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Full Length Research Paper

SEROPREVALENCE OF BOVINE BRUCELLOSIS IN CENTRAL CAMEROON

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ABSTRACT

Brucellosis is a zoonotic disease with a significant economic and public health impact, which particularly affects humans and animal species in developing countries relying on livestock production. This study was conducted to provide asero-prevalence of bovine brucellosis in the central part of Cameroon. Sera from randomly collected blood samples were screened for *Brucella* antibodies using the rapid Rose Bengal Plate Test (RBPT) and the indirect Enzyme Linked Immuno-Sorbent Assay(i-ELISA). A questionnaire was administered to butchers to trace the origin of each animal sampled and know the age and sex of these animals. Statistical significance was determined by Chi-Square test using SPSS v 20 software. A total of 460 cattle (both male and female) were screened. RBPT detected *Brucella* antibodies in 67 (14.63%). With iELISA, 41 (9, 4%) samples tested positive for detecting *Brucella* LPS antibodies for confirmation. Data such as animal ageand their origin were not significant; however, the sexwas statistically significant. Animals from Ngaoundere were found to be more affected than animals from Bertoua. Therefore, the overall sero-prevalence obtained was 67 (14.63%) for RBPT and 41 (9, 4%) for i-ELISA.

Keywords: Brucellosis, bovine, sero-prevalence, i-ELISA, Rose Bengal Test,Cameroon.

INTRODUCTION

Brucellosis also known as Bang's disease or Crimean fever. It is a zoonotic infection and is one of the most virulent infectious and zoonotic diseases in the world. Brucellosis is caused by bacteria of the genus *Brucella.* These bacteria exhibit host specificity and the most studied species include:*Brucella melitensis* and *Brucella ovis*thatcausecaprine brucellosis, *Brucella abortus*thatcauses bovine brucellosis,

Brucella suis that infects pork, and *Brucella canis* known to infect dogs(Fosgate,*et al*., 2002;Ayola *et al*., 2017). Cross species infections often occur; and other host include: humans, camels, dolphins, porpoises, whales, seals, desert wood rats, and common voles (Singh*et al.*, 2015). Brucellosis is the most common bacterial zoonosis worldwide with over 500,000 new human cases annually (Deshmukh*et al*., 2015). The most studied zoonotic *Brucella* species include *Brucella melitensis*, *Brucella abortus*, and *Brucella suis*.Infection in humans generally results from transmission via the gastrointestinal route after consuming unpasteurized dairy products and contaminated meat (Fosgate,*et al*., 2002). Airborne transmission has been reported in animal husbandry exposed to contaminated dust fromaborted animals and in laboratory-associated exposure to aerosols(Inan*et al*., 2010). In the event of a bioterror attack, the preferred method of dissemination would most likely be via aerosols. The major clinical outcomes in animals are abortion and infertility, while in humans, endocarditis, osteomyelitis, undulant fever, and arthritis with complications often leadto a chronic state with neurologic attacks and death(Singh*et al.*, 2015;Inan*et al*., 2010).

The most dependable method of diagnosing brucellosis is by isolating the causative agent from blood or tissues (Alton*et al*., 1988;Serra*et al*., 2014). However, serological methods have been proven useful survey and surveillancetools for diagnosing Brucellosis in developing countries (Baddour*et al*., 2012;Del Pozo*et al*., 2014). Various serological tests have been developed for the screening in humans and animals. One of them,Rose Bengal Plate Test (RBPT) known for its high sensitivity and friendly use is recommended, particularly in areas where animals are not being vaccinated, and where refrigeration is difficult(McGiven*et al*., 2013). However, the World Organisation for Animal Health recommends at least two serological tests (OIE,2009): the competitive ELISA (c-ELISA) and indirect ELISA (i-ELISA) confirmatory assays to the RBPT and the milk ring test (MRT) (Mai *et al*., 2012;Salman*et al*, 2014). ELISA uses the LPS-S (Lipopolysaccharide S) antigen as probe (Percy *et al*., 1998). Brucellosis is considered by the World Health Organization (WHO) as one of the seven Neglected Zoonotic Diseases (NZD) in Africa and is identified as poverty related and poverty inducer, as it affects human health and damages livestock (WHO, 2007).

