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

Kamga Ndefo Rolin Mitterran
University of Dschang
Silatsa Assongo Barberine
Universite de Dschang
Farikou Oumarou
University of Bamenda
Gustave Simo (gsimoca@yahoo.fr)
Universite de Dschang https://orcid.org/0000-0002-0449-818X

Research article

Keywords: Cattle, sheep, B. abortus, B. melitesis, Cameroon

Posted Date: July 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-21575/v2

Abstract

Background: Brucellosis is an infectious disease caused by bacterial of the genus *Brucella*. Although investigations have identified *Brucella* antibodies in many animal species, little attention has been paid towards specific identification of different *Brucella* species in animals in Sub-Saharan African countries. This study was designed to identify *Brucella abortus* and *Brucellla 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 from 597 cattle and 315 sheep from 15 villages of two regions of Cameroon. DNA was extracted from blood samples and primers amplifying the bcsp31 gene locus were used for the identification of *Brucella spp* infections in these animals. Amongst animals found with *Brucella spp* infections, specific primes were used to identify *B. abortus* and *B. melitensis* respectively.

Results: Of the 912 animals analyzed in this study, 159 (17.4%) were infected with *Brucella* spp. This gives a *Brucella* infection rate of 20.9% in cattle and 10.8% in sheep. Of the 159 animals harboring *Brucella spp* infections, 119 (74.8%) were infected by *B. abortus* and 35 (22.0%) by *B. melitensis*. The overall infection rates were 18.4% (110/597) for *B. abortus* and 1.7% for *B. melitensis* in cattle; 2.9% for *B. abortus* and 7.9% for *B. melitensis* in sheep. Co-infections of *B. abortus* and *B. melitensis* were found in 9 (1%) animals; 6 (1%) cattle and 3 (1%) sheep. The *Brucella* infection rate was significantly higher in animals from the Noun division (20.6%) compared to those of Yoko (12.4%). Between animal species, significant differences *(P = 0.0001)* were observed in infection rates of both *abortus* and *B. melitensis*. Within and between sampling sites, significant differences *(P = 0.02)* were observed in the *B. abortus* infection rates.

Conclusion: This study revealed *B. melitensis* and *B. abortus* infections in cattle and sheep from the Noun and Yoko divisions in 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.

Introduction

Brucellosis is one of the most widespread zoonotic infectious diseases in the world [1]. It is amongst the top thirteen neglected zoonotic and contagious diseases [2]. *Brucella* infections are also considered as one of the major threat for livestock production in developing countries [3,4,5]. The economic losses resulting from *Brucella* infections in livestock can be estimated to about 427 million US$ per year in sub-Saharan African countries [6]. Livestock brucellosis has therefore detrimental socioeconomic effects in vulnerable low-income communities and the health of rural population [7].

Brucellosis is caused by bacteria of the genus *Brucella* which contains twelve species [8,9,10]. Amongst the classical *Brucella spp*, *B. abortus* and *B. melitensis* are of paramount zoonotic importance worldwide, with small ruminants and cattle serving as preferential hosts [11]. Human and several animal species including cattle, small ruminants, pigs, rodents and carnivores have been reported to be infected with *Brucella* [8,12]. In animals, *Brucella* infections induce abortion, infertility and decreases milk production, while in humans, they cause long debilitating illnesses [6]. Although human and various domestic animals can be infected by different *Brucella* species, little attention has been paid towards *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) and complement fixation test [12, 13-17]. Although these serological tests have generated epidemiological data on the sero-prevalence of *Brucella* antibodies in humans and domestic animals from several sub-Saharan countries, these tests cannot differentiate different *Brucella* species [9,13-16]. Results generated by these serological tests are of limited epidemiological values. For instance, the vertebrate host species of different *Brucella* species remain poorly characterized. Moreover, the distribution, transmission dynamics and the prevalence of different *Brucella* species remain under-investigated in most sub-Saharan countries.

Accurate identification of *Brucella* species in different hosts is of great importance for updating our epidemiological knowledge of brucellosis. Although culture and isolation of *Brucella* have been considered as the gold standard methods for brucellosis diagnosis [18], these methods are tedious, time consuming and difficult to implement in developing countries [19]. To overcome these challenges, molecular tools have been developed to identify different *Brucella* species in naturally infected hosts [20, 21,22]. These molecular tools appear reliable, simple, easy to perform and able to identify natural infections of different *Brucella* species [12, 20-25]. Their use in West and East Africa has allowed for the identification and characterization of different *Brucella* species. In Zimbabwe, Uganda and Tanzania for instance, molecular tools have been used for the identification and the characterization of *Brucella abortus* and *Brucella melitensis* from cattle, sheep and pig [12, 24-27]. In central Africa, most studies on brucellosis have been focused on serological diagnosis of *Brucella* [14,15,28-30]. Consequently, the *Brucella* species that infects humans and different animal species remain unknown. Species-specific identification of *Brucella* species would undoubtedly shed light on the circulation and spread of different *Brucella* species. Furthermore, such investigations would allow for the establishment and the implementation of control strategies against brucellosis in each setting.

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

Methods

Study zone

This cross-sectional study was conducted in nine villages (Foumban, Foumbot, Njimom, Massangam, Magba, Malentuen, Koutaba, Bangourain and Kouptamo) of the Noun division and six villages (Lena, Ngoun, Yoko Wankou, Megan, Kong and Kounde) of Yoko. The first survey was performed in November 2017 at Yoko and the second 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 (Figure 1). It is situated between the southern green part and the northern Sahelian part of the country and covers about 15 000 km^2. Yoko is known for large scale cattle and small ruminant's production within the center region of Cameroon. The presence of pastures and water favors transhumance of herds not only from the West, East and Adamawa regions of Cameroon, but also from neighboring countries like the Central Africa Republic [31, 32].

The Noun division (4°95' 6°30'N; 10°30'12°E) is located in the western highlands of Cameroon. It covers about 7687 km^2 with a vegetation characterized by the savannah and degraded forest (Figure 1). The Noun division is considered as the main cattle production area of the west region of Cameroon [30]. Animals in this locality move to other regions (Northwest, Center and Adamawa) of Cameroon for trade or transhumance.

In these two localities, the feeding system is essentially free grazing which is sometime associated to stall-feeding. Various animal species including goats, sheep and cattle share the same environment.

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

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 [33].

See formula 1 in the supplementary files.

where i) N is the minimum sample size required; ii) Z (critical value for a given confidence interval) = 1.96; iii) P (expected prevalence) = 0.052; and iv) L (margin of error) = 0.05.

