Detection of *Brucella abortus* and *Brucella melitensis* in cattle and sheep from southern Cameroon

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Abstract

Background: Brucellosis is an infectious disease caused by bacterial of the genus *Brucella*. Although several investigations detected *Brucella* antibodies in many animal species, low attention has been paid for specific identification of different *Brucella* species in animals of Sub-Saharan African counties. It is in this light that this study was designed to identify *Brucella abortus* and *Brucella melitensis* in cattle and sheep of several villages of southern Cameroon in order to improve our epidemiological knowledge on brucellosis in central Africa

Methods: Blood samples were collected in 597 cattle and 315 sheep of 15 villages of two regions of Cameroon. DNA was extracted from blood samples and primers amplifying bcs31 gene enabled the identification of *Brucella spp* infections in domestic animals. Amongst animals found with *Brucella spp* infections, specific primers were used to identify *B. abortus* and *B. melitensis* respectively.

Results: From 912 animals analyzed in this study, 159 (17%) were found with *Brucella* infections. This gives an infection rate of 20.93% (125/597) in cattle and 10.79% (34/315) in sheep respectively. Of the 159 animals harboring *Brucella* infections, 118 (12.93%) were infected by *B. abortus* and 29 (3.17%) by *B. melitensis*. The *Brucella* infection rate (20.56%) was high in animals of the Noun division compared to those (12.35%) of Yoko. Between animal species, significant differences were observed in infection rates of both *abortus* and *B. melitensis*. The difference in the *B. abortus* infection rates remains significant between Yoko and Noun, and especially between villages of the Noun division.

Conclusion: This study revealed for the first time *B. melitensis* and *B. abortus* infections in cattle and sheep of the Noun and Yoko in the southern Cameroon. The identification of *B. melitensis* and *B. abortus* in animals suggests further investigations on human brucellosis. Results of this study highlight the need of developing and implementing control measures against brucellosis in Cameroon, especially in villages presenting high risk for *Brucella* infections.

Introduction

Brucellosis is one of the most widespread zoonotic infectious diseases in the world [1]. It is caused by bacteria of the genus *Brucella* which contains six species notably *Brucella abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* [2, 3]. This infectious disease is among the top thirteen neglected zoonotic and contagious diseases. *Brucella* infections are also considered as one of the major threat for livestock production in developing countries [4, 5, 6]. Human and several animal species including cattle, small ruminants, pigs, rodents and carnivores have been reported with *Brucella* infections [2, 7]. Amongst the six *Brucella* species, *B. abortus*, *B. melitensis* and *B. suis* have impacts on domestic livestock productivity and human health [8]. In animals, *Brucella* infections induce abortion, infertility and the reduction of milk yield while in human, they cause long debilitating illnesses [9]. In sub-Saharan African countries, the economic losses resulting from *Brucella* infections in livestock can be estimated to about 427 million US\$ per year for livestock [9]. Although various human and domestic animals can be infected by different *Brucella* species, little attention has been paid for *Brucella* infections in developing countries due its neglected aspect.

In most sub-Saharan African countries, investigations undertaken on brucellosis have been mainly based on *Brucella* antibodies detection using various serological tests like the Rose Bengal Plate Test (RBPT), Enzyme Linked Immunosorbent assay (ELISA), complement fixation test [7,10, 11, 12, 13, 14]. Even though serological tests have generated epidemiological data on the sero-prevalence of *Brucella* antibodies in humans and domestic animals of several sub-Saharan countries, these tests cannot differentiate different *Brucella* species [7, 10, 11, 13]. Moreover, the host species for each *Brucella* species remain not well known. In consequence, results generated by these immunological tests are of limited epidemiological values. The distribution and the transmission dynamics of different *Brucella* species remain therefore not well understood. Up till now, data regarding the prevalence of different *Brucella* species are still

lacking in most sub-Saharan countries. Accurate identification of strains circulating in different hosts is a prerequisite for the improvement of our epidemiological knowledge of brucellosis.

Although the methods based on the culture and isolation of *Brucella* have been considered as the gold standard for brucellosis diagnosis [15], these methods are tedious, time consuming and difficult to perform in developing countries [16]. To overcome these challenges, molecular tools have been developed to facilitate the identification of different *Brucella* species [17, 18, 19].

