultaneously in a stratified model. For the analysis by zone, variables were first examined individually using a univariable logistic model and selected when $p \leq 0.2$. Selected variables were then included in a logistic model and manual backward elimination was used to obtain a final model, with the least significant variables removed, providing their removal did not alter the odds ratios (OR) of other variables by more than 20% and $p \geq 0.05$. Analyses were also conducted using forward selection, starting with the variables with lowest $p$ values in the univariable analysis. Adjusted OR and 95% confidence intervals (CIs) were obtained, with variables only retained if $p < 0.05$.

For the analysis of all zones simultaneously, stratified logistic regression was used, with herd serological status a binary outcome and zone as stratum. The model was built using the same steps described above for the analysis by zone.

Univariable and multivariable data analyses were carried out using R version 3.5.1 (R Core Team, 2018) and stratified logistic regression

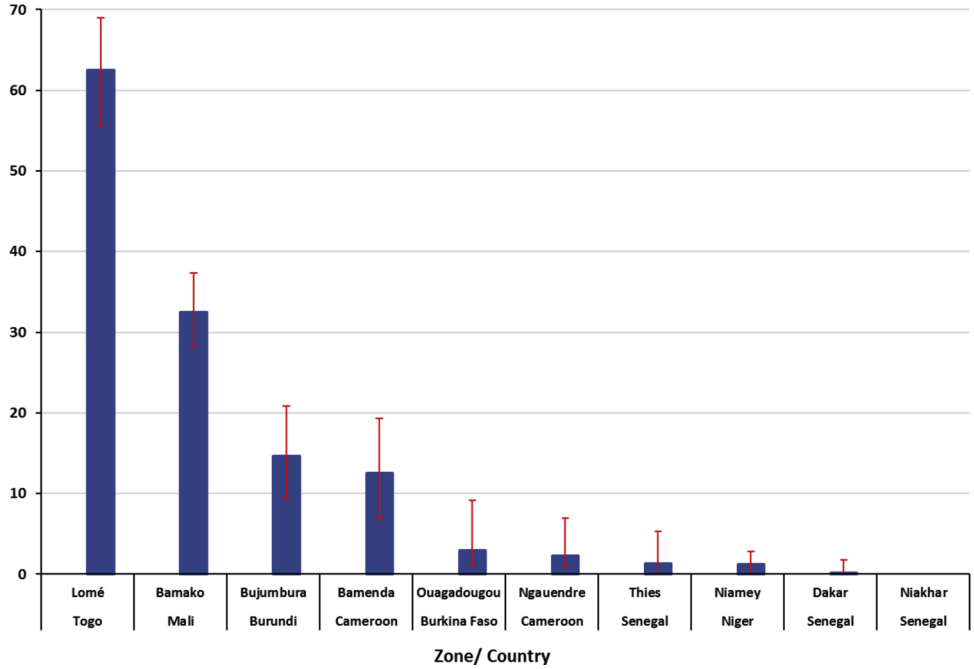


**Fig. 2.** Estimated true herd-level *Brucella* spp. seroprevalence (%) among dairy herds across ten peri-urban areas in seven West and Central African countries. The red bars indicate 95% confidence intervals.

analysis was performed using the function *clogit* implemented in R package *survival*.

## 3. Results

### 3.1. Seroprevalence estimates

Bulk milk samples were collected from 965 herds in 10 study zones and questionnaires completed from 809 herds (84%) (Table 3). Estimated herd-level seroprevalences for the highest four peri- urban zones were: Lomé (Togo) 62.0% (95% CI: 55.0–69.0), Bamako (Mali) 32.5% (95% CI: 28.0–37.0), Bujumbura (Burundi) 14.7% (95% CI: 9.4–20.8) and Bamenda (Cameroon) 12.6% (95% CI: 7.6–21.9), Fig. 2.