For this estimation, the bovine brucellosis prevalence of 5.2% previously reported in the North West Region of Cameroon was used [30]. Additionally, a 95% confidence level and a precision of 5% were also used to determine the sample size. 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 each cattle to be sampled in each chosen herd was done on the basis of systematic random sampling technique as described by Asgedom *et al.* [34]. A total of 37 farms were selected in 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.* [35]. Briefly, frozen samples were thawed and 500µL of whole blood was 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) was 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 1.5 ml micro-tube. DNA was precipitated with 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 *etal*. [23]. This was done using B4 (5'-TGGCTCGGTTGCCAATATCAA-3') and B5 (5'-CGCGCTTGCCTTTCAGGTCTG-3') primers that amplify a 223 bp DNA fragment corresponding to the bcsp31 gene locus of *Brucella genus*. 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 $MgCl_2$, 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 comprised 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 performed 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. The gel was stained with ethidium bromide and DNA bands were visualized under ultraviolet light and photographed. Each PCR positive sample showing an amplicon of approximately 223 bp was considered as positive or having bacteria of the genus *Brucella* (Figure 2). These samples were selected and subsequently subjected to the identification of different *Brucella* species.

Specific identification of different *Brucella* species

All samples that were positive for the bcsp31 gene locus of *Brucella* genus (amplification of 223 bp DNA fragment) were further analyzed to determine the specific infecting *Brucella* species. This identification was done using AMOS PCR as described by Bricker and Halling [36]. 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 (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 $MgCl_2$, 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 comprised an initial denaturation step at 95°C for 5 minutes followed by 40 cycles. Each cycle comprised a denaturation step at 95°C for 30 s, an annealing step at 53°C for 30 s and an extension step at 72°C for 1 min. This was followed by 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 an amplicon size of 495 bp were considered as harboring *B. abortus* while those with 730 bp were considered as positive for *B. Melitensis* (Figure 3).

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. Significance threshold was set at 0.05

Results

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 (Figure 2); giving a *Brucella* infection rate of 17.4% (159/912): 12.4% (43/348) at Yoko and 20.6% (116/564) in the Noun division. This infection rate was significantly (*P = 0.0015*; X^2 = 10.07) higher in the Noun division compared to Yoko (Table 2). The highest infection rate was observed at Foumban (28.8%; 21/73), followed by Magba (28.6%; 28/98) and Bangourain (23.9%; 17/71) (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 differences *(P = 0.03;* X^2 = 22.96) were observed in the overall *Brucella* infection rates. In the Yoko Division, the highest *Brucella* infection rate of 23.52% (4/17) was observed in animals from Kong and the lowest rate of 7.1% (5/70) in those from Megan. Between villages of Yoko, no significant difference (*P = 0.2*; X^2 = 7.22**)** was observed in the overall *Brucella* infection rates.

The *Brucella* infection rates were 20.9% [95%CI: 17-24.95] in cattle and 10.8% [95%CI: 7.45-15.08] sheep (Table 1). This infection rate was significantly higher (*P< 0.0001*; X^2 = 14.71) in cattle (20.9%; [95%CI: 17.43–24.95]) than sheep (10.8%; [95%CI: 7.45-15.08]) (Table 1).

Prevalence of *Brucellaabortus* according to animal species and sampling sites

Of the 159 samples found with *Brucella* infections, 145 (91.2%) were successfully amplified by AMOS-PCR that targets *B. abortus* and *B. melitensis* (Figure 3). From all *Brucella* positive samples, 14 (8.8%) samples were not amplified by the AMOS-PCR and could thus not be identified as *B. abortus* or *B. melitensis*. Amongst the 145 samples that were amplified by AMOS-PCR, 114 (78.6%) were from cattle and 31 (21.4%) from sheep. Amongst the 159 animals harboring *Brucellaspp* infections, 119 (74.8%) were infected by *B. abortus* and 35 (22.0%) by *B. melitensis*.

From the 125 cattle samples found with *Brucella* infections, AMOS-PCR successfully amplified 114 (91.2%) of them. The infecting *Brucella* species could not be identified in the remaining 11 samples that were not amplified by AMOS-PCR. Amongst the 114 samples amplified by AMOS-PCR, 110 (96.5%) were found with *B. abortus* infections. This gives an overall *B. abortus* infection rate of 18.4% (110/597) in cattle.

For the 315 sheep analyzed in this study, 34 (10.8%) were found with *Brucella* infections. Amongst these 34 sheep samples, 31 (91.2%) were amplified by AMOS-PCR. The infecting *Brucella* species could not be also identified in the 3 samples that were not amplified by this PCR. Of the 31 sheep samples that were positive for AMOS-PCR, 9 (29.03%) were found with *B. abortus* infections; giving thus an overall *B. abortus* infection rate of 2.9% (9/315) in sheep. The *B. abortus* infection rate was significantly (*P<0.0001*; X^2= 46.78) higher in cattle than sheep (Table 1).

Between villages of each sampling site, significant differences (*P<0.03*, X^2=11.98 at Yoko; P< *0.04*, X^2=11.94 in the Noun division) were observed in the *B. abortus* infection rates (Table 2). This difference was also significant (*P<0.04*, X^2=5.96) between Yoko and the Noun division (Table 2). The villages found with high number of animals harboring *B. abortus* infections were Magba (20.40%), Foumban (19.7%), Bangourain (18.30%) and Koutaba (15.68%) in the Noun division and Kong (23.52%) at Yoko. The lowest *B. abortus* infection rates were reported at Malentouen (0.00%) in the Noun division and Lena (4%) at Yoko (Table 2).

Prevalence of *Brucellamelitensis* according to animal species and sampling sites

From the 159 samples that were found with *Brucella* infections, the AMOS-PCR successfully amplified 145 of them and revealed *B. melitensis* infections in 35 (22.01%; 35/159) samples. This gives an overall *B. melitensis* infection rate of 3.8% (35/912). Infections due to *B. melitensis* were identified in 10 cattle and 25 sheep. *B. melitensis* infection rates were 1.7% (10/519) in cattle and 7.9% (25/315) in sheep. This infection rate was significantly higher (*P<0.0008*; X^2=29.62) in sheep compared to cattle (Table 1).

Between villages, no significant difference (*P=0.6*; X^2= 0.26) was observed in the *B. melitensis* infection rates within and between sampling sites. Nonetheless, the highest *B. melitensis* infection rate of 6.52% (3/46) was observed in animals from Njimom in the Noun division (Table 2).

