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Bovine Brucellosis in Cattle Production Systems in the Western Highlands of Cameroon

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Abstract

A study was carried out to compare the prevalence of *Brucella abortus* between semi-intensive and extensive managed cattle in the North West Region of Cameroon. A total of 689 cattle were tested for *Brucella* antibodies using the competitive Enzyme-Linked Immunosorbent Assay. Overall prevalence of brucellosis was found to be 5.2% (n = 36). There was strong evidence that cows in the extensive system (6.5%; n=32) had a higher infection rate than those in the semi intensive system (2%; n=4; P<0.0001). Bovine overall brucellosis infection rates were higher in the dry season (67%) than the rainy season (33%), (P<0.05). Healthier cattle (78%; P=0.0009), older cattle (64%; P=0.0003) and cows (75%; P=0.0027) were more infected. The prevalence of *Brucella* in the White Fulani breed was less severe than in other breeds (P=0.0003). Acha had more infected animals than the rest of the region (P<0.0001). The results of this study confirm the endemicity of bovine brucellosis in the North West Region of Cameroon and a moderate seroprevalence rate in extensive cattle management systems in the study area. There is a need for eliminating positive reactors, implementing control measures and raising public awareness of zoonotic transmission of brucellosis, and on improvement of extensive cattle management systems.

Keywords

Brucella, Cattle Systems, ELISA, Seroprevalence

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1. Introduction

Brucellosis is a zoonotic disease (WHO 1986; Sanogo et al 2013), and is perhaps one of the most widespread and economically important diseases affecting cattle in tropical and subtropical regions (Nicoletti 1980; Staak 1990). It is one of the major bacterial infectious diseases, affecting domestic animals in many developing countries (Akakpo and Bornarel 1987; Corbel, 1997; Wastling et al 1999; McDermott and Arimi 2002).

Although brucellosis is almost eradicated from a number of developed countries, it continues to be a major public and animal health problem in many parts of the world, particularly where livestock are a major source of food and income (Mahajan and Kulshreshtha1991; FAO 2003). The disease remains an uncontrolled problem in regions of high endemicity such as Africa, the Mediterranean region, Middle East, parts of Asia and Latin America (Refai 2002). In sub-Saharan Africa, bovine brucellosis remains the most widespread form of the disease in livestock (Akakpo and Bornarel 1987; Corbel 1997; McDermott and Arimi 2002; Bronsvoort et al 2009).It is caused by bacteria of the genus *Brucella* and is considered one of the most widespread

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zoonoses in the world (McDermott and Arimi2002; FAO 2003).

The principal manifestations of brucellosis are reproductive failure such as abortion and birth of unthrifty new born in the female, orchitis and epididymitis with frequent sterility in the male (OIE 2003). Bovine abortion is an important cause of economic loss to the livestock industry. The abortion incidence varies widely, depending on the health status of individual herds (Murray 2006). One clinical sign commonly associated with brucellosis in African cattle herd is the presence of hygroma. In many countries and for years, fluid of hygroma has been used as the sample for biotyping (Thienpont et al 1961; Akakpo and Bornarel 1987; Bankole et al 2010; Sanogo et al 2013).

No outbreaks of the disease have been reported to the World Health Organization for Animal Health, formerly "Office International des Epizooties" (OIE), by Cameroon since 1996 (OIE 2000). Shey-Njila et al (2005) concluded from studies carried out in Cameroon that brucellosis is enzootic in the extensive animal husbandry system (pastoralism) in the Western Highlands and the Adamawa regions of Cameroon. Also, a study carried out by Bayemi et al (2009) on Holstein cattle showed that *Brucella* infection varied with location in Cameroon. It is responsible for considerable economic losses through its negative impacts on livestock production including late term abortion, production of weak calves at birth, placenta retention, metritis, infertility (Olsen and Tatum 2010). It has been suggested that testing and slaughter should be implemented to prevent the further spread of the disease to the larger cattle population in the region. Unfortunately there are no reports on the seroprevalence of the disease in semi-intensive and extensive livestock production systems. Knowing the prevalence in these systems will help in the efficient control of the disease and improvement of the various cattle management systems.