### 3.2. Risk factors

Median adult herd size, minimum, maximum and median number of lactating cows, heifers and bulls in addition to husbandry and management practices for the 10 peri-urban zones are presented in Table 2. Univariable analyses showed the practice of transhumance to be significantly associated with lower risk of positive serological status both in Bamenda and Bujumbura. Also in Bamenda, the regular purchase of new animals during the previous year was significantly associated with higher risk of seropositivity, and the mixing of cattle with other livestock during watering or grazing was marginally associated with higher risk of seropositivity (Table 4).

In the multivariable analyses, transhumance remained a protective factor in both Bujumbura (adjusted OR: 0.24, 95% CI: 0.07- 0.8, $p = 0.02$) and Bamenda (adjusted OR: 0.17, 95% CI: 0.05-0.6, $p = 0.01$). In Bamenda, regular purchasing of new animals in the previous year was also found to be strongly associated with seropositivity (adjusted OR: 5.3, 95% CI: 1.4–25.9, $p = 0.02$). Logistic regression modelling with zone included as a strata revealed transhumance to be a protective factor (adjusted OR: 0.25, 95% CI: 0.13-0.5, $p < 0.01$), no other factor was identified as significantly associated with the risk of seropositivity (Table 5).

## 4. Discussion

Our results confirm that brucellosis is widespread among the peri-urban bovine dairy supply chains of West and Central Africa, which presents a serious public health threat to local populations, particularly those working on dairy farms or consuming raw dairy products. Positive herds were found in all study areas except for one (Niakhar in Senegal). Herd-level seroprevalence values vary considerably between areas.

The high herd seroprevalences found in some areas, such as Lomé and Bamako, are compatible with a high human disease burden, although currently limited, data are available to confirm this (Bonfoh et al., 2003; Akakpo et al., 2009; Dean et al., 2013; Craighead et al., 2017; Kanoute et al., 2017; Awah-Ndukum et al., 2018). Reasons for the large variation in seroprevalence remain unclear, however differences in management practices (e.g. source of replacement cows), farming systems (e.g. intensive vs. traditional) and variations in climate (e.g. the environmental persistence of *Brucella* spp. being influenced by wet and dry seasons lengths) are all likely to be contributory factors (Ducrotoy et al., 2017; Craighead et al., 2017).

For the purpose of sample size calculations we have used a value of 15% for the expected herd-level seroprevalence, based on most recent published data. Considerably higher values of herd-level seroprevalence were obtained in Lomé and Bamako. As a result, the precision of the estimate in these two areas is lower than expected and the confidence intervals are broader (Fig. 2). When comparing our prevalence values with those from other studies it should be noted that the adjustment of apparent prevalence to obtain 'true' prevalence was done assuming sensitivity and specificity values of 0.98 and 0.99 respectively (the values provided by the OIE brucellosis reference centre at the APHA, UK).

Within a given zone, the main factor that emerged as associated with increased risk of seropositive status is the farming system (transhumant vs. sedentary): sedentary herds are at significantly higher risk of infection in two of the four areas (Bujumbura and Bamenda). A potential explanation for this is that sedentary dairy farming creates more favourable conditions for *Brucella* spp. transmission around the calving areas compared to transhumant farming. This is consistent with Ducrotoy's hypothesis that transhumance, among other factors, mitigates the transmission of brucellosis in sub Saharan Africa (Ducrotoy et al., 2017). In this study we focused on dairy herds providing milk to urban areas and located in what we defined as a peri-urban zone. The high proportion of study herds found to practice transhumance in some of the zones may therefore appear to be unexpected. However, this is in agreement with the findings of the Sahel and West Africa Club (SWAC) and the Commission of the Economic Community of West African States (ECOWAS) study where transhumance was recognised as a regional phenomenon across geographic areas and production systems (including peri-urban farming) in West and Central African countries

**Table 4**
Descriptive statistics and univariable associations between potential herd level risk factors and *Brucella spp*. seropositivity in peri-urban dairy herds in Lomé (Togo), Bamako (Mali), Bujumbura (Burundi) and Bamenda (Cameroon). Results from a cross-sectional study conducted between January 2017 and February 2018.