Of the 145 samples that were amplified by AMOS-PCR, single and mixed infections of *B.abortus* and *B.melitensis* were respectively observed in 136 (93.8%; 136/145) and 9 (6.2%; 9/145) samples. Amongst the 136 single infections, 110 (80.88%) were attributed to *B.abortus* and 26 (19.12%) to *B.melitensis* (Table 1). The overall single infection rate was 14.9% (136/912). Single infections were found in 18.4% (110/597) of cattle and 8.3% (26/315) of sheep. Co-infections of *B. abortus* and *B. melitensis* were found in 9 animals; giving an overall co-infection rate of 1.0% (9/912). Six (1%; 6/597) cattle and 3 (1%; 3/315) sheep were co-infected by *B. abortus* and *B. melitensis* (Table 1). Summing single and mixed infections, the infection rates of *B.abortus* and *B. melitensis* were 13.05% (119/912) and 2.83% (35/912), respectively (Table 1).

Although single infections were found in cattle and sheep of most villages of the two sampling sites, co-infections of *B. abortus* and *B. melitenis* were detected in animals of Lena, Ngoun and Yoko wankou of Yoko and, Foumban, Magba, Bangourain and Koutaba of the Noun division (Table 2).

***Brucella* antibodies detection in sample analyzed**

The 912 samples used in the present study have previously been characterized by two serological tests (Rose Bengal test and the enzyme linked immunosorbent assay) as part of a more comprehensive large scale geographical study. Results of these investigations have been recently published by Kamga et al. [28]. Serological test data for animals from the Noun and Yoko division were extracted from the published data, analyzed and compared with molecular identification of *Brucella* infections. The overall sero-prevalence of *Brucella* antibodies was 13.4% (122/912) for the two localities: 10.1% (35/348) at Yoko and 15.4% (87/564) in the Noun division.

Results of *Brucella* antibodies detection and molecular identification of *Brucella* species were compared in the present study to find the strength of agreement between serological and molecular tests (Table 3). Of the 912 samples that were simultaneously analyzed by serological tests and PCR-based test, concordant results were obtained for 841 (92.21%) samples; 736 (80.70%; 736/912) and 105 (11.51%; 105/912) samples were respective negative and positive for both tests. Discordant results were obtained for 71 (7.9%; 71/912) samples; 17 (1.9%; 17/912) samples were positive for serological tests and negative for PCR-based test while 54 (5.92%; 54/912) samples were positive for PCR-based tests and negative for serological tests. The statistical analysis comparing the performance of serological and PCR-based tests revealed a Kappa Cohen coefficient of 0.70 with a significant *p* value ($p < .0001$); highlighting a good strength of agreement between serological and molecular tests.

Discussion

In most sub-Saharan African countries, investigations undertaken on brucellosis have generated epidemiological data that helped to understand the sero-prevalence of *Brucella* antibodies in humans and animals. However, it is well-established that the dominance and overlapping nature of the C epitope of smooth *Brucellae* that is widely used in serological tests does not enable to ascertain the infecting *Brucella* species, [37]. To fill the gap linked to specific identification of *Brucella* species, molecular tools have been used to determine the infection rates of *B. abortus* and *B. melitensis* in cattle and sheep of southern Cameroon.

Although the *Brucella* infection rate of 17.4% revealed by PCR-based tests is higher than its sero-prevalence (13.37%), the value of Kappa Cohen coefficient of 0.70 indicates a good strength of agreement between serological and molecular tests. Our results are in line with previous results; highlighting perfect concordance between these tests [22, 38, 39]. The animals found with *Brucella* DNA, but in which *Brucella* antibodies could not be detected may be carriers of early infections or with antibodies title bellow the detection threshold of the serological tests used in this study. However, we cannot rule out the fact some of these animals could be immune compromised and unable to seroconvert following exposure to *Brucella* infections. It is also important to point out the fact that serological tests could be negative if *Brucella*, which is an intracellular organism, is hidden in some tissues [38,39,40].

The high *Brucella* infection rates revealed by PCR-based test could be explained by the higher sensitivity of the molecular marker used in this study. Previous studies reported that the bcsp 31 gene amplified here for the detection of bacteria of the genus *Brucella* has higher sensibility and specificity compared to other markers like omp 2, Omp28, 16sRNA [41, 42]. Indeed, PCR targeting bcsp 31 gene can detect very small amount of *Brucella* DNA that was extracted from blood [39, 23, 43]. It appears therefore as an appropriate molecular tool for the detection of *Brucella* infections in blood samples.

Our results showing high *Brucella* infection rate in cattle (20.93%) compared to sheep (10.79%) is consistent with results reported in South Africa [26]. The difference observed could be explained by the larger herd sizes of cattle and the management system [4, 44]. The extensive cattle management system and the larger size of herds have been reported as risk factors for brucellosis [45]. Moreover, the relative short life expectancy of sheep compared to cattle is 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 strengthened by observations reporting that as long as infected animals remain in contact with the rest of the herd, the number of infected animals will increase [13, 46, 47, 48].

The identification of *B. abortus* and *B. melitensis* in both cattle and sheep is consistent with results obtained in the South and East Africa [12, 24, 26, 38]. Remarkably, *B. abortus* infection rate was significantly higher (*P <0.0001*; X^2= 46.78) in cattle (17.42%) compared to sheep (1.9%) while *B. melitensis* was significantly (*P =0.0008*; X^2= 29.62) more prevalent in sheep. 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 [49]. Our results showing high *B. abortus* infection rate in cattle are in agreement with data of other African countries [12, 20, 25, 50]. In fact, *B. abortus* has been reported as the most commonly *Brucella* species that has been isolated and characterized in cattle from sub-Saharan countries [12, 16, 50]. The high infection rate of *B. melitensis* in sheep is consistent with results of most sub-Saharan countries [16]. These results indicate that the epidemiological patterns of some *Brucella* species could be similar in sub-Saharan countries. Compared to *B. abortus,* the low *B. melitensis* infection rate reported in domestic animals suggests its low transmission in sub Saharan countries [51]. The small size of sheep' herd is another factor playing for the low transmission of *B. melitensis* and consequently, its low infection rate in domestic animals [14, 30].

The identification of *B. abortus* in sheep and *B. melitensis* in cattle is not common because these animals are not their preferential hosts. Nevertheless, it is important to point out that several *B. abortus* isolates have been obtained from milk and abortion products of sheep and goat [52, 53]; 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 [54, 55]. The detection of *B. melitensis* in cattle is in agreement with previous observations reporting the transmission of *B. melitensis* to cattle [56, 57]. 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. In such context, the transmission of *B. melitensis* to cattle and *B. abortus* to sheep can 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 [56, 57]. Indeed, inter and intra-specific transmissions of different *Brucella* species can occur in larger herds and livestock production system where cattle, goat and sheep are put together [4, 45, 29]. Animals found with *Brucella* infections, but for which *B. abortus* and *B. melitensis* could not be identified may be carriers of bacteria load below the sensitivity threshold of AMOS-PCR. Some of these animals could be infected by other *Brucella* species like *B. ovis* which is a non-zoonotic bacteria species that is restricted to sheep. Although not investigated in the present study, this *Brucella* species has been already reported in sub-Saharan countries [58, 59, 60].

