



## Risk factors associated with brucellosis seropositivity among cattle in the central savannah-forest area of Ivory Coast

Moussa Sanogo<sup>a,b,c</sup>, Emmanuel Abatih<sup>b,\*</sup>, Eric Thys<sup>b</sup>, David Fretin<sup>d</sup>, Dirk Berkvens<sup>b</sup>, Claude Saegerman<sup>c</sup>

<sup>a</sup> LANADA/Central Veterinary Laboratory of Bingerville, P.O. Box 206, Bingerville, Cote d'Ivoire

<sup>b</sup> Unit of Biostatistics and Epidemiology, Department of Animal Health, Institute of Tropical Medicine (ITM), Nationalestraat, 155, B-2000 Antwerp, Belgium

<sup>c</sup> Research Unit of Epidemiology and Risk Analysis Applied to Veterinary Sciences (UREAR-ULg), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster 20, B42, B-4000 Liege, Belgium

<sup>d</sup> Department of Bacteriology and Immunology, Veterinary and Agro-chemical Research Centre (VAR), Uccle, Groeselenberg 99, 1180 Brussels, Belgium

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### ABSTRACT

Serological results obtained from 907 serum samples collected from unvaccinated cattle of at least 6 months of age in the savannah-forest region of Ivory Coast were used to investigate risk factors associated with bovine brucellosis seropositivity. Serum samples were tested using the Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA). Using a parallel interpretation, RBT and iELISA results showed that 10.3% (95% confidence interval (CI): 8.4, 12.4) of the cattle had antibodies against *Brucella* in our study area. The logistic regression analysis indicated that brucellosis seropositivity was associated with age and herd size. Cattle above 5 years of age were found to have a higher chance of being seropositive (odds ratio (OR) = 2.8; 95% CI: 1.3, 6.4) compared to cattle under 3 years of age. Similarly, the odds of brucellosis seropositivity for herds with more than 100 cattle was 3.3 (95% CI: 1.2, 8.9) times higher compared to those with less than 50 cattle.

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## 1. Introduction

Bovine brucellosis is a widespread infectious disease caused by Gram negative bacteria of the genus *Brucella*. The infection is generally caused by one of the *Brucella abortus* biovars (1, 2, 3, 4, 5, 6 and 9). However, some cases of bovine brucellosis have been occasionally related to *Brucella melitensis* and *Brucella suis* (Verger et al., 1989; Godfroid et al., 2005; Olsen and Hennager, 2010).

As in most African countries, bovine brucellosis has been a problem among livestock for many years in Ivory Coast (Gidel et al., 1974; Pilo-Moron et al., 1979; Camus, 1980a; Angba et al., 1987; Thys et al., 2005; Sanogo et al., 2008). The first evidence of the disease was provided by Bohnel

during a serological study in the northern part of the country in 1970 (Pilo-Moron et al., 1979). Thereafter, several studies have been conducted to assess the impact of the disease on livestock production. For example, a national survey estimated the seroprevalence of brucellosis to be around 11.3% (Angba et al., 1987). Estimates obtained during earlier investigations undertaken in the Guinean region were 3.6% and 4.2% in dairy farms and traditional herds, respectively (Thys et al., 2005). In the central savannah-forest region of the country, the true prevalence was found to range between 5 and 16% in traditional herds (Sanogo et al., 2008).

Through its negative impacts such as abortions, decreases in milk production, increases in calf mortality, infertility and veterinary costs, bovine brucellosis is one of the most important pathological constraints for livestock development in Ivory Coast. Angba et al. (1987) estimated the impact of bovine brucellosis to be around 10% of the

\* Corresponding author. Tel.: +32 3 2476392; fax: +32 3 2476268.  
E-mail address: [enjiabatih@itg.be](mailto:enjiabatih@itg.be) (E. Abatih).

annual income of livestock breeders in Ivory Coast. Consequently, vaccination was practised from 1978 to 1982 and also in the 1990s to reduce abortion and mortality rates of brucellosis and limit its impacts (Camus, 1980b, 1995; Angba et al., 1987). Currently, there is no official control or official vaccination programme against brucellosis in Ivory Coast.