| Variables | | Togo (Lome) | | Mali (Bamako) | | Burundi (Bujumbura) | | Cameroon (Bamenda) | |
|---|---|---|---|---|---|---|---|---|---|
| Description | Categories | No. Brucella + ve / total in category (%) | p | No. Brucella + ve / total in category (%) | p | No. Brucella + ve / total in category (%) | p | No. Brucella + ve / total in category (%) | p |
| Adult herd size (median) | ≤ Median | 15/20 (75) | 0.18 | 12/34 (35.3) | 0.45 | 6/46 (13) | 0.33 | 6/49 (12.2) | 0.71 |
| | > Median | 32/57 (56.1) | | 15/32 (46.9) | | 8/41 (19.5) | | 8/47 (17) | |
| Transhumance | No | 1/2 (50) | 0.16 | 23/49 (46.9) | 0.16 | 9/31 (29) | 0.03 | 9/26 (34.6) | 0.002 |
| | Yes | 46/75 (61.3) | | 4/17 (23.5) | | 5/56 (9) | | 5/70 (7.1) | |
| Regular mixing with other flocks for water or grazing | No | 12/16 (75) | 0.21 | 16/43 (37.2) | 0.44 | 8/61 (13.1) | 0.21 | 2/36 (5.6) | 0.07 |
| | Yes | 35/61 (57.4) | | 11/23 (47.8) | | 6/26 (23.1) | | 12/60 (20) | |
| Insemination method | AI | 0 | NA | 2/6 (33.3) | 0.69 | 1/6 (16.7) | 0.7 | 0 | NA |
| | NI | 48/77 (62) | | 25/60 (41.7) | | 13/81 (16) | | 14/96 (14.6) | |
| Borrowing bulls for service | No | 44/72 (61.1) | 0.93 | 20/50 (40) | 0.79 | 6/35 (17.1) | 0.79 | 14/82 (17.1) | 0.21 |
| | Yes | 3/5 (60) | | 7/16 (43.8) | | 8/52 (15.4) | | 0/14 (0) | |
| Regular purchase of new animals. | No | 25/36 (69.4) | 0.16 | 12/28 (42.9) | 0.81 | 6/38 (15.8) | 0.81 | 3/55 (5.5) | 0.01 |
| | Yes | 22/41 (53.7) | | 15/38 (39.5) | | 8/49 (16.3) | | 11/41 (26.8) | |

AI = Artificial insemination; NI = Natural insemination.

**Table 5**
Results of a multivariable logistic regression model (A) and multivariable logistic regression with zone included as strata (B) on serological status of cattle herds against *Brucella* spp. in peri-urban zones of Bujumbura (Burundi) and Bamenda (Cameroon).

A Results of multivariable logistic regression by zone with stepwise elimination of variables

| Variable (Category) | Burundi (Bujumbura) | | | Cameroon (Bamenda) | | |
|---|---|---|---|---|---|---|
| | Adjusted odds ratio | 95% CI | *p* | Adjusted odds ratio | 95% CI | *p* |
| Transhumance (Yes) | 0.24 | 0.07 - 0.8 | 0.02 | 0.17 | 0.05–0.6 | 0.01 |
| Regular purchase of new animals (Yes) | | | | 5.3 | 1.4–25.9 | 0.02 |

B Results of multivariable logistic regression with zone included as a strata with stepwise elimination of variables

| | Adjusted odds ratio | 95% CI | *p* |
|---|---|---|---|
| Transhumance (Yes) | 0.25 | 0.13–0.5 | < 0.01 |

(SWAC-OECD/ECOWAS, 2008).

Regular purchase of new animals into the herd was found to be a significant risk factor for herd seropositivity in Bamenda, highlighting the risk of introduction of animals of unknown disease status in endemic areas and the need to test animals coming onto the farm and promote the use of home bred replacement cows where possible (Kanoute et al., 2017). Exploration of the relationship between the proportion of positive herds in an area and the density of dairy herds and animals within the area did not show any clear pattern, although the area with highest prevalence (Togo) had also the highest density at both, herd and individual animal level.