These molecular tools appeared reliable, simple, easy to perform and are able to identify natural infections of different *Brucella* species [7,17,18, 20, 21, 22]. Their use in West and East Africa enabled the identification and characterization of different *Brucella* species [7, 21, 23, 24]. However, most studies performed in central Africa have been focused on *Brucella* antibodies detection and consequently, the *Brucella* species that infects humans and different animal species are still unknown. Specific identification of different *Brucella* species could help to understand the circulation and spread of each species, and also the current epidemiological situation of brucellosis in each setting.

The present study was designed to identify *Brucella abortus* and *Brucella melitensis* in cattle and sheep of several villages of southern Cameroon in order to improve our epidemiological knowledge on brucellosis in central Africa.

Materials And Methods

Study design and description of the study areas

This cross-sectional study was conducted in nine villages (Foumban, Foubot, Njimom, Massangam, Magba, Malentuen, Koutaba, Bangourain and Kouptamo) of the Noun division of the west region and six villages (Lena, Ngoun, Yoko Wankou, Megan, Kong and Kounde) of Yoko in the center region of Cameroon. The first survey was performed in November 2017 at Yoko and the second one from April to June 2018 in the Noun Division.

Yoko (5°31'60"N; 12° 18' 57"E) is located in the "Mbam et Kim" Division of the center region of Cameroon. At about 270 km in the northeast of Yaoundé, (capital of Cameroon), this locality covers about 15 000 km² (Figure 1). Yoko is located between the southern green part and the northern Sahelian part of the country. It is recognized as the large basin for cattle and small ruminant's production of the center region of Cameroon. Moreover, the presence of pasture and water for animals breeding favors transhumance of herds originating from Western, Eastern and Adamawa regions of Cameroon. This transhumance phenomenon occurs also for herds from neighboring countries like the Central Africa Republic [25, 26].

The Noun division (4°95' 6°30'N; 10°30'12"E) is located in the western highlands of Cameroon. It covers about 7687 km² with a vegetation characterized by the savannah and degraded forest (Figure 1). Due to its climatic conditions that are favorable for cattle and small ruminant breeding, the Noun division is considered as the main cattle production area of the west region of Cameroon [27]. For trade or transhumance purposes, animals of these localities can move to other regions such as the Northwest, the Center and the Adamawa regions of Cameroon.

The cattle reared at Yoko and in the Noun division are indigenous breeds made of Zebu Goudali, Zebu White Fulani and Zebu Red Fulani with few cross breeds [27]. In these two localities, various animal species including goats, sheep and cattle share the same environment. The feeding system observed in these localities is essentially free grazing and sometime, associated to stall-feeding.

Ethics statement

The protocol of this study was approved by the Ministry of livestock, fisheries and animals Industries of Cameroon with the reference number N°015/16/L/DDEPIA.NN. The review board of the molecular parasitology and entomology subunit of the Department of Biochemistry of the Faculty of Science of the University of Dschang gave also its approval. The local administration and traditional authorities of each sampling site were informed and their approvals were obtained. Verbal consent was obtained from each owner, after detailed explanation of the objective of the study.

Sample size estimation

The prevalence of *B. abortus* and *B. melitensis* was determined in cattle and sheep of 15 villages of Southern Cameroon. For this study, a stratified sampling strategy was applied to select herds and individual cattle per herd. The sample size was estimated for cattle using a standard formula for cross-sectional studies as described by Thrusfield [28];

$$n = Z^2 P_{exp} (1 - P_{exp}) / L^2$$

where n = the minimum sample size required, Z = the critical value for a given confidence interval which is 1.96 at 95% confidence interval, P = expected prevalence and L = margin of error (the margin of error is 0.05)).

The prevalence of brucellosis was assumed to 5.2% as reported from study previously undertaken in the North West Region of Cameroon [27]. In each herd and depending on its size, at least 20% of cattle were sampled. More than 20% of cattle of some herds were sampled for specific reasons like the interests and cooperation of some farmers and advices from veterinarians. Selection of individual cattle to be sampled from each chosen herd was based on a systematic random sampling technique as described by Asgedom *et al.* [29]. A total of thirty-seven (37) farms were enrolled in fifteen (15) villages for a sample size of 597 cattle.