This study was conducted to compare the prevalence of bovine brucellosis in semi-intensive and extensive managed cattle, to provide data for the control of the disease and create awareness of brucellosis among the cattle farmers in the North West Region of Cameroon.

2. Materials and Methods

2.1. Study Area

The study was carried out in the North West Region of Cameroon. This region is located in the mid to high altitude zone of the country that lies between latitudes 5º20' and 7º00' North and longitudes 9º40' and 11º10' East. Altitudes range from 300 to 3000 metres above sea level. The rainy season runs from mid-March to mid-November and a short dry season of 4 months from mid-November to mid-March (MINEPIA 2010). Annual rainfall varies between 1300 and 3000 mm, with a mean of 2000 mm. Daily minimum and maximum temperatures were 15.5ºC and 24.5ºC, respectively, although temperatures can occasionally exceed 30ºC. The human population is estimated at 1.82 million inhabitants with an annual growth rate of 3.1% (Winrock International 1992). The North West Region is agriculturally based with 72% of its population involved in agriculture. The main vegetation is savannah. Pastures are dominant with *Sporobolus africanus*, but the following species can also be encountered: *Pennisetum clandestinum*, *P. purpureum*, *Loudetia spp*, *Hyparrhenia spp*, *Urelytrum fasciculatun*, *Panicum phramitoides* and *Paspalum arbiculare*. Some improved species have been introduced such as *Brachiaria* spp, *Trypsacum laxum*, *Stylosanthes* spp and tree legumes (Merlin et al 1986; Njoya et al 1999).

2.2. Cattle Population and Survey

The samples used for the study were collected from animals in the cattle market at Bamendankwe (Bamenda), cattle farms in and around Momo Division, and from the cattle herds in IRAD Bambui. Verbal consent was obtained from cattle owners and herds' men before the blood samples were collected.

Animals used for this study were selected from February to August, and samples were collected in the morning before the animals went out for grazing. During the study, animals were identified by giving serial numbers to each of them. The coding system included the management system, stock composition, sex, age, date of collection of blood sample, health condition of the animal, collection site, owner, and the breed. Animals considered as poor in body condition were those with dry udder, swollen knees (hygromas), cows that had aborted or registered still birth, and were sterile, animals with scabies and rough coat, bloody urine and worms (pot belly).

A semi structured questionnaire was completed by 20 farmers with the aim of having additional information about the management systems. The interviews were conducted in Pidgin English and Fulfude (through an interpreter). The questionnaire focused on level of education of the farm owner, knowledge on brucellosis, records on abortion or stillbirth, vaccination against brucellosis and disposal of sick animals at the verge of death. Secondary information was obtained from reports of Ministry of Livestock. The study was conducted on both extensively and semi-intensively managed herds, using samples from animals of all ages, breeds and sex. The extensively managed herds were traditionally managed. Livestock composition consisted of cattle as dominant stock and variable number of small

ruminants kept in crop-livestock mixed farming systems. The breeds used for the study were the White Fulani (WF), Red Fulani (RF), Gudali (GU), and crossbreeds of indigenous and exotic breeds (Hosltein X Gudali and Holstein X Red Fulani).

2.3. Blood Samples

Venous blood (5ml) was collected using vacutainer blood collection tubes from the tail vein. The blood samples were carefully packed to avoid any possibility of cross-contamination, and transported in a cooler with ice packs to the Animal Physiology and health Laboratory in IRAD Bambui for analysis. In the laboratory, the samples were left at room temperature overnight to allow the blood to clot. The serum formed was collected using pasteur pipettes into sterile cryovials and stored at -20ºC for serological testing.

2.4. Sample Processing and Serological Analysis

The screening was done using *Brucella*-Ab C-ELISA kit (SVANOVA, Sweden2011).The SVANOVIR® competitive Enzyme Linked Immunosorbent Assay (C-ELISA) for detection of serum antibodies to *Brucella abortus* and *B. melitensis* is a multi-species assay, allowing detection of *Brucella* specific antibodies in both domestic and wildlife species.

Pre-coated microtitre plates with *B. abortus* smooth LPS coated wells on microtitre plates together with a mouse monoclonal antibody (mAB) specific for an epitope on the o-polysaccharide portion of the smooth LPS antigen were used.The technique used was C-ELISA for detection of *B. abortus* and *melitensis* specific antibodies. The kit procedure is based on a solid phase competitive Enzyme Linked Immunosorbent Assay.

