

# Prevalence of *Brucella abortus* antibodies in serum of Holstein cattle in Cameroon

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**Abstract** Holstein cattle of a small scale dairy production systems were screened for *Brucella abortus* antibodies in 21 villages in Cameroon by ELISA. Results show a general seroprevalence of 8.4% in Holstein cattle. Of the 192 cows tested, 14 were infected giving a within-sex seroprevalence of 7.3% while 6/74 bulls were infected with a seroprevalence of 8%. There was no evidence ( $P=0.11$ ) of differences in seroprevalence between age groups although animals above one year and below three years accounted for nearly half of the infected animals. 64% of infected animals were found in three locations ( $P=0.015$ ): Kutaba (32%), Bamendankwe (16%) and Finge (16%). A specific control programme should be organized at these locations. Measures should be taken to ensure the eradication of the disease within the population and sound control measures adopted to avoid a further spread of the disease to larger cattle populations. Infected animals should be slaughtered systematically. All farmers should be advised to boil milk before consumption. Vaccination against *Brucella abortus* should be instituted and use of artificial insemination propagated. In order to ensure a productive and healthy population of Holstein cows within the dairy production scheme, regular *Brucella* testing should be instituted.

**Abbreviations** OIE World organization for animal health - ELISA enzyme-linked immunosorbent assay

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## Introduction

Brucellosis is a major zoonosis that occurs worldwide. It is caused by small nonmotile coccobacilli of the genus *Brucella*. Although the reported incidence and prevalence of the disease vary widely from country to country, bovine brucellosis caused mainly by *B. abortus* is still the most widespread form. Even though many countries have tried to eradicate *B. abortus* from cattle, it appears that bovine *B. melitensis* infection is emerging as an increasingly serious public health problem. Although it is anticipated that brucellosis is present in the major animal producing areas, the current status of brucellosis in Cameroon is not clear and it is becoming a major concern to veterinarians and physicians alike.

According to OIE, official measures are in place in Cameroon to control bovine brucellosis through movement control of cattle at the borders and within the country but since 1996 no outbreaks have been reported to OIE. This however is not indicative of the absence of the disease but rather of an underestimation of the brucellosis in Cameroon (Shey-Njila et al. [2005](#)).

Peri-urban small scale dairy farms in the Western Highlands of Cameroon use Holstein cows in a zero grazing system. The milk produced is home consumed, sold in informal markets or collected by a processing plant. However, the health status of this milk is unknown. There is a need to assess its quality in terms of health hazards and ensure the safety of consumers. The main objectives of this work therefore were to carry out a sero-epidemiology of Holstein cattle in the Western Highlands in order to establish their brucellosis status, create public awareness of the epidemiology of brucellosis in Cameroon and make concrete proposals to the appropriate authorities for adequate control measures to be put in place.

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## Materials and methods

298 Holstein cattle were screened in a 4 by 2 factorial design age by sex, with samples from animals from 21 locations representative of villages where purebred Holstein cows are kept. However, only 266 animals were fit for the analysis. Age was divided into the following groups: less than one year, one to two years, three to five years and more than five years. Blood samples were collected and immediately put on ice. At the laboratory, the samples were centrifuged at 1500 rpm for 15 min and the serum kept in a freezer until the day of analysis.

### Principle of the test

The screening was done using Brucella-Ab C-ELISA kit (SVANOVA, Sweden, [2005](#)). The competitive Enzyme Linked Immunosorbent Assay (C-ELISA) for detection of serum antibodies to *Brucella abortus* and *melitensis* is a multi-species assay allowing detection of *Brucella* specific antibodies in both domestic and wildlife species. In cattle, this assay can distinguish between *Brucella* infected animals, *Brucella* strain 19 vaccinated and animals infected with cross-reacting gram-negative bacteria. Briefly, the

samples are exposed to *Brucella abortus* smooth lipopolysaccharide (S-LPS) coated wells on microtiter plates together with a mouse monoclonal antibody (mAb) specific for an epitope on the o-polysaccharide portion of the S-LPS antigen. After an incubation period the microplate is washed and goat anti-mouse IgG antibody conjugate with horseradish peroxidase is added which binds to the mAb's. Unbound materials are removed by rinsing before the addition of substrate solution. The chromogen tetramethyl-benzidine with H<sub>2</sub>O<sub>2</sub> as substrate leads to a colour change which is read within 15 minutes with an ELISA reader at 450 nm. Samples were tested in duplicate.

## Statistical analysis

The Chi-square analysis in SAS was used to test differences of *Brucella* prevalence amongst age groups, sex and locations.