Although present in many countries, brucellosis is still highly underreported because incidence of the disease in cattle remains unknown worldwide. The epidemiology of cattle brucellosis is influenced by several factors includingthoseassociated with disease transmission between herds and those influencing the maintenance and infection spread within herds (Crawford*et al*., 1990). Understanding the epidemiology of brucellosis is therefore vital for setting up evidence-based disease control measures. However, such information is inadequate in Sub-Saharan Africa. Consequently, appropriate preventive measures have not been undertaken in this part of the world (McDermott*et al*., 2002).

Cameroon is one of the African countries where livestock production is high. The current literature doesn't indicate recent reports on the prevalence and incidence of both human and animal brucellosis in major parts of Cameroon. For instance, the sero-prevalence percentages in cattle were, respectively, reported: 9.64% in 2004 and 10% in 2005in the West region (Shey-Njila, 2004;Shey-Njila*et al*., 2005), 16% in 2016 in the South-West region (Ojong*et al*., 2016) and 6.1% and 11.3% in 2018 in the North and Adamawa regions of Cameroon respectively (Awah-Ndukum*et al*., 2018). However, these are limited to few regions in Cameroon.Therefore, this

study aims at determining the sero-prevalence of *Brucella* spp. in cattle in the Centre region of Cameroon in order to provide useful information for the setting up of an effective biosurveillance measure against this disease.

MATERIALS AND METHODS

Description of the study site: This study was conducted in the Centre Region of Cameroon precisely in Yaounde (Figure 1). Yaounde, also known as the "Seven hills city", is the political capital of Cameroon. Yaounde is a 180 km^2 surface area city, located 3° 52 '0 North Latitude and 11° 31' 0 East Longitude. With a population of 2,440,462 inhabitants, it is the second most populous city in the country, after Douala. It is also the headquarters of the CentralRegion. It has a mildclimate, with an average temperature of 31°C, and North-East wind at 5 km/h, with 48% humidity.

Generally, the Yaounde climate, in which several watersheds are located, is an equatorial climate of Guinean type with four well-marked seasons: long rainy season from mid-August to mid-November; long dry season from mid-November to mid-March; brief rainy season from mid-March to mid-June; and another brief dry season from mid-June to mid-August.This city is highly covered with hills of which the highest are the Mbam Minkom mountains (1,295m) and Mount Nkolodom (1,221m) in the North-West; and Mount Eloumden (1,159m) in the South-West. Theyharbour the Mvog-Betsi Zoo which serves as habitat for primates rescued from the wild game trade. Livestock and agriculture are quite popular sectors of activity in this city including the breeding of cattle, goats, and sheep, amongst other livestock. Cattle are mainly brought in from the Adamawa region of Cameroon.