Reagents and serum samples were equilibrated to room temperature (18 to 25º C). Serum samples were first analysed in batches of 10, chosen serially in order of collection. Four µl (4 µl) of each from a batch of 10 samples was pooled in a vial i.e., each pool had 10 serum samples in one, in order to analyse for the presence of antibodies against *Brucella* using cELISA. Positive batched samples were identified and individual sera of batch samples were analysed for identification of the particular animals that were seropositive for *Brucella* infection.

Forty-five µl (45 µl) of Sample Dilution Buffer was put into each of the wells on the microtitre plate (having 96 wells) after which 5 µl of Serum Controls (positive, weak positive and negative) were added into appropriate wells on microtitre plates. Each control was run in duplicate. Five µl (5 µl) of Sample Dilution Buffer was put into each of two appropriate wells designated as Conjugate Control (Cc), after which 5 µl of each test sample was added to appropriate wells in duplicates. Fifty µl (50 µl) of mAB-Solution was added to all wells used for controls and samples. The plate was sealed and the reagents mixed thoroughly by shaking the plate for 5 minutes on a shaker. The plate was incubated at room temperature (25ºC) for 30 minutes, after which the plate was rinsed 4 times with PBS-Tween Buffer. One hundred µl (100 µl) of Conjugate Solution (goat anti-mouse IgG horse-radish peroxidase) was added to each well; the plate was sealed and incubated for 30 minutes at room temperature. The plate was rinsed again 4 times with PBS-Tween Buffer. Into each well was added 100 µl of Substrate Solution (tetramethylbenzidine in substrate buffer containing H_2O_2) and the plate was incubated at room temperature for 10 minutes. The reaction was stopped by adding 50 µl of Stop Solution to each well and the plate was shaken for 5 seconds using a shaker and the optical density (OD) was read within 15 minutes. The OD of the controls and samples was measured at 450 nm in a microplate photometer (Model; Absorbance microplate reader ELx800™BioTek) using air as a blank.

2.5. Statistical Analysis

Mean OD values for each of the controls and samples were calculated and percent inhibition (PI) values for controls and samples calculated using the following formula:

PI = 100 - (Mean OD samples and control X 100) / (Mean OD conjugate control)

Samples with PI values ≥ 30% were defined as seropositive.

Statistical analyses were performed using Chi square in SAS (2002). One of the ten areas, Acha, was found to have 72% infection rate and so was also analyzed separately.

3. Results

The results of the survey revealed that in all the study areas, a high proportion of the farmers were uneducated or have attended only primary education (63.2%; Table 1). More than half of the farmers (89.5%) were not aware of bovine brucellosis. Most animals (70 %) sold were at the verge of death. Just 10% of the farmers buried the carcasses of their dead animals while 20% of them slaughtered their terminally ill animals and no cattle were vaccinated against brucellosis.

An overall prevalence of 5.2% was found in sera from cattle sampled in of the North West Region. There was strong evidence that the extensive system (6.5%; n=32) had more infected animals than the semi intensive system (2%; n=4; $P<0.0001$). Animals were more infected in the dry season (67%) than the rainy season (33%), ($P<0.05$). Healthier animals (78%; $P=0.0009$), older cattle (64%; $P=0.0003$) and

cows (75%; P=0.0027) were more infected. The prevalence of *Brucella* in White Fulani breed was less severe than in other breeds (P=0.0003). Acha hosted more infected animals than the rest of the study sites (72%; P<0.0001). When a separate analysis was done within this area, it was found that the results of this study were strongly influenced by the level of infection in this area. As a result, there was no evidence that any of the factors studied (Season, management system, sex, body condition score, breed, age group and location)influenced *Brucella* inflection in the remaining region (P > 0.05). The study also showed that antibodies to *Brucella* infection varied with location (P<0.0001), Acha having a higher prevalence than Chupm, Mbengwi and Ngie.

Table 1. Percentage of infected cattle in all areas.