For the sheep, where there is no published data on the brucellosis prevalence in Cameroon, a prevalence of 50% was used for the sampling size estimation. Due to the small number of sheep in each village, all of them were sampled irrespective of the number of animals presented by each household. In consequence 315 sheep were sampled from the two agro-ecological zone of southern Cameroon.

Blood collection and DNA preparation

After approval from each herd owner, the farm characteristics and information regarding each animal including the name of the village (where each sample was collected), the geographical coordinates of each sampling site, the animal species found in farm (cattle, goat, sheep), the origin, sex, age, breed and the feeding system were recorded. Thereafter, about 5 ml of blood were collected from the jugular vein of sheep and cattle into EDTA coated tubes by a veterinarian. The tubes were labelled and carefully packed to avoid crossed contamination. In the field, the blood samples were stored at 4°C in an electric cooler before being transported to the laboratory where they were kept at -20°C.

Genomic DNA was extracted from whole blood using the cetyl trimethylammonium bromide (CTAB) method adopted from Navajas *et al.* [30]. Briefly, frozen samples were thawed and 500µL of whole blood were pipetted into a micro-tube containing 1 mL of sterile water. The micro-tube was vigorously vortexed and then, centrifuged at 10.000 rpm for 5 min. To the resulting pellet, 500 µL of CTAB buffer (CTAB 2%; 1 M Tris, pH 8; 0.5 M EDTA pH 8; 1.4 M NaCl) were added. The pellet was re-suspended and incubated in a water bath at 60°C for 30 min. To the content of each micro-tube, 600 µL of chloroform/isoamyl alcohol (24/1) mixture was added. Each micro-tube was slowly homogenized for 15 min and the upper aqueous phase was removed and transferred to a new micro-tube of 1.5 mL. DNA was precipitated by adding 600 µL of isopropanol. The mixture was gently homogenized for 5 min and then incubated overnight at -20° C. Thereafter, each micro-tube was centrifuged at 13,000 rpm for 15 min. DNA pellet was then washed twice with cold 70% ethanol and dried overnight at room temperature. The resulting DNA pellet was re-suspended in 50 µL of sterile nuclease free water and then stored at -20° C until use.

Detection of *Brucella* spp

The identification of bacteria of the genus *Brucella* was performed as described by Mitka *et al.* [20]. This was done using B4 (5'-TGGCTCGGTTGCCAATATCAA-3') and B5 (5'-CGCGCTTGCCTTTCAGGTCTG-3') primers that amplify a DNA fragment of 223 bp. This latter corresponds to bcs31 gene encoding an immunogenic outer membrane protein of 31 kDa which is conserved in all *Brucella* species. PCR reactions were performed in a total volume of 25 µL containing 10 pmol of each primer, 2.5 µL of 10X PCR buffer, 2 mM MgCl₂, 200 mM of each dNTP, 5 µL of DNA template and 0.5 Unit of Q5 high fidelity Taq polymerase (New England Biolab 5 U/µL). The amplification program consisted of an initial denaturation step at 95°C for 5 min followed by 40 amplification cycles made up of a denaturation step at 95°C for 30 s, an annealing step at 60°C for 30 s and an extension step at 72°C for 45s. A final extension step was done at 72°C for 5 min.

The amplified products were resolved by electrophoresis on 1.5% agarose gel. The separation was done at 100 volts for 30 min. Subsequently, the gel was stained with ethidium bromide and DNA bands were visualized under ultraviolet light and photographed. Each sample showing a PCR product of approximately 223 bp was considered as positive or having bacteria of the genus *Brucella*. These samples were selected and subsequently subjected to the identification of different *Brucella* species.