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## Results and discussion

Results obtained in this study show a seroprevalence of bovine brucellosis of 8.4% in the sera tested. This prevalence is within 4.88 to 9.64% reported by Shey-Njila (2004) in a survey conducted at the abattoir of Dschang, Cameroon. Similarly, the samples collected at the abattoir of Yaounde in Cameroon, indicated a seroprevalence of bovine brucellosis of between 7.2 and 8.8% (Shey-Njila et al. 2005). It was concluded that brucellosis was still enzootic in zebu cattle in areas where the cattle came from. Already in 1986 a survey carried out at the Institute of Animal Research at Bambui, Cameroon showed a prevalence of over 20% in cattle reared at ranches and 4% in those from traditional systems (IRZ 1985 and 1986). In the northern part of Cameroon, Bornarel et al. (1987) found a brucellosis seroprevalence of 12.5%. Several other studies reported seroprevalences ranging from 7 to 31% (Domenech et al. 1980, 1982a, 1982b, 1985; Bornarel et al. 1987, Akakpo and Bornarel 1987). Lefèvre (1991) concluded 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 Mali the prevalence was 22% (Toukara et al. 1994).

Animals selected for this work were Holstein cows. The *Brucella* seroprevalence in this population was quite high probably because they were kept in zero-grazing systems. In Eritrea however, Omer et al. (2000) found that only 8.2% of cattle infected with *Brucella* were kept in an intensive system. Cows used in the Heifer International Cameroon scheme are quite isolated from each other since the farms are quite remote. But it may be possible that milk used to feed calves in an infected herd could be sold to other dairy farmers for the feeding of calves. Otherwise, it could be possible that proximity with other farm animals (sheep, goats, dogs and pigs) serves as reservoirs for these herds. It will be interesting then to also screen the prevalence of *Brucella* in other farm animals. Furthermore the 'passing-on-the-gift' scheme used in this Non Governmental Organization, whereby farmers receiving a Heifer pay-back with another heifer which will be given to another farmer may also contribute to spreading the infection. In case a

cow is infected, the heifer calf will carry the infection to the neighbouring farm. A proportion of animals may also have been infected because of the common bull scheme whereby farmers take cows for breeding to the village bull although in this study, mature breeding bulls were not infected.

Of the 192 cows tested, 14 were infected giving a within-sex seroprevalence of 7.3% while 6/74 bulls were infected with a seroprevalence of 8%. In Nigeria, Ocholi et al (1996) also found no difference between sexes although the overall seroprevalence was 6.6%. There was no evidence ( $P=0.11$ ) of differences in seroprevalence of different age groups although animals above one year and below three years accounted for nearly half of the infected animals (Table 1).

**Table 1** Frequency and percentage of infected animals by age groups

Age (years)	Sex	Frequency (Infected/Tested)
≤1	Females	1/9
	Males	2/13
≥1 and ≤3	Females	4/51
	Males	4/36
≥3 and ≤5	Females	6/56
	Males	0/20
≥5	Females	3/76
	Males	0/5

NB 32 Animals did not have any labelled sex and 3 of such animals were infected.

The study also showed that *Brucella* infection varied with location ( $P=0.015$ ) with 3 out of 21 locations accounting for 64% percent of infected animals namely Kutaba (32%), Bamendankwe (16%) and Finge (16%). These results indicate that a specific control programme should be organized at these locations. Schelling et al. (2003) working in Tchad found that 3.8% of people working with livestock were infected with *Brucella*. This is an alarmingly high value which emphasizes that the dairy farmers selected for this study should take special measures to avoid infection by *Brucella*. Measures should be taken to ensure the eradication of the disease within the population and a sound testing and control system instituted to prevent the further spread of the disease to the larger cattle populations in the zone. 1) All farmers should be advised to boil milk before consumption. 2) Another test should be done to confirm positive animals, after which all infected animals should be slaughtered. 3) Care should be taken in areas of high prevalence to determined the possible causes of the spread of the infection. 4) Vaccination against *Brucella* should be instituted. This will be done every 3 years for the same animals as the vaccine lasts for that period of time. The vaccinated animals should be ear tagged or marked. However, care should be taken not to vaccinate pregnant animals. 5) To ensure a sound population of Holstein cows within the HPI scheme, this ELISA test should be repeated annually since it is a more sensitive test compared to the Rose Bengal, standard agglutination and Coombs test in the diagnosis of brucellosis (Saz et al. 1987). 6) Artificial insemination with healthy semen should be imperative for the

Heifer International scheme to avoid contamination of these valuable animals from infected bulls.

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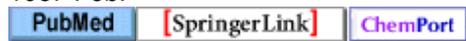
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