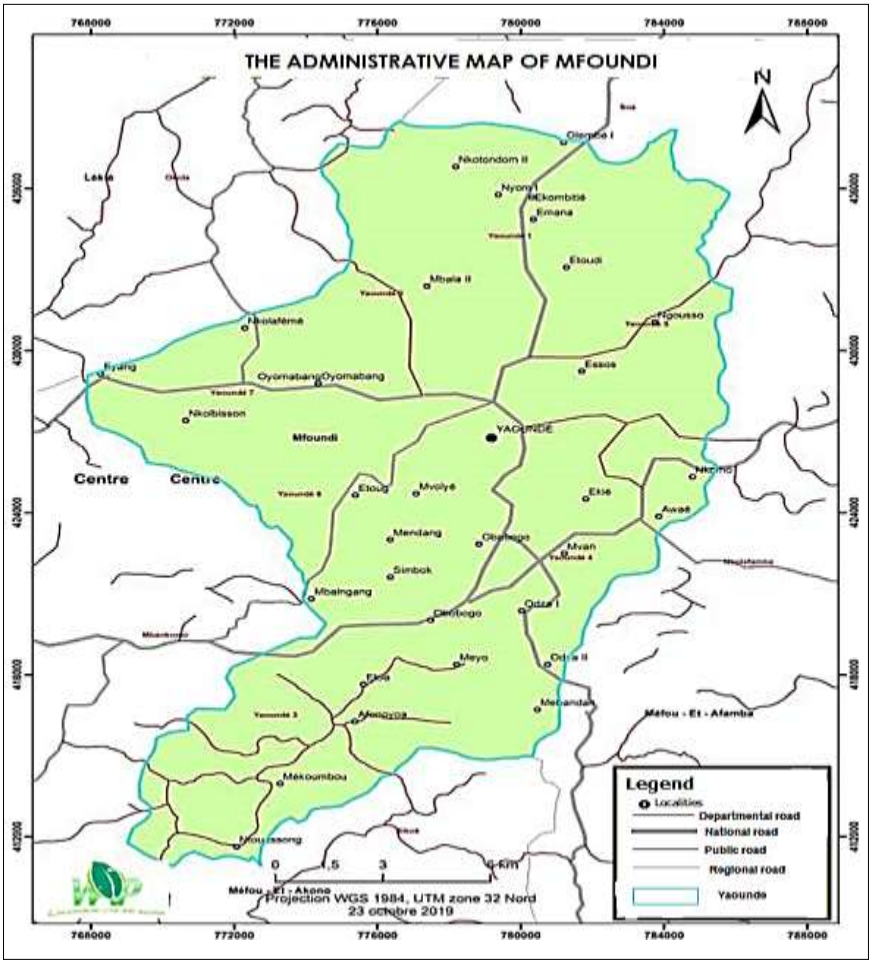

Figure 1: Map of Mfoundi division in the Centre Region of Cameroon (CRESA; 2017)

Sample and data collection: A total of 460cattle were screened at the slaughterhouse of Yaoundéfrom July 2016 to December 2016. A systematic random sampling technique was used for the animal selection. Ten milliliters of blood was collected when slaughtering the animals and the meat was stored frozen. After taking the blood samples to the National Veterinary Laboratory Annex Yaoundé, all blood were then kept at room temperature (25-30°C) for 24 hours. They were centrifuged at 3000 rpm for five minutes to collect serum in pre-labeled tubes. The sera collected were stored at -20 ° C in freezers prior to analysis.A questionnaire-based survey with open and close ended questions was carried out and questionnaires were administered to the shepherds whose flocks were to be sampled. The questions were specifically designed to get information from the Shepherd. The following data were collected on every animal under investigation: the origin of animal, the sex and age.The animal age was estimated by carrying out a dental inspection for toothed animals and an examination of horn rings for animals without teeth. Based on its biological relevance, age was stratified into three categories as follows: ≤4 years, 5–8 years, and ≥9 years.

Sample Analysis.

Rose Bengal Plate Test: All serum samples were analyzed using the Rose Bengal Plate Test (RBPT) to search *Brucella* antibodies in the sera. The test procedure recommended by the manufacturer (Alton*et al.*, 1988) was followed. An equal volume (30μL) of antigen and serum were placed side by side on the plate, mixed well, rotated for 4 minutes and then checked for the presence of agglutination. The samples showing agglutination within the first and thefourth minute were considered positive. Samples for which the appearance of agglutination was beyond the fourth minute or with no agglutination were considered negative.

Indirect Enzyme Linked Immuno-sorbent Assay(i-ELISA)

The i-ELISA was performed according to the manufacturer's instructions and was used to detect Lipopolysaccharide S (LPS-S)antibodies in all sera (Limet,1988). The i-ELISA kit (IDVET) was brought to room temperature before use. The dilution buffer 2 (190µl) was added to all wells, and 10µl samples were added in wells E1 to H12. Each of Wells A1 and B1 received 10 µl of negative control, while wells C1 and D1 received 10µl of positive control. The microplate was incubated for 45 min (±4 min) at 21°C (±5°C). The wells were emptied and washed 3 times with approximately 300 µl of the wash solution. The conjugate was prepared by diluting the concentrated conjugates 10X to 1/10 in dilution Buffer 3. The conjugate 1X (100 µl) was added to each well. It was incubated for 30 min ±3 min at 21°C (±5°C). The wells were emptied and washed 3 times with approximately 300 µl of the wash solution. A hundred microliter of the substrate solution was added to each well. The whole plates were incubated for 15 min (±2 min) at 21°C (±5°C) in the dark. Hundred microliter of the stop solution was added to each well. The OD (Optical Density) of the microplate was read and recorded at 450 nm by an automatic ELISA reader (LEDETECT 96) and for each sample *S*um/*P*roduct % was calculated as follows:

$$\frac{S}{P}\% = \frac{(OD\ sample - OD\ nc)}{(OD\ pc - OD\ nc)}\ x\ 100$$

where OD sample, ODnc, and ODpc are the readings of optical densities for the sample, negative control, and positive control, respectively. The samples were classified as positive if $\frac{S}{P}\% \geq 120$, negative if $\frac{S}{P}\% \leq 110$, and doubtful if $110 < \frac{S}{P}\% < 120$. Additionally, the fact that OD pc>0.350 and $\frac{ODpc}{ODnc} > 3$ indicated that the test is working properly.