Specific identification of different *Brucella* species

All samples that have shown a DNA fragment of 223 bp corresponding to bcs31 gene of *Brucella* genus were subjected to the molecular identification of different *Brucella* species. This identification was done using AMOS PCR as described by Bricker and Halling [31]. For this identification, three primers were used: the IS711-specific primer (5'- TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT-TGC-3') hybridizes the IS711 element that is common to all *Brucella* species while the two other primers hybridize an adjacent region outside the IS711. The primer with sequence 5'-GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC-3' was specific to *B. abortus* while the third one (5'- AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA-3') was specific to *B. melitensis*. During the identification of the different *Brucella* species, PCR reactions were carried out in a final volume of 25µL containing 10 pmol of each primer, 2.5µL of 10X PCR buffer, 2 mM MgCl₂, 0.5 µL (200 mM) of each dNTPs, 5 µL of DNA template and 0.5 units of Taq DNA polymerase (New England Biolab 5 U/IL). The amplification program consisted of an initial denaturation step at 95°C for 5 minutes followed by 40 cycles of denaturation step at 95°C for 30 s, an annealing step at 53°C for 30 s; an extension step at 72°C for 1 min and a final extension step at 72°C for 5 min.

The amplified products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide. This was done at 100 V for 45 min. After the electrophoresis, the gel was visualized under ultraviolet light and photographed. Samples showing a DNA fragment of 495 bp were considered as harboring *B. abortus* while those with 730 bp were considered as positive for *B. Melitensis*

Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows® version 22.0 (SPSS Inc., Chicago, Illinois). Chi-squared tests were used to compare the infection rates of different *Brucella* species. The difference was considered significant if the p-value was lower than 0.05.

Results

1. Prevalence of *Brucella* spp infections

A total of 912 domestic animals including 597 (65.46%) cattle and 315 (34.54%) sheep were investigated for the presence of *Brucella* infections (Table 1). Five hundred and sixty four (61.84%) of these animals were from the Noun

division and 348 (38.16%) from Yoko (Table 2). From the 912 animals tested, the DNA fragment corresponding to the *bcsp 31* gene of *Brucella* was successfully amplified in 159 blood samples; giving thus a *Brucella* infection rate of 17.43% [95%CI:14.83 – 20.36]: 12.35% (43/348) at Yoko and 20.56% (116/564) in the Noun division. This infection rate was significantly ($P = 0.0015$; $X^2 = 10.07$) high in Noun (20.56%) compared to Yoko (12.35%) (Table 2). The highest infection rate was observed at Foumban (28.76%), followed by Magba (28.57%) and Bangourain (23.94%) (Table 2). The lowest infection rate was observed at Malentouen where no animal was found with *Brucella* infection (Table 2). Between villages of the Noun division, significant difference ($P = 0.03$; $X^2 = 22.96$) was observed in the overall *Brucella* infection rates. In villages of Yoko, the highest *Brucella* infection rate of 23.52% was observed in animals of Kong and the lowest rate of 7.1% in those of Megan. Between villages of Yoko, no significant difference ($P = 0.35$; $X^2 = 5.52$) was observed in the overall *Brucella* infection rates.

The *Brucella* infection rate was 20.93% [95%CI: 17-24.95] in cattle and 10.79% [95%CI: 7.45-15.08] sheep. This infection rate was significantly high ($P < 0.0001$; $X^2 = 14.71$) in cattle (20.93%; [95%CI: 17.43–24.95]) than sheep (10.79%; [95%CI: 7.45-15.08]) (Table 1).

2. Prevalence of *Brucella abortus* and *Brucella melitensis* according to animal species

From the 159 animals found with *Brucella* infections, the AMOS-PCR targeting *B. abortus* and *B. melitensis* revealed 12.93% [95%CI: 10.71 – 15.49] with *B. abortus* infections and 3.17% [95%CI: 2.04 – 4.43] with *B. melitensis* (table 1). Of the 125 cattle reported with *Brucella* infections, 112 (89.6%) were infected by *B. abortus* and 10 (8.0%) by *B. melitensis*. The remaining five (2.4%) animals found with *Brucella* infections were probably carriers of other *Brucella* species that have not been investigated in this study. Between the infection rates of *B. abortus* and *B. melitensis*, significant difference ($P = 0.001$, $X^2 = 95.81$) was observed in cattle. From 34 sheep found with *Brucella* infections, 6 (17.6%) were infected by *B. abortus* and 19 (55.9%) by *B. melitensis*; giving thus the infection rate of 1.9% (6/315) and 6.03% (19/315) for *B. abortus* and *B. melitensis* respectively. Between the infection rates of *B. abortus* and *B. melitensis*, significant difference ($P = 0.008$, $X^2 = 7.04$) was also observed in sheep.