Factor	Characteristic	Within factor percentage of infected cattle	Frequency (infected/tested)	Significance level
Season	Dry season	67	24/290	*
	Rainy season	33	12/399	
Management system	Extensive	89	32/492	***
	Semi intensive	11	4/177	
Sex	Male	25	9/221	*
	Female	75	27/466	
Condition score	Poor	22	8/72	***
	Good	78	28/617	
Breed	RF	42	15/207	**
	WF	3	1/66	
	GU	55	20/310	
Age group	<1 year	11	4/62	***
	>1 to <3years	25	9/287	
	>3years	64	23/338	

* = P<0.05; ** = P<0.01; *** = P<0.001

4. Discussion

The results from this study indicate that bovine brucellosis has an overall prevalence of 5.2% in the study sites North West Region of Cameroon. This is similar to the findings of Shey-Njila (2004) who reported a seroprevalence of 4.88 to 9.64% in a survey conducted at the abattoir of Dschang in Cameroon. Bayemi et al (2009) obtained a prevalence of 8.4% in Holstein cattle in the same region. However, sample collection was carried out only in small scale zero grazing dairy animals. In Cameroon, *Brucella* infection has been found to be higher in ranches compared to the traditional systems (IRZ, 1986). The level of infection is lower in this study because more animals were suckler cattle. Lefèvre (1991) had already stated that in Cameroon, the prevalence of bovine brucellosis exceeds 5%. In neighbouring Tchad, *Brucella* seroprevalence was found to be 7% (Schelling et al 2003) while in Nigeria the prevalence was 5.82% (Cadmus et al 2006). In Tanzania, Swai and Schoonman (2010) reported similar overall brucellosis prevalence of 5.3%. These results indicated that the prevalence of brucellosis has remained unchanged over the years and the disease is in the North West Region of Cameroon.

Bovine brucellosis in cattle of the extensive management was 6.5% while that of the semi-intensive system was lower 2.0%. This is not in accordance with previous work, perhaps because most infected animals came from the same area (26 infected over 36 total infected cattle).This finding is in accord with Gebretsadik (2005) in Northern Ethiopia where a higher prevalence of brucellosis was also reported in cattle in extensive management. Shirima et al (2007) depicted that pastoral (extensive) animals were three times more likely of being exposed to *Brucella* infection compared to animals in agro-pastoral farming systems. Megersa et al (2011) concluded in their study that bovine brucellosis is widely prevalent in cattle herds of most villages with higher seroprevalence in pastoral than mixed farming areas. It was explained that mobile herds have a greater opportunity to come into contact with other potentially infected herds during their movement into the different areas (Omer et al 2000). Cattle farms close to stock route and access to surface water emerge as the most important risk factors. Additionally, pastoral household often keep a diverse composition of livestock species as part of a coping mechanism for uncertainties and risks. Such conditions certainly increase aggregation and interaction of different animals at villages, grazing fields and water points, thus facilitating transmission of the disease. The dynamics and frequent migration of pastoral herds might increase the chances of coming into contact with other potentially infected herds and exposure to geographically limited or seasonally abundant diseases (Mergesa et al 2011). Extensive cattle management system is the most common cattle management system in the North West Region of Cameroon. Indiscriminate movements of transhumant animals from one area to the other or using them as escort for other animals to be sold at the cattle markets, expose them to diseases, not only brucellosis. These results also suggest inadequate management of extensive animals in the North West Region.

When the incidence of brucellosis in the most infected area was removed, it was revealed that sex did not influence seroprevalence of *Brucella* antibodies (P>0.05). The absence of a significant difference between brucellosis and sex is in agreement with other authors in Cameroon (Bayemi et al 2009). In neighbouring Nigeria, Ocholi et al (1996) found no difference in prevalence between sexes although the overall seroprevalence was 6.6%. In Tanzania, Swai and Schoonman (2010) reported no significant association between *Brucella* antibodies and sex. But Mergesa et al (2011) reported a difference between sexes (P=0.040) in traditional livestock husbandry practice in Southern and Eastern Ethiopia. In the most infected area (Acha) however, female were more infected (P=0.0004). This may be due to the smaller number of male cattle (30%) compared to females (70%) sampled in this study. A similar result was obtained by Abdirahim (2009) in Swaziland. The possibility of venereal transmission being rare limits the extent of spread of brucellosis in males even when the prevalence is high in females (McDermott et al 2002).