Data Analysis

Statistical analysis was done using SPSS, version 20. Chi-square test was used to assess the significant difference between the factors and test outcome. A positive *Brucella* sample was defined as a sample that was positive on RBPT and confirmed by i-ELISA, while a negative sample was defined as a sample that was negative on RBPT, as well as i-ELISA. The level of statistical significance was considered at ($p < 0.05$).

RESULTS

Individual sero-prevalencerates of Bovine Brucellosis

The overall sero-prevalence of brucellosis in 460 cattle revealed 14.56% (67) for RBPT and 9.35% (43) for i-ELISA.In all these positive results 43(9. 35%) were only for RBPT, 19 (4.13%) for i-ELISA, and24 (5. 21%) for RBPT and i-ELISA (Table 1).

Table 1: The RBPT and i- ELISA positive cases

Serological tests on cattle (n= 460)	No.	%
RBPT (Positive)	67	14.56
i-ELISA (Positive)	43	9.35
RBPT (Positive), i-ELISA (Positive)	24	5.21
RBPT (Positive), i-ELISA (Negative)	43	9.34
RBPT (Negative), i-ELISA (Positive)	19	4.13

Factors for brucellosis sero-positivity by i-ELISA test and RBPT in cattle(n = 460) in the Central region of Cameroon are shown in Tables 2 and 3, respectively. These results show that Ngaoundere have the highest positive result (52/67 by RBPT and 34/43 by ELISA).The Chi-Square test for i-ELISA prevalence by sex revealed a significant difference (P<0.05) (Table 2). However, there was no significant difference (P>0.05) between sero-prevalence for locality and age of cattle These same results for RBPT (the prevalence by age, sex and locality using RBPT)showed that, there was no significant difference (P>0.05) between sero-prevalence for locality, sex, and age (Table 3)

Table 2: Factor for brucellosis sero-positivity by i-ELISA test in cattle(n = 460) in the Central region of Cameroon.

Factor / Variable	**Categories**	**Cattle Tested**	**ELISA + result**	**Sero-positive%by iELISA**	***P*-value**
Region	Central	460	43	9.35	
Locality	Ngaoundere	329	34	10.3	0.094
	Maroua	64	5	7.8	
	Garoua	3	0	0.0	
	Central African Republic	5	0	0.0	
	Ngaoundal	15	1	6.2	
	Meiganga	3	0	0.0	
	Tchad	37	2	5.4	
	Bertoua	1	1	100.0	
Sex	Male	364	22	6.0	≤0.001
	Female	94	21	22.3	
Age(years)	≤ 4	79	6	7.6	0.331
	5 – 8	307	33	10.7	
	≥ 9	72	4	5.6	

Table 3: Factor for brucellosis sero-positivity by RBPT in cattle(n = 460) in the Central region of Cameroon.

Factor/Variable	Categories	Cattle tested	RBPT Positive result	Sero-positive Percentage by RBPT	*P*-value
				%	
Region	Central	460	67	13.56	
Locality	Ngaoundere	327	52	15.9	0.591
	Maroua	65	10	15.4	
	Garoua	3	1	33.3	
	Central African Republic	4	0	0.0	
	Ngaoundal	16	2	12.5	
	Meiganga	4	0	0.0	
	Tchad	38	2	5.3	
	Bertoua	1	0	0.0	
Sex	Male	365	48	13.2	0.076
	Female	93	19	20.4	
Age(years)	≤ 4	78	13	16.7	0.287
	5 – 8	310	48	15.5	
	≥ 9	70	6	8.6	

DISCUSSIONS

The results obtained in this study showed that bovine brucellosis is still present in the Central Region of Cameroon and the sero-prevalence was 14.63% for the RBPT and 9.4%forthe i-ELISA methods of diagnosis. These results are not the same but look similar to those obtained elsewhere in the country. Awah-Ndunkum *etal.* (2018) reported a sero-prevalence of 11.3% and 4.1%, respectively, in the Adamawa and North regions of Cameroon using competitive ELISA.Ojong*et al*. (2016) reported lower sero-prevalence of 4.6% using the RBPT in cattle in North-West region (Scolamacchia et *al.*,2010). Bayemi*et al*. (2008) also reported asero-prevalence of 8.4% in the Northwest Region while Shey-Njila (2004)had reported the prevalence of 9.64% in a study done at the abattoir of Dschang, Cameroon. Similarly, the samples collected at the abattoir of Yaounde in Cameroon (Shey-Njila*et al*., 2005)indicated a sero-prevalence of bovine brucellosis between 7.2 and 8.8%.However, the same interval of sero-prevalence has been recorded in many other countries in Africa such as in Nigeria (7.8%) by Ayola *et al*. (2017], Ethiopia (3.4%) byAsgedom*et al*. (2016) and Niger (1.3%) by Del Pozo*et al*. (2014). Interestingly, Mazeri*et al*. (2012) reported lower rates of 20% in the Adamawa Region. Higher rates (15.6%) have been reported in Namibia (Mufinda*et al*., 2017), 22.9% from Ghana (Tasiame*et al*., 2016), 23. 8%fromEgypt (Mohamed *et al*., 2016) and 31% from South Soudan (Madut*et al*., 2018).

