SITUATION OF BRUCELLOSIS IN BEEF-TYPE CATTLE IN CAMEROON

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SITUATION OF BRUCELLOSIS IN BEEF-TYPE CATTLE RAISED UNDER DIFFERENT HUSBANDRY SYSTEMS IN CAMEROON

INTRODUCTION

Caused by Gram-negative non-spore-forming coccobacilli of the genus *Brucella*, brucellosis is a contagious systemic disease primarily affecting cattle, sheep, goats, pigs, horses and dogs. It is characterised by inflammation of the genital organs and foetal membranes, abortion, sterility, and formation of localised lesions in the lymphatic system and joints[18]. Besides domestic animals, the disease has also been associated with a wide range of wild animal species[6]. There is an increasing evidence of infection in marine mammals such as seal, whale, and dolphins[8,9,29].

Bovine brucellosis is a well-known zoonosis which also has profound negative impacts on cattle productivity[10]. It leads to loss of foreign exchange earnings; as it compounds international trade[25]. It represents one of the most important cause of zoonoses across the globe[32,3], increasingly so in developing countries, with grave economic, veterinary and public health consequences[12,20, 28,].

Despite significant advances in the control of brucellosis in some countries, it has remained endemic in many other countries[5]. Recent assessment of the global status of brucellosis indicates that it is a neglected and emerging zoonosis in some parts of the world[31]. The impact of brucellosis on a wide spectrum of domestic animals, its widespread distribution, and debilitating effect on man; is a priority disease for control and prevention in sub-Saharan Africa[14,23,30].

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Studies carried out in the early 1990s indicated that brucellosis is endemic in the bloc of countries that make up Central Africa; estimating the incidence to be greater than 30%[7]. More recent findings on the prevalence of *Brucella abortus* antibodies among Holstein cows in periurban dairy activities in the Western Highland Plateau Savannah (WHPS) of Cameroon by competitive ELISA puts its seroprevalence at 8.2%[2]. The WHPS is situated between latitude $4^{o}54'$ to $6^{o}36'$ North and longitudes $9^{o}18$ to $11^{o}24'$ East of the equator while the Guinea Highland Savannah (GHS) lies between latitude $5^{o}42`$to $8^{o}36`$North and longitude $11^{o}24`$to $14^{o}36`$ East of the equator. The ecologies of these areas have been described by several authors[21-25] and also the various cattle husbandry systems[26].

In this study we investigated the seroprevalence of bovine brucellosis and its risk determinants in these agro-pastoral ecologic zones in Cameroon.

MATERIALS AND METHODOLOGY:

Target Population

The unit of interest was all intact male and female cattle in villages covered by the GHS and WHPS ecologic zones. A small part of the GHS found in the Centre province (*Mbam et Kim* and *Mbam and Inubu* divisions) was not considered in this study because these two divisions are in the transition area into the bimodal forest ecology. Given that animals in this area graze both in the relatively small area of savannah as well as the forest, this study avoided ambiguity in the interpretation of results by deliberately leaving out animals found in these locations.

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The village herd, which was considered as a collection of herds sharing the same environment (village communal grazing land, watering points and zooveterinary infrastructure), risk of infection, and can potentially contaminate each other, was the epidemiological unit. In this situation animals grazing in a common transhumance area were considered to be a village herd.

Study Design

Even though precise data on cattle population in Cameroon are unavailable, data on village herds extracted from recent vaccination campaigns that were used by the Government`s Ministry of Livestock, Fisheries and Animal Industries'collaborative work with OIE for the 2008 study for the seroprevalence of rinderpest were used for this study.

The basic administrative unit in Cameroon; the subdivision, and village herds were considered as clusters for a two-stage cluster sampling procedure. To enable us sample animals by probability proportional to size (PPS), cattle population of each village (cluster) was noted against the corresponding village. This way, each beef-type cattle in Cameroon was assigned to a single cluster (village). This village list was entered into Microsoft Excel(R) 2007 version and by the use of C-survey(R) version 2.0 it was shuffled and a final list of villages to be visited was established with their corresponding herd sizes.