The *B. abortus* infection rate was significantly higher ($P = 0.0001$; $X^2 = 51.98$) in cattle (18.76%; [95%CI: 15.45-22.57]) than sheep (1.9%; [95%CI: 0.69 – 4.14]) (Table 1). Regarding *B. melitensis*, its infection rate was significantly higher ($P = 0.0004$, $X^2 = 12.73$) in sheep (6.03%; [95%CI: 3.63-9.41]) than cattle (1.67%; [95%CI: 0.08-3.08]) (table 1). Amongst the 159 domestic animals found with *Brucella* infections, 9 (5.6%) were co-infected by *B. abortus* and *B. melitensis* while 130 (81.71%) were single infections of *B. abortus* (68.55%: 109/159) or *B. melitensis* (12.57%: 20/159).

3. Prevalence of *Brucella abortus* and *Brucella melitensis* according to sampling sites

Of the 43 animals found with *Brucella* infections at Yoko, 33 (76.7%) harbored *B. abortus* and 8 (18.6%) *B. melitensis*, giving thus an overall infection rate of 9.48% (33/348) for *B. abortus* and 2.29% (8/348) for *B. melitensis* respectively. Between the infection rates of *B. abortus* and *B. melitensis*, significant difference ($P < 0.001$; $X^2 = 16.21$) was observed at Yoko. However, no significant difference was observed between villages of this locality neither for the overall infection rate of *B. abortus* ($P = 0.05$, $X^2 = 10.84$) nor for *B. melitensis* ($P = 0.7$; $X^2 = 3.002$) (Table 2). In animals of Yoko, the highest infection rate of 23.52% [95% CI: 6.41-60.24] for *B. abortus* was found at Kong and the lowest rate of 4.2% [95% CI: 0.08-12.52] at Megan.

From 116 animals reported with *Brucella* infections in the Noun division, 85 (73.3%) were due to *B. abortus* and 21 (18.1%) to *B. melitensis*. This gives the overall infection rates of 15.07% (85/564) for *B. abortus* and 3.71% (21/564) for *B. melitensis*. Between these two *Brucella* species, significant difference ($P < 0.001$; $X^2 = 0.00$) was observed in their infection rates in the Noun division. The overall *B. abortus* infection rates vary significantly ($P = 0.04$, $X^2 = 16.34$)

between villages of this locality. The highest *B. abortus* infection rate of 21.42% was found at Magba followed by 20.57% at Foubman. The *B. melitensis* infection rates vary between villages of the Noun division with the highest rate (9.18%) at Magba. Between villages of the Noun division, no significant difference ($P = 0.106$; $X^2 = 13.17$) was observed in the *B. melitensis* infection rates (Table 2).

In general, the *B. abortus* infection rate varies within and between the two localities (Table 2). The infection rate of 15.07% [95%CI: 12.04-18.64] reported for *B. abortus* in the Noun division was significantly higher ($P=0.014$, $X^2= 5.96$) than the 9.48% [95%CI: 6.52-13.31] recorded at Yoko (Table 2). Between Yoko and the Noun division, no significant difference ($P =0.23$; $X^2= 1.42$) was observed for the *B. melitensis* infection rates. No *B. mellitensis* infection was recorded in animals of Malentouen of the Noun division, Kong and Megan of Yoko (table 2).

Discussion

During the last decades, several investigations have been undertaken to determine the sero-prevalence of *Brucella* antibodies in sub-Saharan African countries [7, 10, 11, 13, 14]. Although most of these investigations demonstrated contacts between *Brucella* and various hosts including humans and different animal species, the identity of bacteria species that are responsible for the production of *Brucella*' antibodies has not been deeply investigated. To fill these gaps, molecular tools were used to determine the prevalence of *B. abortus* and *B. melitensis* in cattle and sheep of southern Cameroon.