Seroprevalence rate obtained in this study is believed to be due to the massive entry of infected animals into herds across border areas, transhumance and community pasture research, livestock markets and veterinary interventions and lack of hygiene in livestock areas (Ibrahim*et al*., 2009;Muma*et al*., 2007;Mazeri*et al*., 2012). Slaughter sites are areas of intense activity that could be responsible for this increase in prevalence (Ayola *et al*., 2017). However, differences in prevalence in Cameroon and other parts

of Africa may also be associated with the protocol adopted, such as the type and number of diagnostic tests used.

In this study, the number of male cattle was higher than females. This can be explained by the fact that males are mainly slaughtered whereas females are often kept in sheepfolds for procreation and milk production. Those brought for slaughter are sterile or infected cows. This corroborates with the study carried out byAyola *et al*. (2017) who explained that bulls are usually kept for shorter duration in breeding herds than cows and thus, lowering the exposure of males as compared to females. Furthermore, cows are not sold for slaughter by herdsmen unless they are not doing well and the parameter of "not doing well" coincides with poor reproductive performance. This study revealed the presence and resurgence of brucellosis in females and bulls aged between 5 and 8 years of age, unlike younger cattle. These results corroborate previous studies (Alton*et al*., 1988;Limet,1988;Ojong*et al*., 2016) that explained that the economic and reproductive life of female cattle is much longer than that of bulls and that the older the animal, the longer the potential exposure to the disease. Practices on these animals on farms may also play a role in different seropositive status because of sex and age. However, Bayemi*et al*.(2008) showed that animals aged 3 or older accounted for nearly half of the seropositive animals in small-scale farms, while Ayola *etal*. (2017) andAlton*et al*. (1988] did not observe differences in sero-positivity due to sex.

Results concerning the localities showed that there is no significant difference between them, but the most seropositive result was obtained in Ngaoundere in the Adamawa region. This part of the country is known for its high-breeding activities linked to the climatic conditions which are favourable for breeding. In fact, the Adamawa region is an area with a favourable humid climate for breeding, which will also favour the high prevalence of the disease in this area. This is in line with the work done previously byBayemi*et al*. (2008) which showed that sero-prevalence is higher in Adamawa. The climate of Adamawa region is favorable to the appearance of brucellosis than the one of the North region.

Brucellosis continues to be a major public and animal health problem in Cameroon. However, there is no national control program in place due to economic crisis and the lack of information on the disease at the national level. Therefore, it is necessary to devise and implement an integrated-disease control approach involving inter-sectorial collaborative teams including the veterinary and human health sectors as well as the environmental sector, non-governmental and governmental institutions to minimize the burden of the disease.

Conclusion

It is clear that brucellosis is endemic at a high prevalence level in Cameroon. In this study, we found a very considerable level of sero-prevalence in the slaughterhouse of Yaounde. However, the obtainedresults indicated the widespread distribution of the disease in the area. In all studied factors, only sex had an influence on the sero-positivity of *Brucella* species. This study provides important information on the epidemiology of brucellosis in cattle in the study area and highlights the need to implement control measures and raise public awareness on potential zoonotic transmission of brucellosis.

Conflicts of Interest

No potential conflicts of interest have been showed by the authors.

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Authors' contributions
The conception and the planning of this project were done by all the authors including Takanouo Toukem Doriane, Wade Abel, Boda Maurice, Mbacham Wilfred and Etoa François-Xavier. The leading was done by Takanouo Toukem Doriane, Wade Abel and Boda Maurice. The Data collection, analyses and preparation of manuscripts was by Takanouo Toukem Doriane. All authors read and approved the final edition for publication.

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