Sample Size

Recent information on the prevalence of brucellosis in Cameroon are lacking. Following findings by Domenech *et al*., on the incidence of bovine brucellosis in Cameroon[7], the sample size was designed to expect a prevalence of 30% for the ecological zones under study. The probability

desired for expressing the seroprevalence at 95% confidence with an accepted risk of 5% which resulted a values of 323 herds being obtained (see Table 1).

Table 1 *Table 1: Distribution of Sample Size proportional to Cattle density in the different subdivisions and villages (*Cattle Population obtained from OIE/PACE Rindepest Study conducted in Cameroon in the year 2008)*

Region	**Division**	***Cattle Population**	**% of Village herds**		**Number of herds**	
Adamawa	Vina	379,565	25.24	25	80.75	81
Adamawa	Mbere	66,700	4.43	4	12.92	13
Adamawa	Mayo Banyo	346,356	23.03	23	74.29	74
Adamawa	Djerem	101,967	6.78	7	22.61	23
Adamawa	Faro et Deo	74,559	4.96	5	16.15	16
East	Lom et Djerem	55,000	3.66	4	12.96	13
East	Kadey	30,000	1.99	2	6.46	6
Northwest	Mezam	64,169	4.27	4	12.92	13
Northwest	Bui	56,349	3.75	4	12.92	13
Northwest	Boyo	66,187	4.4	4	12.92	13
Northwest	Ngoketunjia	12,310	0.82	1	3.23	3
Northwest	Momo	26,463	1.76	2	6.46	6
Northwest	Donga Mantung	129,654	8.62	9	29.07	29
Northwest	Menchum	54,231	3.61	4	12.92	13
West	Noun	32,053	2.13	2	6.46	6
West	Bamboutos	8,500	0.57	0	0	1
4	***16***	***1,504,063***		***100***		***323***

Potential Risk Factors

To investigate into intrinsic (animal factors) and extrinsic (husbandry and environmental) factors that can have bearings with *Brucella* sero-status of the target population, a field questionnaire was designed and administered in the course of sampling. Intrinsic factors considered in the investigation were age, sex and breed. The following extrinsic factors; ecological zone, herd size, herd management system, history of third trimester abortion, interaction with wildlife, and interaction with sheep and goats during grazing., were investigated as potential risk factors. In addition to popular grazing systems like free range without a fixed perimeter; free range within a fixed perimeter, free range with transhumance, tethering, and zero grazed options were also included in the investigation.

Sample Collection

About eight millilitres of blood was collected aseptically from the external jugular veins from adequately restrained animals by the use of vacutainer tubes devoid of coagulants. On a slanting position, in order to prevent haemolysis of red blood corpuscles and encourage serum separation, the samples were allowed to clot overnight at room temperature and then decanted to obtain serum which was transferred into sterile Eppendoff tubes. The filled Eppendoff tubes were labelled with indelible markers and then stored at -20^{o}C. For samples collected from areas without refrigeration facility, the samples were transported in a portable car refrigerator (Mr Hyde GEO'STYLE (R) Professional Cooler) that had been earlier packed with ice blocks before transportation to the Laboratory for Emerging Infectious Diseases of the University of Buea,

Cameroon where they were stored at -20^{o}C until tested. The field questionnaire was also administered on every herd visited.

Diagnostic Procedures

Before screening, the sera were identified from the labels and then given serial numbers and entered into a sample register with entries related to the corresponding questionnaire. The samples were screened using the Rose Bengal Plate Test (RBPT) in consonance with method described by Alton *et al.*, [1].