The significant difference in the *Brucella* infection rates reported between villages of the Noun division; but not at Yoko highlights some variations occurring within and between sampling sites. This difference results probably from environmental factors and/or livestock management system that vary between villages and that have impacts on the transmission pattern of *Brucella* species. Within and between sampling sites, our results showing no significant difference (*P =0.6*; X^2=0.26) in the infection rates of *B. melitensis* could be explained by the low size of herds and the limited movement of sheep. In such context, 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. For *B. abortus* where the infection rates vary significantly (*P =0.02*; X^2=5.96) between villages of the two localities, it is likely that high transmission of *B. abortus* occur in some villages like Kong in Yoko, Foumban, 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 [29,31, 61]. 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 risk factors linked to brucellosis transmission 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 [62, 63, 64].

The main limitations of this study rely on the fact that no *Brucella* species was isolated and characterized and specific identification of *Brucella* species was based on band size. Consequently, 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 also remain unknown. With these limitations, the transmission dynamics within and between villages, and also between different animal species remains 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 from the villages of the Yoko and Noun division of southern Cameroon. Results of this study have allowed for the identification of villages with high risk of *Brucella* infections and where control operations could be implemented. They highlight the need for 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.

Funding

This project received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The Molecular Parasitology and Entomology subunit of the Faculty of Science University of Dschang of Cameroon provided reagents required for lab experiments that scheduled in this project.

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.

Acknowledgements

We are grateful for the support of inhabitants of different localities as well as the staff of different divisional delegations of the Ministry of livestock, fisheries and animals Industries of Cameroon. We also thank the anonymous reviewers for helpful comments on the manuscript

Authors' Information

[1]: Molecular Parasitology and Entomology Unit, Department of Biochemistry, Faculty of Science, University of Dschang, PO. Box 67, Dschang, Cameroon.
[2]: University of Bamenda, Cameroon.

Abbreviations

RBPT: Rose Bengal Plate Test; ELISA: Enzyme Linked Immunosorbent assay; PCR: Polymerase chain reaction; CI: confidence interval; CTAB: cetyl trimethylammonium bromide; Omp: Outer membrane protein; BCSP31: *Brucella* cell surface protein 31; AMOS PCR: *abortus*, *melitensis*, ovis and *suis* polymerase chain reaction; EDTA: Ethylene diamine tetraacetic acid; dNTP: Deoxyribonucleotide trisphosphate; DNA: Deoxyribonucleic acid

References

1. Corbel M, Leberg S, Cosivi O. Brucellosis in humans and animals. Geneva: WHO/CDS/EPR/2006.7:Available:http://www.who.int/csr/resources/publications/Brucellosis.pdf. Accessed: 15 february 2020.