The low *Brucella* infection rate of 17.43% obtained in this study is lower than 35% reported in India [22]. This could be explained by some variations of environmental factors and livestock management systems which are risk factors for brucellosis transmission [32]. The significant ($P<0.0001$; $X^2= 14.71$) high *Brucella* infection rate in cattle (20.93%) compared to sheep (10.79%) is consistent with results reported in South Africa where more cattle were found with *Brucella* infections than sheep [23]. This high infection rate could result from the larger herd sizes of cattle and their management system [5, 33]. Indeed, the extensive cattle management system and the larger size of herds have been reported as risk factors for brucellosis [34]. Moreover, the relative short life expectancy of sheep compared to cattle is an additional factor explaining the low *Brucella* infection rate in sheep. In fact, cattle remain in the herd for many years and therefore, are more exposed to *Brucella* infections than sheep. This hypothesis is in agreement with previous observations reporting that as long as infected animals remain in contact with the rest of the herd, the number of infected animals will increase [10, 35, 36, 37].

The identification of *B. abortus* and *B. melitensis* in both cattle and sheep corroborates results obtained in Tanzania and Uganda [7, 21]. Remarkably, the *B. abortus* infection rate was significantly higher ($P <0.0001$; $X^2= 53.54$) in cattle (18.76%) compared to sheep (1.9%) while *B. melitensis* was significantly ($P =0.0004$; $X^2= 26.58$) more prevalent in sheep (6.03%) than cattle (1.67%). These results could be explained by the fact that *B. abortus* and *B. melitensis* are the main etiologic agents of brucellosis in cattle and sheep, respectively [38]. Our results showing high *B. abortus* infection rate in cattle are in agreement with those previously reported in Asia and other African countries where *B. abortus* was more prevalent in cattle [7, 17, 22, 38]. Moreover, *B. abortus* has been reported as the most commonly *Brucella* species that has been isolated and characterized in cattle from sub-Saharan countries [13]. The high *B. melitensis* infection rate in sheep is also consistent with observations reporting this bacteria species as the most prevalent in sheep in most sub-Saharan countries [13]. Compared to *B. abortus*, the low *B. melitensis* infection rate in domestic animals suggests low transmission of *B. melitensis* in sub-Saharan countries [39]. Known as the etiologic agent of brucellosis in sheep [13], the small size of sheep' herd compared to cattle is another factor playing for the low transmission of *B. melitensis* and consequently, its low infection rate in domestic animals [11, 27].

The identification of *B. abortus* in sheep and *B. melitensis* in cattle is not common because these animals are not their main hosts. Nevertheless, it is important to point out that several isolates of *B. abortus* have been obtained from milk and abortion products of sheep and goat [40, 41]; highlighting the probability of sheep to be infected by *B. abortus*. In addition, other animal species like sheep has been reported to be susceptible to *B. abortus* infections [42, 43]. The detection of *B. melitensis* in cattle is in agreement with previous observations reporting the transmission of *B. melitensis* to cattle [44, 45]. The cross-transmission of *B. abortus* to sheep and *B. melitensis* to cattle could be explained by the fact that these animals share the same environment. As these animals live and share together the same breeding sites in villages of Yoko and the Noun division, the transmission of *B. melitensis* to cattle and *B. abortus* to sheep can therefore occur. This hypothesis is strengthened by previous observations reporting the transmission of *B. melitensis* to cattle, especially where both cattle and small ruminants share the same breeding sites [44, 45]. Indeed, the presence of larger herds and livestock production system that put together, and in the same place, cattle, goat and sheep favor inter and intra-specific transmission of different *Brucella* species [5, 33, 46]. The animals found with *Brucella* infections, but for which *B. abortus* and *B. melitensis* have not been identified were certainly infected by other *Brucella* species. Searching for other *Brucella* species, like for instance *B. ovis* which is a non-zoonotic bacteria species that is restricted to sheep, will be of great interest, especially because its existence has been reported in sub-Saharan countries [47, 48, 49].