On each day of test, an ample quantity of standardised *Brucella abortus* antigen (S 99W), test sera and positive and negative control sera, obtained from *Istituto Zooprofilattico Sperimentale (IZS) Del l'Abruzzo e del Molise "G. Caporale"* Italy, estimated to suffice the quantity of samples to be tested was removed from refrigeration at +4 to +6 ^{o}C and allowed to assume room temperature of 24^{o}C. Before each screening session the RBPT was validated by using a previously known positive and negative control sera. For a test, 30 µl each of test serum and Rose Bengal-stained antigen were placed into one of the wells of a previously cleaned polystyrene plate specially designed for the test, by use of different sterile microtitre pipettes to produce a circular zone of mixture of approximately 2 cm in diameter. A positive serum produced agglutination after gentle rocking at six rotations per minute on an orbital shaker (Cole Pomer$^{(R)}$ 51300 series) for four minutes at room temperature. All tests were read under good lighting conditions that could detect agglutinating particles which varied from being relatively coarse with relative ease of detection to a fine floccular form.

Data Analysis

The data generated from the field questionnaire were entered in Microsoft Excel(R) 2007 (non-commercial Version) and then tested for significance of differences between administrative units, herd management system practised, breed, sex, age, history of third trimester abortion, ecological zone, interaction with wildlife, interactivity with sheep and goats herdsize, against seropositivity by the use of ANOVA and Chi-Square in R-Software(R).

RESULTS

Out of 1562 heads of cattle screened from the village-herds in the study population, 72 heads were seropositive, indicating a prevalence of 4.61%. At the herd level, of the 250 villages effectively screened, 40 village-herds had at least one seropositive case representing a herd seroprevalence rate of 16%. None of the village herds visited had a previous history of vaccination against brucellosis. It was observed that all the herds have annual vaccinations against contagious bovine pleuropneumonia (CBPP), and blackquarter. Other vaccines recorded were anthrax spore vaccine and lumpy skin disease (contagious dermatitis) vaccine.

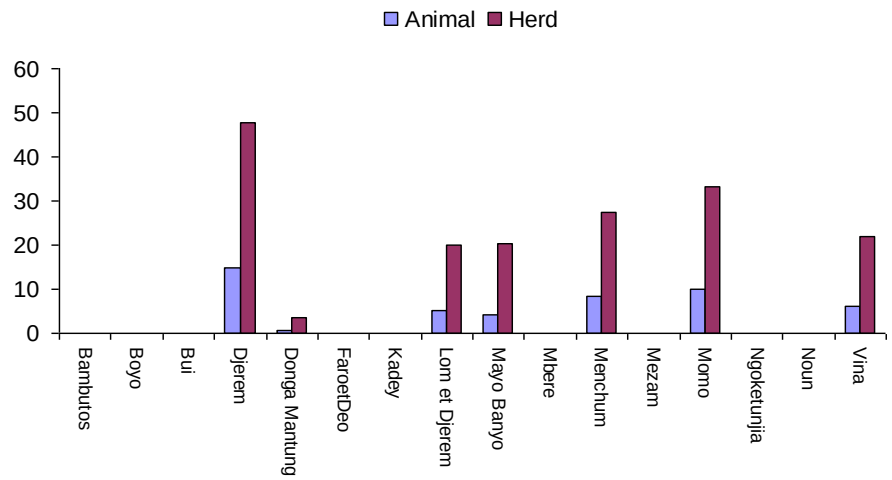
Figure 1: A Google map of Cameroon with the seropositive divisions marked with orange balloons and the seronegative divisions marked in blue

Analysis of Potential Risk Factors (ANOVA) using R-Software

Extrinsic Factors

Association between Seropositivity and Administrative Divisions

Figure 2 shows the seroprevalence of brucellosis in relation to divisions at both the animal and herd levels.