In our study we did not explicitly address the urban-rural interface, however, given how common transhumance is in some of the areas, it is likely that there is frequent contact and potentially transmission between herds providing milk and dairy products to urban areas and herds with production destined for home consumption in rural areas. Some studies previously conducted in the region have shown higher prevalence of bovine brucellosis in rural areas compared to urban and peri-urban areas, this was explained by the free animal movement in the rural area (Boukary et al., 2013). In East Africa, prevalence estimates of human brucellosis in rural, peri-urban and urban areas of Kampala, Uganda revealed higher number of human cases in the urban areas resulting from the consumption of raw milk transported from peri-urban and rural areas (Makita et al., 2008).

By relying on tests that detect antibodies against *Brucella* spp. it is not possible from this study to confirm which *Brucella* species is responsible for dairy cow infection in each zone. However, given the cattle host preference of *B. abortus* and findings from previous studies that isolated *Brucella* from cattle in the region (Akakpo et al., 2009; Dean et al., 2013) it seems reasonable to assume that *B. abortus* is circulating in these zones. There is no brucellosis control program in place in any of the study areas, therefore, the status of dairy herds supplying milk and dairy products to the nearby urban populations is not expected to improve and on the contrary, may become worse in the future as farms trade animals between them. The widespread presence of brucellosis in the peri-urban dairy chains of the region calls for the design and implementation of appropriate control programs. In those areas in which the prevalence is high, cattle vaccination may be justified as a means to reduce the incidence of infection in cattle and the risk for farmers and for the general population as consumers of dairy products. The most widely used vaccine in cattle is the S19 *B. abortus* live attenuated vaccine, which has been used successfully to reduce the prevalence of infection in dairy herds in different countries (CFSPH, 2009). It seems reasonable to assume that its application in peri-urban dairy farms in Sub-Saharan Africa would yield similarly positive results. This measure should be accompanied by awareness campaigns aimed at dairy herd owners and workers (e.g. avoiding introduction of animals of unknown infection status and hygienic and safe disposal of abortions and handling of parturitions) and the general population (boiling milk before consumption or processing into dairy products). Disease elimination by means of test and slaughter is not realistic unless resources are available to compensate farmers for the animals lost and to prevent re-introduction of infection through uncontrolled movement of animals. Currently, this strategy does not seem feasible in the study areas and therefore, in the absence of vaccination, dairy farmers should be encouraged and assisted to implement sanitary measures that minimize the risk of *Brucella* being introduced into their herds and transmitted between their animals. Establishment of local diagnostic capacity will be a key pillar for control, regardless of the specific pathway that is adopted, and this has been one of the objectives of this project in which diagnostic laboratories of seven countries carried out indirect diagnostic tests as per international standards.

In conclusion, our findings confirm that *Brucella* spp. circulates among dairy cattle supplying milk to urban consumers in West and Central Africa, posing a serious public health concern. There is an urgent need for control programs, in particular in high prevalence areas such as Lomé or Bamako, where more than 30% of the herds we studied showed evidence of infection. Intensification and replacement of the traditional practice of transhumance by systems where cattle are confined all year round increases the risk of infection and is likely to contribute to the sustained circulation of *Brucella* spp. in peri-urban dairy settings in the absence of control programs. The current study provides much-needed prevalence estimates and adds to the current knowledge on brucellosis in West and Central Africa, demonstrating that brucellosis is endemically established among dairy herds of these areas, in some cases at very high levels. In light of previous studies, our results show that there are areas where brucellosis has remained endemic among dairy cattle for decades. In this context, vaccine-based control programs, which have been proved effective at markedly reducing prevalence of brucellosis in cattle in different countries, seem to be an urgent need for the region. Further steps to facilitate action towards vaccine-based control include improvements in local diagnostic capability, strain isolation and characterization and vaccination trials.

## Conflict of interest

The authors report no conflict of interests.