While no significant difference ($P = 0.35$; $\chi^2 = 5.52$) was observed in the overall *Brucella* infection rates between villages of Yoko, significant difference ($P = 0.003$; $\chi^2 = 22.96$) was reported between villages of the Noun division; highlighting some variations in the *Brucella* infection rates according to sampling sites. This could result from variations of environmental factors and/or livestock management system that may have impacts on the transmission pattern of different *Brucella* species.

Although significant differences were reported in the *B. abortus* infection rates in villages of the two localities, no significant difference ($P = 0.23$; $\chi^2 = 1.42$) was observed for *B. melitensis* neither in villages of Yoko nor in those of the Noun division. These results suggest that the infection rates of *B. melitensis* do not seem to vary with the sampling sites. This could be explained by the low size of herds and the limited movement of sheep. In such context, it is likely that the transmission of *B. melitensis* from infected to uninfected sheep is limited because animals of different herds cannot mix together and most of time, sheep are regularly sale and killed for different ceremonies. The situation is completely different for *B. abortus* because its infection rates vary significantly ($P = 0.014$; $\chi^2 = 5.96$) between villages of the two localities (Table 2). These results suggest high transmission of *B. abortus* in some villages like Kong in Yoko, Fouban, Magba, Bangourain and Koutaba in the Noun division. These villages can be considered as hot spots where particular attention must be paid during the implementation of control measures. They are also of great epidemiological interest for further investigations on human brucellosis. The reasons explaining the high infection rate, and probably the high transmission of *B. abortus* in these villages are not well understood. However, we can speculate about: i) the large size of herds in these villages, ii) the environmental factors that could be more favorable for brucellosis transmission, iii) the poor cattle management systems, iv) the regular mixing of animals from different herds. Most of these factors have been recognized as important contributors for the transmission and the spread of brucellosis in livestock [25, 46, 50]. Viewed the high prevalence of *B. abortus* recorded in these villages and the zoonotic nature of this bacterium, measures should be taken to prevent the transmission of *B. abortus* to humans.

Results of this study highlight the need to raise awareness for brucellosis control and to design control measures that could be implemented to fight this neglected zoonotic disease. While waiting for investigations that will enable to identify the risk factors responsible for the transmission of brucellosis in villages showing high *Brucella* infection rates, the feeding and management systems must be improved; the sharing of common grazing areas as well as the mixing of animals of different herds must be limited because these factors have been recognized elsewhere as risk factors for brucellosis transmission [51, 52, 53].

The main limitations of this study rely on the fact that no *Brucella* species was isolated and characterized. In consequence, the genetic structure of *Brucella* circulating within and between the two localities remains unknown. Moreover, all *Brucella* species for which cattle and sheep are susceptible have not been investigated and consequently, other *Brucella* species that could be found in animals of Yoko and the Noun division remain also unknown. With these limitations, the transmission dynamics within and between villages, and also between different animal species is not well understood for efficient planning of control operations against brucellosis.

Conclusion

This study revealed the presence of *B. melitensis* and *B. abortus* in cattle and sheep of villages of Yoko and the Noun division of southern Cameroon. Results of this study enabled to identify villages showing high risk for *Brucella* infections and where control operations could be implemented. They highlight the need of developing control measures to fight *Brucella* infections. The identification of *B. melitensis* and *B. abortus*, which are zoonotic bacteria, suggests investigations on human brucellosis, especially in villages showing high risk for *Brucella* infections. Such investigations could help to design control measures for both human and animal brucellosis.

Declarations

Ethics approval and consent to participate

The protocol of this study was approved by the Ministry of livestock, fisheries and animals Industries of Cameroon with the reference number Ref N°015/16/L/DDEPIA.NN. The local administrative and traditional authorities of each sampling site were also informed and gave their approval. Subsequently, the review board of the molecular parasitology and entomology subunit of the Department of Biochemistry of the Faculty of Science of the University of Dschang gave its approval. Verbal consent was obtained from each owner, after detailed explanation of the objective of the study.

Consent for publication

Not applicable

Availability of data and materials

All data generated and/or analyzed are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KNRM helped in the sample collection, identification of *Brucella* species and the drafting of the manuscript. SAB participated in the collection of samples, the study design and the drafting of the manuscript. FO helps in the collection of samples. GS participated in the conception, data collection and the drafting of the manuscript. All authors read and approved the final version of the manuscript.