2. World Bank, 2011. World Livestock Disease Atlas: A Quantitative Analysis of Global Animal Health Data (2006-2009). pp. 11. Available: http://documents.worldbank. org/curated/en/323671468179364909/World-livestock-disease-atlas-aquantitative-analysis-of-global-animal-health-data-2006-2009. Accessed: 15 february 2020.
3. Corbel, M.J. Brucellosis: an overview. *Emerging Infectious Diseases*. 1997; 3: 213-221.
4. Ariza, Javier, Mile Bosilkovski, Antonio Cascio, Juan D. Colmenero, Michael J. Corbel, Matthew E. Falagas, Ziad A. Memish, Mohammad Reza, Hasanjani Roushan, Ethan Rubinstein, Nikolaos V Sipsas, Javier Solera, Edward J. Young, and Georgios Pappas. Perspectives for the Treatment of Brucellosis in the 21st Century : The Ioannina Recommendations. *PLoS Med.* 2007; 4(12): 4 e317.
5. Nielsen, K. and Yu WI. serological diagnosis of brucellosis. *Contributions, Sec. Biol. Med. Sci.* 2010; 89: 65–89.
6. Mangen, M.J., Pfeiffer, D., Chilonda, P. Bovine Brucellosis in Sub-Saharan Africa: Estimation of Meat and Milk off Take Potential. 2002; Available: www.fao.org/3/a-ag274e.pdf. Accessed: 15 february 2020.
7. Godfroid J., Scholz,H., Barbier, T.,Nicolas,C., Wattiau, P.,Fretin, D.,& Moriyon, I. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive Veterinary Medicine*. 2011; 102(2): 118–131.
8. Hull NC and Schumaker BA. Comparisons of brucellosis between human and veterinary medicine. *Infect Ecol Epidemiol*. 2018; 8(1):1500846.
9. Akakpo, A. J., A. Têko-Agbo, and P. Koné. The Impact of Brucellosis on the Economy and Public Health in Africa. *Compendium of Technical Items Presented to the OIE World Assembly of Delegates or to OIE Regional Commissions.* 2010; 71–84.
10. Michaux-charachon, Sylvie, Vincent Foulongne, David O. Callaghan, and Michel Ramuz. Brucella à l ' Aube Du Troisième Millénaire : Organisation Du Génome et Pouvoir Pathogène. *Pathol Biol.* 2002; 50: 401-412.
11. Poester FP, Samartino LE, Santos RL. Pathogenesis and pathobiology of brucellosis in livestock. *Rev Sci Tech*. 2013; 32(1):105–115.
12. Assenga, Justine A., Lucas E. Matemba, Shabani K. Muller, Joseph J. Malakalinga, and Rudovick R. Kazwala. Epidemiology of Brucella Infection in the Human , Livestock and Wildlife Interface in the Katavi-Rukwa ecosystem, Tanzania. *BMC Veterinary Research.* 2015;11: 1–11.
13. Madut, Nuol Aywel, Adrian Muwonge, George William Nasinyama, Bwalya Muma, Jacques Godfroid, Ambrose Samuel Jubara, James Muleme, and Clovice Kankya. The Sero-Prevalence of Brucellosis in Cattle and Their Herders in Bahr El Ghazal Region , South Sudan. *BMC Infectious Diseases.* 2018; 19:450.
14. Awah-Ndukum, J., Mouiche, M.M., Bayang, H.N., Ngu Ngwa, V., Assana, E., Feussom, K.J.M., Manchang, T.K., & Zoli, P.A. Factors of Brucellosis among Indigenous Cattle in the Adamawa and North Regions of Cameroon. *Journal of Hindawi Veterinary Medicine International*, 2018. https://doi.org/10.1155/2018/3468596
15. Awah-ndukum, Julius, Mohamed Moctar, Mouliom Mouiche, Lucy Kouonmo-ngnoyum, Houli Nicolas Bayang, Tanyi Kingsley Manchang, Rodrigue Simonet, et al. Seroprevalence and Risk Factors of Brucellosis among Slaughtered Indigenous Cattle , Abattoir Personnel and Pregnant Women in Ngaoundéré , Cameroon. *BMC Infectious Diseases.* 2018; 18: 611 1–13.
16. Ducrotoy, M., Bertu, W.J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A.M., Welburn, S., Ocholi, R., Blasco, J.M., & Moriyón, I. Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta Tropica*. 2017; 165: 179-193.
17. Mazeri S., Scolamacchia F., Handel, Kenton, I.G., Morgan, L.V.N., Tanya, B.M., & De Bronsvoort, C. Risk factor analysis for antibodies to *Brucella*, Leptospira and C. burnetii among cattle in the Adamawa Region of Cameroon: a cross-sectional study. *Tropical Animal Health and Production.* 2012; 45: 617–623.
18. Bovine brucellosis, In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 2013; pp.1–35.
19. Kaynak-Onurdag, F; Okten, S and Sen, B. Screening Brucella spp. in bovine raw milk by real-time quantitative PCR and conventional methods in a pilot region of vaccination, Edirne, Turkey. *Dairy Sci.* 2016; 99: 3351-3357.
20. Karthik, K., P. Thomas, Elamurugan Appavoo, Tamil Nadu Veterinary, and Arun Remesh. Serological and Molecular Detection of Brucella Abortus from Cattle by RBPT , STAT and PCR , and Sample Suitability of Whole Blood for PCR. *Asian Journal of Animal and Veterinary Advances*. 2014; 9 (4): 352-359.
21. Wang, Ying, Zhanli Wang, Yaxian Zhang, Liyun Bai, Yue Zhao, Chunfang Liu, An Ma, and Hui Yu. Polymerase Chain Reaction – Based Assays for the Diagnosis of Human Brucellosis. *Annals of Clinical Microbiology and Antimicrobials* 2014;13 (31): 1–8.
22. Mahajan, V.; Banga, H. ; Filia, G.; Gupta, M. P. and Gupta, K. Comparison of Diagnostic Tests for the Detection of Bovine Brucellosis in the Natural Cases of Abortion. *IJVR*, Vol. 2017; 18 (3): 183-189.
23. Mitka, Stella, Constantine Anetakis, Efimia Souliou, Eudoxia Diza, Athina Kansouzidou, and J. C. L. I. N. M. Icrobiol. Evaluation of Different PCR Assays for Early Detection of Acute and Relapsing Brucellosis in Humans in Comparison with Conventional Methods. *journal of clinical microbiology*. 2007; 45 (4):1211–1218.
24. Hoffman, Tove, Kim Rock, Denis Rwabiita, and Shaman Muradrasoli. Molecular Detection and Characterization of Brucella Species in Raw Informally Marketed Milk from Uganda. *Infection Ecology and Epidemiology.* 2016; 6: 32442
25. Khamesipour, Faham, Abbas Doosti, and Shahrekord Branch. Molecular Detection of Brucella Spp. in the Semen, Testis and Blood Samples of Cattle and Sheep Molecular Detection of Brucella Spp . in the Semen , Testis and Blood Samples of Cattle and Sheep. *journal of pure and applied microbiology.* 2013; 7: 495-500.
26. Caine Lesley-Anne, Uchechukwu Uchechukwu Nwodo, Anthony Ifeanyin Okoh, Ezekiel Green. Molecular characterization of Brucella species in cattle, sheep and goats obtained from selected municipalities in the Eastern Cape, South Africa. *Asian Pac J Trop Dis* 2017; 7(5): 293-298.