Bovine brucellosis indiscriminately affected all age categories, because the disease is spread through various ways like ingesting contaminated feed, water, or milk, suckling or licking an infected placenta, newborn or foetus, or the genitalia of an infected female soon after it has aborted or after birth (Godfroid et al 2004 a, b). Brucellosis is essentially a disease of the sexually mature animals, the predilection site being the reproductive tract, especially the gravid uterus. Salihu et al (2011) also reported from a study in Nigeria that there was a significant difference in prevalence between animals of different age groups.

Dairy herds were uninfected by brucellosis. This can be explained by the small-size units and stall feeding of dairy herds, which minimises contact between herds and other animals (Swai and Schoonman, 2010). One of the ranches had also culled animals that previously tested positive for *Brucella*.

The prevalence of brucellosis was higher (P=0.0009) in animals with good body condition. It may be due to the very low number of animals of poor body condition score. Kungu et al (2010) observed in a study on risk factors for brucellosis in cattle in Northern Uganda that the difference in occurrence of brucellosis in cattle of various body conditions was not significant (P>0.05).

Among the potential risk factors considered in the present study, the breed of cattle was shown to have a significant effect on the serological prevalence of bovine brucellosis. The serological prevalence was higher in Red Fulani and Gudali than any other breed. Salihu et al (2011) in a study of brucellosis in breeding herds showed that there was a strong association between *Brucella* infection and breed. They observed that Sokoto Gudali had a prevalence of 29.59% and White Fulani had a prevalence of 6.45%. There were no positive reactors among the exotic crossbred animals in the present study. In Tanzania Shirima et al (2007) reported a higher prevalence of brucellosis in indigenous cattle than in crossbred animals kept by smallholder dairy farmers. Jergefa et al (2009) also found a significant difference with prevalence amongst breeds (P<0.05), but the serological prevalence was higher in crossbred animals than in indigenous ones. Similarly, Kungu et al (2010) reported a higher seroprevalence in exotics and crosses. In contrast to these findings, Radostits et al (2000) reported no association between the breed of cattle and the seroprevalence of bovine brucellosis. The differences in the results obtained in these studies could be due to the effects of management systems. All the four positive reactors from the first ranch are GU and RF (indigenous breeds), which were under semi-intensive management along with exotic crossbred cattle but still came up with the disease or were brought in infected.

The study also showed that antibodies to *Brucella* infection varied with location (P<0.0001). Nine out of 21 herds accounted for 42.9% of positivity for *B. abortus*. Absence of seropositive animals in some locations may be due to low prevalence of brucellosis or to the number of samples collected not sufficient to come up with positive reactors. In the study area, frequent contact with livestock, low awareness on importance of brucellosis as a zoonoses and consumption of raw milk probably increased the risk of animal and human exposure to *Brucella* infection. The high prevalence in some areas can be attributed to the fact that animals taken to the cattle markets are usually brought back to their respective herds since some of them serve as escorts.

There was a very high significant seasonal difference in *Brucella* infection, with the dry season having a higher prevalence. This high prevalence could be due to the fact that animals always meet at watering points and share the same pasture field during drought periods when they go on transhumance.

5. Conclusion

This study has confirmed the presence of bovine brucellosis in the North West Region of Cameroon and the moderate seroprevalence in extensive cattle management systems in the study area. Cameroon has not been able to react adequately and the disease continues to be a major animal (and thus public) health problem. As livestock brucellosis control intervention by immunization has never been attempted in the North West Region, there is no history of vaccination against brucellosis in the study area. The

presence of *Brucella* shows the reality of a potential public health threat, in such an epidemiological context where close contact may occur between animals and people, where hygienic conditions are usually poor, where customs favour consumption of raw milk, partially roasted meat and where no control strategies are implemented. The low prevalence of only 2% under semi intensive management would even allow sanitation to culling out. Acute brucellosis in humans might even be misdiagnosed and missed out in cases of febrile illness similarly encountered in others endemic human diseases like malaria or typhoid. Hence, there is need for implementing control measures and raising public awareness on zoonotic transmission of brucellosis, and on improvement of extensive cattle management systems. It would be interesting to also study the prevalence of the disease in marketed milk and in the human population of those practicing cattle rearing.

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