## Acknowledgments

Research funding was provided by Zoonoses and Emerging Livestock Systems (ZELS), a joint research initiative between; Biotechnology and Biological Sciences Research Council (BBSRC), Defence Science and Technology Laboratory (DSTL), Department for International Development (DFID), Economic and Social Research Council (ESRC), Medical Research Council (MRC) and Natural Environment Research Council (NERC). This project represents one of 11 programmes funded in total, and was funded under the title,

‘Establishment of a multi-sectoral strategy for the control of brucellosis in the main peri-urban dairy production zones of West and Central Africa. Authors acknowledge all farmers who participated in this study, field technicians and laboratory technicians for their help and support. We thank Dr Yu-Mei Chang for providing statistical advice.

## References

Akakpo, A., Teko-Agbo, A., Kone, P., 2009. The Impact of Brucellosis on the Economy and Public Health in Africa. pp. 85–98.

Al Dahouk, S., Kohler, S., Occhialini, A., Jimenez de Bagues, M.P., Hammerl, J.A., Eisenberg, T., Vergnaud, G., Cloeckaert, A., Zygmunt, M.S., Whatmore, A.M., Melzer, F., Drees, K.P., Foster, J.T., Wattam, A.R., Scholz, H.C., 2017. Brucella spp. of amphibians comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts. Sci. Rep. 7, 44420.

Alonso, S., Dohoo, I., Lindahl, J., Verdugo, C., Akuku, I., Grace, D., 2016. Prevalence of tuberculosis, brucellosis and trypanosomiasis in cattle in Tanzania: a systematic review and meta-analysis. Anim. Health Res. Rev. 17, 16–27.

Awah-Ndukum, J., Mouiche, M.M.M., Bayang, H.N., Ngwa, V.N., Assana, E., Feussom, K.J.M., Manchang, T.K., Zoli, P.A., 2018. Seroprevalence and associated risk factors of brucellosis among indigenous cattle in the Adamawa and north regions of Cameroon. Vet. Med. Int., 3468596.

Bonfoh, B., Fane, A., Steinmann, P., Hetzel, M., Traore, A., Traore, M., Simbe, C., Alfaroukh, I., Nicolet, J., Akakpo, J., 2003. Qualité microbiologique du lait et des produits laitiers vendus au Mali et leurs implications en santé publique. Etudes et recherches sahéliennes 8, 19–27.

Boukary, A.R., Saegerman, C., Abatih, E., Fretin, D., Alambedji Bada, R., De Deken, R., Harouna, H.A., Yenikoye, A., Thys, E., 2013. Seroprevalence and potential risk factors for Brucella spp. infection in traditional cattle, sheep and goats reared in urban, peri-urban and rural areas of Niger. PLoS One 8, e83175.

CFSPH, 2009. Ovine and Caprine Brucellosis: Brucella melitensis. Available:. CFSPH, Iowa State University, Iowa, USA. http://www.cfsph.iastate.edu/Factsheets/pdfs/brucellosis_melitensis.pdf.

Corbel, M.J., 2006. Brucellosis in Humans and Animals. World Health Organization.

Craighead, L., Meyer, A., Chengat, B., Musallam, I., Akakpo, J., Kone, P., Guitian, J., Hasler, B., 2017. Brucellosis in West and Central Africa: a review of the current situation in a changing landscape of dairy cattle systems. Acta Trop. 179, 96–108.

Dean, A.S., Crump, L., Greter, H., Hattendorf, J., Schelling, E., Zinsstag, J., 2012. Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS Negl. Trop. Dis. 6, e1929.

Dean, A.S., Bonfoh, B., Kulo, A.E., Boukaya, G.A., Amidou, M., Hattendorf, J., Pilo, P., Schelling, E., 2013. Epidemiology of brucellosis and q Fever in linked human and animal populations in northern togo. PLoS One 8, e71501.

Doganay, M., Aygen, B., 2003. Human brucellosis: an overview. Int. J. Infect. Dis. 7, 173–182.

Ducrotoy, M., Bertu, W.J., Matope, G., Cadmus, S., Conde-Alvarez, R., Gusi, A.M., Welburn, S., Ocholi, R., Blasco, J.M., Moriyon, I., 2017. Brucellosis in Sub-Saharan Africa: current challenges for management, diagnosis and control. Acta Trop. 165, 179–193.