27. Betty, Maphuti, Ledwaba Id, Calvin Gomo, Kgaugelo Edward Lekota, Philippe Le, Ayesha Hassim, Gilles Vergnaud Id, and Henriette Van Heerden. Molecular Characterization of Brucella Species from Zimbabwe. *PLoS Negl Trop Dis.* 2019; 13(5): e0007311.
28. Kamga Rolin M., Barberine Silatsa A., Farikou Oumarou, Jules-Roger Kuiate and Gustave Simo. Detection of Brucella antibodies in domestic animals of southern Cameroon: implications for the control of brucellosis. V*et Med Sci.* 2020; 00:1–11.
29. Musallam, Imadidden, Andrée Prisca, Damitoti Yempabou, Cha-ah Crystella Ngong, Müller Fotsac, Mohamed-moctar Mouiche-mouliom, Jean Marc, Kameni Feussom, Jean Bosco, Désiré Ntakirutimana, Adama Fane, Elisabeth Dembele, Adama Doumbia, Amah Akpemdo, Paul-henri Siméon Ayih-akakpo, Pindemnewe Pato, Magnoudewa Pali, Arnaud S. R. Tapsoba, Germaine Minougou, Haladou Gagara, Aïda Issaka, Bhagyalakshmi Chengat, Laura Craighead, Emi Takahashi, John Mcgiven, Patrick Nguipdop-djomo, Punam Mangtani, Rrianatou Alambédji-bada, Ayayi Justin, and Javier Guitian. Acta Tropica Brucellosis in Dairy Herds : A Public Health Concern in the Milk Supply Chains of West and Central Africa. *Acta Tropica.* 2019; 197:105042.
30. Bayemi, P. H., G. D. Mah, K. Ndamukong, V. M. Nsongka, I. Leinyuy, H. Unger, N. M. Ndoumbe, E. C. Webb, M. D. Achukwi, F. Hakoue, and N. D. Luogbou. Bovine Brucellosis in Cattle Production Systems in the Western Highlands of Cameroon. *Int J Animal Biology*. 2015; 1(2):38–44.
31. Motta, Paolo, Thibaud Porphyre, Saidou M. Hamman, Kenton L. Morgan, Victor Ngu Ngwa, Vincent N. Tanya, Eran Raizman, Ian G. Handel, and Barend Mark Bronsvoort. Cattle Transhumance and Agropastoral Nomadic Herding Practices in Central Cameroon. *BMC Veterinary Research*. 2018;14: 214.
32. Seignobos, C. La Question mbororo. Réfugiés de la RCA au Cameroun, Yaoundé/Paris (HCR/SCAC/IRD) 2008.
33. Thrusfield M., Blackwell Science Ltd, a Blackwell, *Veterinary epidemiology*, Oxford, UK 2007; 610.
34. Asgedom H., D. Damena, and R. Duguma. Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia, *SpringerPlus*. 2016; 5 (1):
35. Navajas, M., Lagnel, J., Gutierrez, J., Bourset, P. Species wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite Tetranychus urticae contrasts with extensive mitochondrial COI polymorphism. *Heredity*. 1998; 80: 742–752.
36. Bricker, Betsy J. and Shirley M. Halling. Melitensis , Brucella Ovis , and Brucella Suis Bv . 1 by PCR. *J Clin Microbiol.* 1994; 32 (11): 2660–6.
37. Alonso-Urmeneta B., Marin C., Arago N V., Blasco J. M., R. Diaz, And Moriyo N I. (1998). Evaluation of Lipopolysaccharides and Polysaccharides of Different Epitopic Structures in the Indirect Enzyme-Linked Immunosorbent Assay for Diagnosis of Brucellosis in Small Ruminants and Cattle. *Clinical and diagnostic laboratory immunology.*1998; 5 (6) 749–754.
38. Kolo Francis B., Abiodun A. Adesiyun, Folorunso O. Fasina, Charles T. Katsande, Banenat B. Dogonyaro, Andrew Potts, Itumeleng Matle, Awoke K. Gelaw, Henriette van Heerden Seroprevalence and characterization of Brucella species in cattle slaughtered at Gauteng abattoirs, South Africa. *Vet Med Sci.* 2019; 5:545–555.
39. Keid LB, Soares RM, Vasconcellos SA, Chiebao DP. A polymerase chain reaction for detection of Brucella canis in vaginal swabs of naturally infected bitches. *Theriogenol*. 2007; 68:1260–70.
40. Radostits, O. M., Gay, C. C., Hinchcliff, K. W., & Constable, P. D. A textbook of the diseases of cattle, horses, sheep, pigs and goats. *Veterinary Medicine*. 2006; 969–984.
41. Wang, Y.; Wang, Z.; Zhang, Y.; Bai, L.; Zhao, Y.; Liu, C.; Ma, A.; Yu, H. Polymerase chain reaction-based assays for the diagnosis of human brucellosis. *Clin. Microbiol. Antimicrob.*2014*;* (13), 31.
42. Manal M. B. and Dalal H. Alkhalifa. Evaluation of three polymerase chain reaction technique for the detection of Brucella DNA in peripheral human blood. *J. Microbiol.* 2008; 54: 352-357.
43. Baily GG, Krahn J B, Drasar B S and Stoker N G. Detection of Brucella melitensis and Brucella abortus by DNA amplification. *J trop med Hyg*. 1992; 95: 271-275.
44. Gul, S.T., Khan, A., Rizvi, F., & Hussain, I. Sero-prevalence of brucellosis in food animals in the Punjab, Pakistan. *Pakistan Veterinary Journal.* 2014; 34: 454-458.
45. Mohamed, Eman Mohamed-ahmed, Abdelhamid Ahemd, Mohamed Elfadil, and Enaam Mohamed El-sanousi. Seroprevalence and Risk Factors of Caprine Brucellosis in Khartoum State , Sudan. *Veterinary World, EISSN*. 2018; 223 (11):511–518.
46. Kaindi, D. W., Schelling, E, Wangoh J. M., Imungi, J. K., Farah, Z. and Meile, L.. Risk factors for symptoms of gastrointestinal illness in rural town Isiolo, Kenya. *Zoonoses Public Health.* 2012; 59 (2):118–125.
47. Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W., Chapter18, Diseases associated with bacteria-lll. In: Veterinary Medicine: A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses, 9th ed. W.B. Saunders Company Ltd., Sydney, 2000; 963–994.
48. Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. Disease caused by Brucella spp. A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses. 10th ed. ELBS Bailliere Tindall, London, UK 2007; 870-871.
49. Kelly, Robert F., Saidou M. Hamman, Kenton L. Morgan, Egbe F. Nkongho, Ngu Ngwa, Vincent Tanya, Walters N. Andu, Melissa Sander, Lucy Ndip, G. Handel, Stella Mazeri, Adrian Muwonge, and Barend M. De C. Bronsvoort. Knowledge of Bovine Tuberculosis , Cattle Husbandry and Dairy Practices amongst Pastoralists and Small-Scale Dairy Farmers in Cameroon. *PLoS ONE.* 2016; 11(1): e0146538.
50. McDermott, J., Grace, D., Zinsstag, J. Economics of brucellosis impact and control in low-income countries. *Sci. Tech*. 2013; 32: 249-261.
51. Acha, N.P., Szyfres, B., Zoonoses and Communicable Diseases Common to Man and Animals, third ed., vol. 1. Pan American Health Organization (PAHO), Washington, DC. 2003.

52. Okoh, A.E. An investigation of abortion in sheep in Rano L.I.B.C. near Kano, Nigeria. *Anim. Health Prod. Afr.* 1980; 28: 135–138.
53. Bertu, W.J., Ducrotoy, M.J., Mu˜noz, P.M., Mick, V., Zú˜niga-Ripa, A., Bryssinckx, W., Kwaga, J.K.P., Kabir, J., Welburn, S.C., Moriyón, I., Ocholi, R.A. Phenotypic and genotypic characterization of Brucella strains isolated from autochthonous livestock reveals the dominance of B. abortus biovar 3a in Nigeria. *Microbiol.* 2015; 180(1-2):103-8.
54. Aparicio, E. Díaz. Epidemiology of Brucellosis in Domestic Animals Caused by Brucella Melitensis , Brucella Suis and Brucella Abortus. *sci. tech. Off. int. Epiz.*, 2013;32 (1): 53-60.
55. Lilenbaum, W., G.N. De Souza, P. Ristow, M. Cortez, M. Cortez Moreira, S. Fráguas, V. da Silva Cardoso, and W. Martin Roland Oelemann. A serological study on Brucella abortus, caprine arthritis–encephalitis virus and Leptospira in dairy goats in Rio de Janeiro, Brazil. *The Vet. J.* 2007; 173: 408-412.
56. Benkirane, A. Ovine and caprine brucellosis: world distribution and control/eradication strategies in West Asia/North Africa region. *Small Rumin. Res.* 2006; 62: 19–25.
57. Refai, M. Incidence and control of brucellosis in the Near East region. *Microbiol.* 2002; 90: 81–110.
58. Van Rensburg, S.W., Van Heerden, K.M., Le Roux, D.J., Snyders, A.J., van Heerden, K.M., Infectious infertility in sheep. *S. Afr. Vet. Assoc.* 1958; 29: 223–233.
59. Cameron, R.D., Carles, A.B., & Lauerman, L.H.J. The incidence of Brucella ovis in some Kenya flocks and its relationship to clinical lesions and semenquality. *Veterinary Record*, 1971; 89: 552–557.
60. Ate, I.U., Bello, A., Nenshi, P.M., Allam, L. Rashidat, M. Foetal maceration associated with Brucella ovis infection in a Yankassa ewe. *REDVET Rev. Electron. Vet.* 2011;12: 1–6.
61. Motta, P., Porphyre, T., Handel, I., Hamman, S.M., Ngu Ngwa, V., Tanya, V., Morgan, K., Christley, R., Bronsvoort, B.M. Implications of the cattle trade network in Cameroon for regional disease prevention and control. *Sci Rep.* 2017; 7: 43932.
62. Fèvre, E.M., Bronsvoort, B.M.d.C., Hamilton, K.A., Cleaveland, S., Animal movements and the spread of infectious diseases. *Trends in Microbiology.* 2006; 14: 125- 131.
63. Awa, Daniel Ndzingu and Mbunkah Daniel Achukwi. Livestock Pathology in the Central African Region : Some Epidemiological Considerations and Control Strategies. *Animal Health Research Reviews.* 2010; 11(2): 235–244.
64. Volkova, Victoriya V, Richard Howey, Nicholas J. Savill, and Mark E. J. Woolhouse. Sheep Movement Networks and the Transmission of Infectious Diseases." *PLoS ONE* 2010; 5(6): e11185.