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At the animal level the seroprevalence of the seven divisions showing seropositives ranged from 0.7% (Donga Mantung) to 14.8% (Djerem). The other nine divisions did not show any seropositive cattle. Analysis showed that division had a significant effect on seropositivity at the animal level and was analysed further with Djerem set as the division that the other divisions would be analysed against. Djerem was significantly different from four divisions, these were Donga Mantung (0.7%; p=0.002), Lom et Djerem (5%; p=0.021), Mayo Banyo (4.3%; p<0.001) and Vina (6.2%; p=0.005). Of the other two divisions, Djerem was not significantly different from Menchum (8.3%; p=0.210) and Momo (10%; p=0.409). The seronegative divisions came out as non significant. This is because they did not have positives.

At the herd level seroprevalence ranged from 3.4% (Donga Mantung) to 47.8% (Djerem). Analysis showed that division had a significant effect (p<0.001) on seropositivity at the herd level and was analysed further with Djerem set as the division that the other divisions would be analysed against. Djerem was significantly different from three of the other divisions, these were Donga Mantung (3.4%; p=0.003), Mayo Banyo (20.3%; p=0.013) and Vina (21.9%; p=0.047). There was no significant difference between Djerem and the other three districts Djerem was not significantly different from Lom et Djerem (20%; p=0.090), Menchum (27.2%; P= 0.261) and Momo (33.3%; p=0.528).

Seropositivity and Ecological Zone

The seroprevalence rate in the GHS at the head level was 5.7% which was significantly (p<0.001) higher than the seroprevalence of 2.0% in the WHPS. While at herd level the seroprevalence in the GHS was 21.8% which was significantly higher than the value of 6.4% obtained in the WHPS.

Seropositivity and Interaction with wildlife

In total 27.2% of herds had access to wildlife. The seroprevalence at the herd level was significantly ($p<0.001$) higher in herds that had access to wildlife (38.2%) compared to those that did not (7.7%)

Seropositivity and Interaction with Small Ruminants

In total 43.6% of herds had access to small ruminants. The seroprevalence at the herd level was significantly ($p<0.001$) higher in herds that had access to small ruminants (24.8%) compared to those that did not (9.2%).

Seropositivity and History of Third Trimester Abortion

In total 68.0% of herds had a history of third trimester abortion. The seroprevalence at the herd level was significantly ($p<0.001$) higher in herds that had a history of third semester abortion (21.2%) compared to those that did not (5.0%).

Seropositivity and Herd Size

Herd size ranged from 10 to 411 with a mean of 74.25. The smallest herd size with a seropositive case was a herd of 22 while the largest herd size that registered a positive case was a herd of 122 heads. The average size of a seronegative herd was 71.5 while that of a herd containing at least one seropositive animal was 88.6, this difference was significant ($p<0.001$).

Seropositivity and Herd Management System

Of the three herd management systems studied, 41 of the 72 seropositive cases registered in the study area were animals managed under free range with seasonal migration on transhumance. This herd management system showed the highest seroprevalance both at animal level (2.6%), and herd level (9.2%). Seventeen of the total seropositive heads were animals managed free range without a fixed perimeter; giving animal level and herd level seroprevalences of 1.09% and 2.8% respectively.

Ten of the seropositive animals were from herds managed free range within a fixed perimeter all year round to the exclusion of external animals. This gave an animal level seroprevalence of 0.9% and a herd seroprevalence of 4%.