FAO, 2010. Brucella melitensis in Eurasia and the Middle East. 2009. Proceeding of a Joint Technical Meeting FAO/WHO/OIE. Available at: http://www.fao.org/docrep/012/i1402e/i1402e00.pdf.

Guneralp, B., Zhou, Y., Urge-Vorsatz, D., Gupta, M., Yu, S., Patel, P.L., Fragkias, M., Li, X., Seto, K.C., 2017. Global scenarios of urban density and its impacts on building energy use through 2050. Proc. Natl. Acad. Sci. U.S.A. 114, 8945–8950.

Jennings, G.J., Hajjeh, R.A., Girgis, F.Y., Fadeel, M.A., Maksoud, M.A., Wasfy, M.O., El-Sayed, N., Srikantiah, P., Luby, S.P., Earhart, K., Mahoney, F.J., 2007. Brucellosis as a cause of acute febrile illness in Egypt. Trans. R. Soc. Trop. Med. Hyg. 101, 707–713.

Kanoute, Y.B., Gragnon, B.G., Schindler, C., Bonfoh, B., Schelling, E., 2017. Reprint of "epidemiology of brucellosis, Q Fever and Rift Valley Fever at the human and livestock interface in northern Cote d'Ivoire. Acta Trop. 175, 121–129.

Kirk, M.D., Pires, S.M., Black, R.E., Caipo, M., Crump, J.A., Devleesschauwer, B., Dopfer, D., Fazil, A., Fischer-Walker, C.L., Hald, T., Hall, A.J., Keddy, K.H., Lake, R.J., Lanata, C.F., Torgerson, P.R., Havelaar, A.H., Angulo, F.J., 2015. World health organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. PLoS Med. 12, e1001921.

Makita, K., Fevre, E.M., Waiswa, C., Kaboyo, W., De Clare Bronsvoort, B.M., Eisler, M.C., Welburn, S.C., 2008. Human brucellosis in urban and peri-urban areas of Kampala, Uganda. Ann. N. Y. Acad. Sci. 1149, 309–311.

McDermott, J., Grace, D., Zinsstag, J., 2013. Economics of brucellosis impact and control in low-income countries. Rev. Sci. Tech. 32, 249–261.

Ogugua, A.J., Akinseye, V.O., Cadmus, E.O., Jolaoluwa Awosanya, E.A., Alabi, P.I., Idowu, O.S., Akinade, S.A., Dale, E.J., Perrett, L., Taylor, A., Ignocio, M., Cadmus, S.I.B., 2018. Prevalence and risk factors associated with bovine brucellosis in herds under extensive production system in southwestern Nigeria. Trop. Anim. Health Prod. 50, 1573–1582.

Oseguera Montiel, D., Bruce, M., Frankena, K., Udo, H., van der Zijpp, Rushton, J., 2015. Financial analysis of brucellosis control for small-scale goat farming in the Bajío region, Mexico. Prev. Vet. Med. 118, 247–259.

SWAC-OECD/ECOWAS, 2008. Livestock and Regional Markets in the Sahel and West Africa: Potentials and Challenges. Sahel and West Africa Club/OECD, Paris, France P: 127.

Whatmore, A., Davison, N., Cloeckaert, A., Al Dahouk, S., Zygmunt, M., Brew, S., 2014. Brucella papionis sp. nov. isolated from baboons (Papio spp.). Int. J. Syst. Evol. Microbiol. 64 (4120), 4128.

WHO, 2005. The Control of Neglected Zoonotic Diseases. Geneva; Report of a Joint WHO/DFID-AHP. P: 54. Available:. . http://www.who.int/zoonoses/Report_Sept06.pdf.

WHO, 2015. WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015. Available:. . http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en.

World Bank, 2011. World Livestock Disease Atlas: A Quantitative Analysis of Global Animal Health Data (2006-2009). Available:. pp. 11. http://documents.worldbank.org/curated/en/323671468179364909/World-livestock-disease-atlas-a-quantitative-analysis-of-global-animal-health-data-2006-2009.