Tables

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

Animal species	*N*	*Brucella spp* positive (%)	*95% CI*	*Brucella spp* negative (%)	*95% CI*	Single infections				MI (%)	*95% CI*	BN (%)
						B. abortus (%)	95% CI	*B. melitensis* (%)	95% CI			
Cattle	597	125 (20.9)	17-24.95	472 (79.1)	72.09-86.53	104 (17.4)	14.23-21.11	4 (0.7)	0.1-1.71	6 (1.0)	0.3-2.18	114 (19.1)
Sheep	315	34 (10.8)	7.45-15.08	281 (89.2)	79.08-100	6 (1.9)	0.06-4.14	22 (7.0)	4.37-10.57	3 (1.0)	0.19-2.78	31 (9.8)
Total	912	159 (17.4)	14.83-20.36	753 (82.6)	76.77-88.68	110 (12.1)	9.91-14.54	26 (2.9)	1.86-4.17	9 (1.0)	00.45-1.87	145 (15.9)
X^2		14.71				46.78		29.62				
P value		0.0001*				0.0001*		0.0008*				

N: number of animal tested; CI: Confidence interval; (%): *Brucella* infection rate; MI: mixed infections; *significance difference; BN: total number of animals in which *B. abortus* and *B. melitensis* have been identified.

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

sites	Villages	N	*Brucella* ssp positive (%)	95% CI	*Brucella* ssp Negative (%)	*95% CI*	*single infections*				MI (%)	95% CI	BN (%)
							B. abortus (%)	95% CI	*B. melitensis* (%)	95% CI			
Yoko	Lena	50	5 (10.0)	3.24-23-33	45 (90.0)	65.65-100	2 (4.0)	0.48-14.45	1 (2.0)	0.05-11.14	1 (2.0)	00.15-11.14	4 (8.0)
	Ngoun	100	17 (17.0)	9.9-27.22	83 (83.0)	66.22-100	14 (14.0)	7.65-23.49	1 (1.0)	0.02-5.57	1 (1.0)	00.02-5.57	16 (16.0)
	Yoko wankou	40	6 (15.0)	5.5-32.65	34 (85.0)	58.86-100	4 (10.0)	2.72-25.6	1 (2.5)	0.00-13.92	1 (2.5)	0.00-13.92	6 (15.0)
	Megan	70	5 (7.1)	2.31-66	65 (92.9)	71.67-100	3 (4.3)	0.08-12.52	0 (0.0)	/	0 (0.0)	/	3 (4.3)
	Kong	17	4 (23.5)	6.41-60.24	13 (76.5)	40.72-100	4 (23.5)	6.41-60.24	0 (0.0)	/	0 (0.0)	/	4 (23.5)
	Kounde	71	6 (8.4)	3.1-18.39	65 (91.6)	70.66-100	4 (5.6)	1.53-14.42	2 (2.8)	0.03-10.17	0 (0.0)	/	6 (8.5)
	Total	348	43 (12.4)	8.9-16.64	305 (87.6)		31 (8.9)	6.52-13.31	5 (2.3)	00.99-4.53	3 (0.9)		39 (11.2)
	x^2		7.22				11.98		2.78				6.91
	P value		0.2				0.03*		0.73				0.07
Noun	Foumban	73	21 (28.8)	17.81-43.97	52 (71.2)	53.2-93.41	13 (17.8)	9.48-30.45	3 (4.1)	0.84-2.01	2 (2.7)	0.33-9.89	18 (24.7)
	Foumbot	56	11 (19.6)	9.81-35.15	45 (80.4)	58.61-100	9 (16.1)	7.35-30.51	2 (3.6)	0.43-12.90	0 (0.0)	/	11 (19.6)
	Njimom	46	9 (19.6)	8-95-37.14	37 (80.4)	56.63-100	6 (13.0)	4.79-28.39	3 (6.5)	13.45-19.05	0 (0.0)	/	9 (19.6)
	Massangam	62	9 (14.5)	6.64-27.56	53 (85.5)	64.03-100	7 (11.3)	4.54-23.26	2 (3.2)	0.39-11.65	0 (0.0)	/	9 (14.5)
	Magba	98	28 (28.6)	18.99-41.29	70 (71.4)	55.68-90.25	18 (18.4)	10.89-29.03	5 (5.1)	1.65-11.06	2 (2.04)	00.24-7.37	25 (25.5)
	Malentouen	51	0 (0.0)	/	51 (100)	/	0 (0.0)	/	0 (0.0)	/	/	0 (0.00)	0 (0.0)
	Bangourain	71	17 (23.9)	13.95-38.34	54 (76.1)	57.14-99.24	12 (16.9)	8.73-29.52	2 (2.8)	0.03-10.17	1 (1.4)	0.03-7.84	15 (21.1)
	Kouptamo	56	9 (16.1)	7.35-30.51	47 (83.9)	61.67-100	7 (12.5)	5.03-25.75	2 (3.6)	0.43-12.9	0 (0.00)	/	9 (16.1)
	Koutaba	51	12 (23.5)	12-41.1	39 (76.5)	54.38-100	7 (13.7)	5.52-28.28	2 (3.9)	0.47-14.16	1 (2.0)	0.05-10.92	10 (19.6)
	Total	564	116 (20.6)	17-24.67	448 (79.4)	72.25-87.14	79 (14.0)	11.09-17.46	21 (3.7)	2.30-5.69	6 (1.1)	0.39-2.31	106 (18.8)
	x^2		22.96				11.94		3.74				17.67
	P value		0.003*				0.04*		0.87				0.024*
Total		912	159 (17.4)	14.83-20-36	753 (82.54)	76.77-88.68	110 (12.1)	9.91-14.54	26 (2.9)	1.86-4.17	9 (0.98)	0.45-1.87	145 (15.9)
X^2			10.07				5.96		0.26				10.43
value**			0.0015*				0.02*		0.6				0.001*