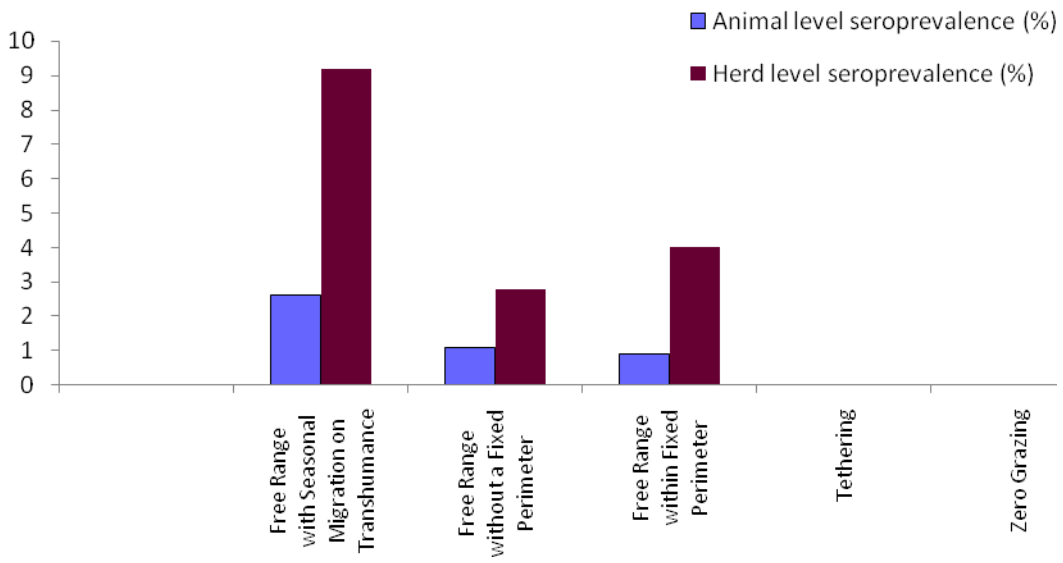

Figure 4 *Percentage Distribution of Seroprevalence with Herd Management System*

Analysis of variance showed that the management system had a significant effect (p=0.03) on the seropositivity and was further analysed, with free range with seasonal migration on transhumance which showed the highest seropositivy set as the system of reference. Free range with seasonal migration on transhumance was significantly different from Free range within a fixed perimeter all-year-round to the exclusion of other animals (0.9%; p=0.014) and free range without a fixed perimeter (2.8%; p=0.16).

Analysis of Potential Intrinsic Factors

Association between Age Group and Seropositivity

Five age groups were investigated; these were less than 12 months old, 12 to 23 months old, 24 to 36 months old, 37 to 48 months old and greater than 48

months old. The RBPT test showed that seroprevalence was 3.5% in animals less than 12 months old, 3% in animals 12 to 23 months old, 5.2% in animals 24 to 36 months old, 4.1% in animals 37 to 48 months old and 5.5% in animals greater than 48 months old.

Analysis show that age group had no significant effect on seropositivity (p=0.61). The effect of age continued to be non significant if the cattle were grouped as 23 months and under and 24 months and older (p=0.22)

Association between Sex and Seropositivity

The seroprevalence rate in female cattle was 4.9% (n=1285) while it was 3.2% (n=277) in males.

This difference was not significant (p=0.21).

Association between Breed and Seropositivity

Table 2 shows the relative proportion of seropositivity obtained from the six different breeds under study.

Table 2: Percentage distribution of Seropositivity with respect to Breed and Sex

Breed	No. Screened	No. Seropositive	%	Animal level seroprevalence (%)	Herd level seroprevalence (%)
White Fulani	526	39	54.1	2.5	15.6
Red Bororo	363	12	16.7	0.77	4.8
Boran	12	0	0	0	0

Goudali	617	21	29.2	1.34	8.4
Namnchi	27	0	0	0	0
Brahman	16	0	0	0	0

Analyses showed that breed had a significant effect on seropositivity ($p<0.05$). The animal level seroprevalences of the breeds that had positive cases were as follows: White Fulani 2.5%, Red Bororo 0.77%, and Goudali 1.34%. These can be seen in Figure 9 which shows the percentage distribution of seroprevalence among the different breeds studied.

Further analysis of seroprevalence at herd-level showed that the White Fulani (15.6%; $p<0.01$) was significantly different from the Goudali (8.4%; $p<0.003$) and the Red Bororo (4.8%; $p<0.012$).