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Tables

Table 1: Prevalence of *Brucella abortus* and *Brucella melitensis* according to animal species

Animal species	N	<i>Brucella</i> genus (%)	95% CI	<i>B. abortus</i> (%)	95% CI	<i>B. melitensis</i> (%)	95% CI
Cattle	597	125 (20.93)	17-24.95	112 (18.76)	15.45-22.57	10 (1.67)	0.08-3.08
Sheep	315	34 (10.79)	7.45-15.08	6 (1.9)	0.06-4.14	19 (6.03)	3.63-9.41
Total	912	159 (17.43)	14.83-20.36	118 (12.93)	10.71-15.49	29 (3.17)	2.13-4.56
X ²		14.71		51.98		12.73	
P value		0.0001*		0.0001*		0.0004*	

N: number of animal tested; CI: Confidence interval; (%): *Brucella* infection rate; *significance difference

Table 2: Prevalence of *Brucella abortus* and *Brucella melitensis* according to sampling sites and villages

sites	Villages	N	<i>Brucella</i> ssp (%)	95% CI	<i>B. abortus</i> (%)	95% CI	<i>B. melitensis</i> (%)	95% CI
Yoko	Lena	50	5(10)	3.24-23.33	3 (10)	1.23-17.53	2 (4)	0.48-14.44
	Ngoun	100	17 (17)	9.9-27.22	14 (17)	7.65-23.49	3 (3)	0.06-8.76
	Yoko wankou	40	6 (15)	5.5-32.65	5 (12.5)	4.06-29.17	1 (2.5)	0.00-13.92
	Megan	70	5 (7.1)	2.31-66	3 (4.2)	0.08-12.52	0 (0.00)	/
	Kong	17	4 (23.52)	6.41-60.24	4 (23.52)	6.41-60.24	0 (0.00)	/
	Kounde	71	6 (8.4)	3.1-18.39	4 (7.04)	1.53-14.42	2 (2.81)	0.03-10.17
	Total	348	43 (12.35)	8.9-16.64	33 (9.48)	6.52-13.31	8 (2.29)	00.99-4.53
	x ²		5.52		10.84		3.002	
Noun			P value	0.35	0.05		0.70	
	Foumban	73	21 (28.76)	17.81-43.97	15 (20.54)	11.5-33.89	4 (5.47)	1.4-14.03
	Foumbot	56	11(19.64)	9.81-35.15	9 (16.07)	7.35-30.51	1 (1.78)	0.00-9.94
	Njimom	46	9 (19.56)	7.71-34.27	6 (13.04)	4.79-28.39	1 (2.17)	0.00-12.11
	Massangam	62	9 (14.51)	6.64-27.56	6 (9.6)	3.55-21.06	1 (1.61)	0.00-8.98
	Magba	98	28 (28.57)	18.99-41.29	21 (21.42)	13.26-32.76	9 (9.18)	4.19-17.43
	Malentouen	51	0 (0.00)	/	0 (0.00)	/	0 (0.00)	/
	Bangourain	71	17 (23.94)	13.95-38.34	13 (18.30)	9.75-31.31	2 (2.81)	0.03-10.17
	Kouptamo	56	9 (16.07)	7.35-30.51	7 (12.5)	5.03-25.75	1(1.78)	0.00-9.94
	Koutaba	51	12 (23.52)	12-41.1	8 (15.68)	6.77-30.91	2 (3.92)	0.00-10.92
	Total	564	116 (20.56)	17-24.67	85 (15.07)	12.04-18.64	21 (3.71)	2.30-5.69
	x ²		22.96		16.34		13.17	
	P value		0.003*		0.04*		0.106	
Total		912	159 (17.43)	14.83-20-36	118 (12.93)	10.71-15.49	29 (3.17)	2.13-4.56
X ²			10.07		5.96		1.42	
P value**			0.0015*		0.014*		0.23	

N: number of animal tested; CI: Confidence interval; (%): *Brucella* infection rate; *significance difference; **: P values resulting from the comparison of infection rates between Yoko and Noun.

Figures

Figure 1

Map showing villages where animals were sampled