N: number of animal tested; CI: Confidence interval; (%): *Brucella* infection rate; MI: mixed infections; *significance difference; **: P values resulting from the comparison of infection rates between Yoko and Noun; BN: total number of animals in which *Brucella* species (*B.abortus* and *B.melitensis*) has been identified

Table 3: Compared results of serological and molecular tests for the detection of *Brucella* infections

		Molecular test			Kappa Cohen coefficient
		Positive	Negative	Total	
Serological tests	Positive	105	17	122	$K = 0.70$
	Negative	54	736	790	$P = 0.0001$
Total		159	753	912	

Serological tests: Rose Bengal test and the enzyme linked immunosorbent assay; Molecular tests: PCR targeting bcsp 31 gene of bacteria of the genus *Brucella; K*: Kappa Cohen coefficient; P = P value

Figures

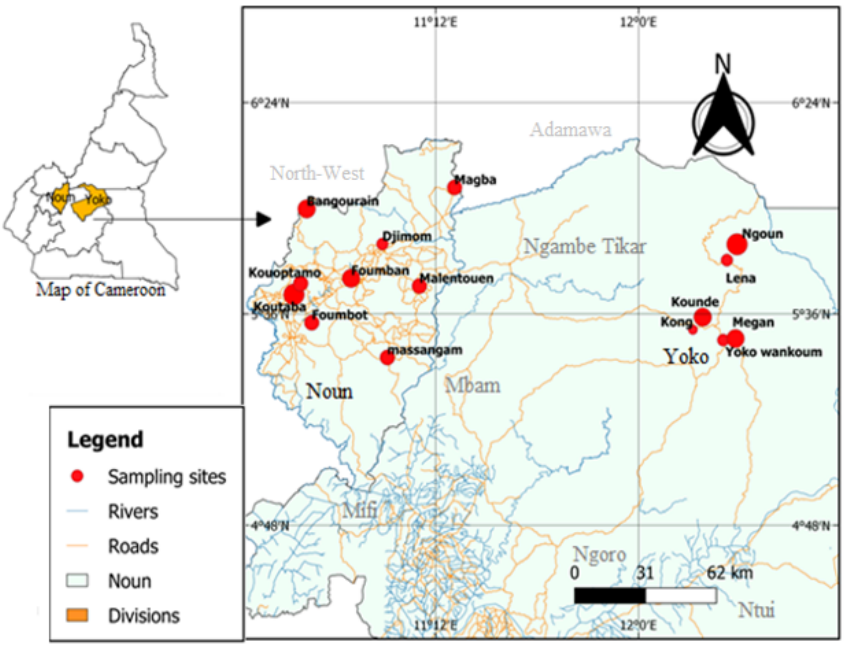

Figure 1

Map showing villages where domestic animals were sampled at Yoko and in the Noun division. The size of each circle is proportional to the number of animals (sampling intensity) that was sampled in this village (map was created with QGIS v. 3.8 software available at https://www.qgis.org)

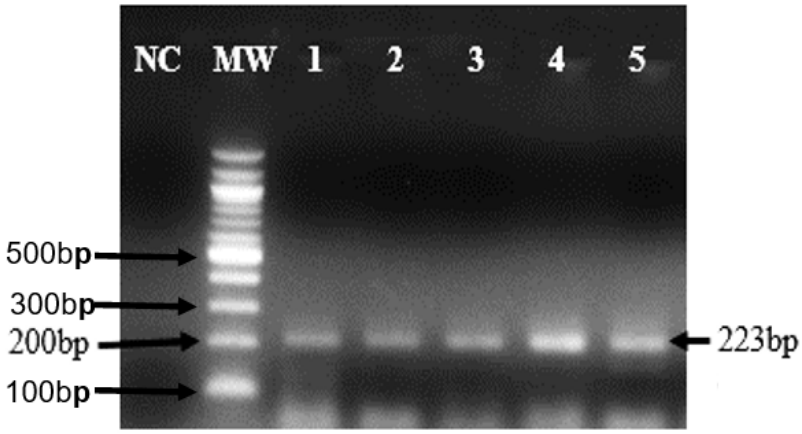

Figure 2

Electrophoretic profile of an agarose gel showing DNA fragments resulting from the amplification of bcsp31 gene of Brucella spp. NC: negative control; MW: 100bp DNA ladder (New England Biolabs); Lane 1-5: samples showing a specific band at 223bp indicating the presence of Brucella infections.

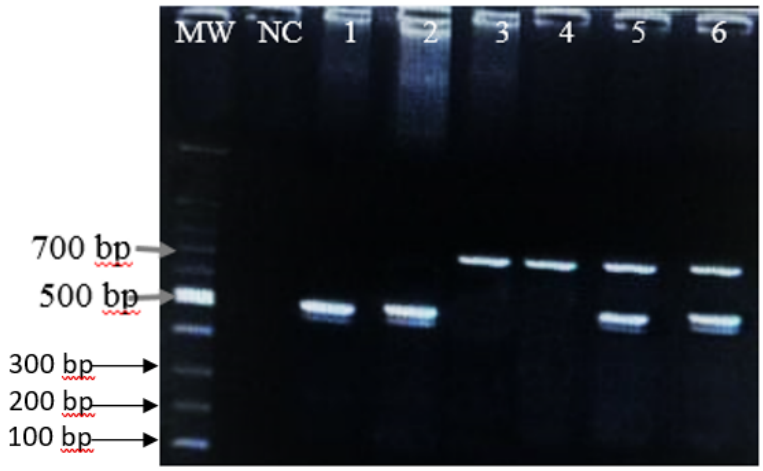

Figure 3

Electrophoretic profile of an agarose gel illustrating DNA fragments resulting from the amplification of IS711 gene using AMOS-PCR. Lanes 1 and 2: samples with B. abortus infections; Lanes 3 and 4: samples with B. melitensis infections; Lanes 5 and 6: samples harboring co-infections of B. abortus and B. melitensis; NC: negative control; MW: 100 bp DNA ladder (New England Biolabs).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfiles.docx
- formula.docx