DISCUSSION

A significant proportion (54%) of Cameroon's beef-type cattle are reared in the two ecologic areas studied. The results showed, scientifically, that bovine brucellosis is endemic in Cameroon. With an uneven but wide distribution the study revealed a 4.61% and 16% seroprevalence at the animal-level and herd-level respectively. There was a preponderance of seropositivity in the GHS (87.5%) over the WHPS (12.5%). This 4.6% animal-level seroprevalence is largely consistent with 4.88%, using RBPT, observed in an abattoir study in West Region of Cameroon[21]. Albeit the absence of an identification traceability system for animals presented at

the abattoirs in the country, the authors hold that these animals were mostly bred and reared in the GHS and WHPS ecologies.

This seroprevalence, however, deviates widely from a 30% prevalence estimated for the Central African sub-region[7].

On potential bovine intrinsic factors that may militate in the seroprevalence of *Brucella*, this study showed that sex, and age group do not have significance in the epidemiology of the disease. These findings concur with recent studies on Holstein cattle in peri-urban intensive dairy farms of the WHPS[2]. However, this absence of collinearity between age and seropositivity contrasts with results of a crossectional study of risk factors of brucellosis in the extensive cattle production system of Tigray region of Ethiopia[11]. With respect to breeds, these findings are equally in agreement with results of a survey of livestock in Ibadan, Nigeria[4]

Of the six beef-type breeds investigated, the results show that the White Fulani is more likely to be seropositive (2.5%) and is significantly different from those of the Red Bororo and the Goudali breeds.

The extrinsic risk factors investigated were all positively correlated with seropositivity. It is interesting to note that cattle interactivity with sheep and goats (small ruminants), wildlife, herd size, administrative division, history of third trimester abortion, ecological zone, and herd management systems had significant effects on seropositivity.

It is quite plausible to think that the positive correlation between bovine *Brucella* seropositivity and interaction with small ruminants is attributable to the susceptibility of cattle to *Brucella ovis*

and *Brucella melitensis*. This is so because a high prevalence of these infections in sheep and goats of sub Saharan Africa has been demonstrated by several authors[24,13].

The link between seropositivity and interaction with wildlife animals could be due to the fact that when free range cattle share drinking points and grazing grounds mostly during the calving season the environment could be contaminated by *Brucella* organisms from reservoir hosts such as the buffaloes and other wild ruminants that are abundant in the two ecosystems studied. The effects of transhumance on diseases such as brucellosis has been previously emphasised[15]. In this study we noticed clustering of seropositivity in the Djerem division (GHS) where the Mbakaou reservoir and the Djerem National Parks are found. However, this phenomenon was not observed in herds screened in close proximity with other wildlife reserves such as the Kimbi and Mbi crater reserves in the WHPS. It would therefore be of interest to investigate the prevalence of *Brucella* in wildlife from these reserves to see if there are any differences. Such investigations would better situate the role of wildlife in the epidemiology of brucellosis in these ecologies.

Another interesting observation is the clustering of seropositivity in herds that are greater than 75 heads; 75% of the seropositive cases were derived from such herds.

Analysis of the response on history of third trimester abortion and seropositivity showed a significant effect ($p<0.001$). This finding can be logically related to the fact that third trimester abortion is a prominent clinical feature of brucellosis in farm animals.

Much of transboundary implications, we observed a clustering of positive cases along the border divisions of the Northwest region (Wum and Donga-Mantung) which are contiguous with the Federal Republic of Nigeria (Figure 1). It is possible to expect that the high level of informal cattle trade between the two countries is responsible for this.

CONCLUSION

To the best of our knowledge, these findings provide the first factual insight into the seroprevalence of bovine brucellosis and some of its associated risk factors in Cameroon. The absence of a widespread practice of vaccination against *Brucella spp* in livestock in Cameroon as revealed by responses from the questionnaire that showed absence of a history of vaccination in all the two hundred and fifty herds screened and the seropositivity registered in all age groups in this study, implies that *Brucella* is naturally circulating in beef-type cattle herds in the WHPS and GHS ecologic zones. The uneven but wide distribution and the paucity of animal-level seroprevalence observed in this study are strong incentives for diagnostic capacity building, control and eradication.

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