Previous investigations mainly demonstrated the presence of antibodies against *Brucella* in different areas of Ivory Coast and/or characterized circulating strains of *B. abortus* (Sanogo et al., unpublished data) but potential risk factors associated with bovine brucellosis have not been reported. The aim of this study was therefore to assess and describe potential risk factors associated with its seropositivity among cattle in the central Soudano-Guinean regions of Ivory Coast. The results of this study might be helpful for developing and implementing control measures aimed at sensitizing farmers, regulating herd management practices and ultimately on abating the prevalence of brucellosis among livestock in Ivory Coast.

## 2. Materials and methods

### 2.1. Study area and data

Ivory Coast is a West African country located between 3–9° West and 5–11° North. It is divided into three main agro-ecological areas: *The Guinean zone*, which is the most humid with annual rainfall generally above 1500 mm, covers almost the whole forest region in the south. *The Soudanean zone* is in the northern savannah regions and registers between 900 and 1200 mm of rainfall per year. *The Soudano-Guinean zone* covers the central savannah-forest regions and serves as a transition zone between the south and the north with annual rainfall of 1200–1500 mm. The northern savannah and central savannah-forest regions are the main livestock breeding areas of the country with nearly 80% of the one million and a half cattle heads of the country (FAO, 2009). Four cattle breeds belonging to two main types are encountered in the country: The *N'Dama*, the *Baoulé*, the *Lagunaire* which are West African humpless shorthorn breeds (*Bos taurus* type) and the longhorn humped zebu of *Bos indicus* type. In addition, various cross-bred of these two main types (*B. taurus* × *B. indicus*) are also met. All types are met in the central Soudano-Guinean zone where samples used in this study were obtained.

The data of this study are composed of two datasets obtained during two surveys undertaken, respectively three (dataset 1) and seven years (dataset 2) after the socio-political crisis of 2002 on sedentarily managed cattle herds in the savannah-forest areas of Ivory Coast. The first dataset comprised data collected in 2005 as part of a sero-epidemiological survey on rinderpest. Forty-four cattle herds out of 214 herds of the savannah-forest area were randomly selected from a list of 1265 herds. Fifteen blood samples were taken in each selected herd. The second dataset was built from data collected in 2009 during a survey of brucellosis. Six cattle herds with a history of abortion were conveniently chosen and sampled. At least

25 cattle were blooded within each herd. In both cases, only cattle above six months of age were included.

A total of 1036 blood samples were collected and submitted to serological assays among which were 660 and 376 samples, respectively for 2005 and 2009. Each blood sample was collected with information on the date of collection, the locality of origin, the type of breed, age, sex and size of the herd of origin. Since the early 1990s, there is no official control program or official vaccination against brucellosis in Ivory Coast.

### 2.2. Serological analysis

After collection, blood samples were allowed to clot at ambient temperature and centrifuged at 3000 rpm for 10 min. Sera samples were collected and stored at –20 °C until testing. Each serum sample was screened for anti-*Brucella* antibodies by Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA). RBT was applied as described by Alton et al. (1988) by mixing 30 µL of serum with 30 µL of antigen (*B. abortus* strain Weybridge 99) on a glass plate. After 4 min of reaction, the test was recorded as negative when no agglutination occurred and considered positive whenever a level of agglutination was noticed. For the indirect ELISA, the test was done as previously described by Limet et al. (1988) and Godfroid et al. (2002) using *B. abortus* biotype 1 (Strain Weybridge 99, A epitope) as antigen, protein G-Horseradish Peroxidase (G-HRP) as bovine anti-IgG conjugate and serum No. 1121 as positive reference. Briefly, 50 µL of serum were diluted in duplicate on plates and incubated at room temperature. After 1 h, antibodies binding were revealed by protein G-HRP conjugate. Conjugate was also incubated for 1 h at room temperature. Then, protein G activity was revealed by a citrate-phosphate buffer containing 0.4% of O-phenylenediamine and 2 mM of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Optical densities (OD) were read at 492 nm with a differential wavelength of 620 nm. Seropositivity was determined at a cut-off value of 2 unities, defined as threshold.

Serological status for a given sera was given by a parallel test interpretation of the results of the two tests. Thus, a serum was regarded as serologically positive when a positive result was recorded for one of both RBT and iELISA. Assuming the sensitivity (Se) and specificity (Sp) values for both RBT and iELISA to be: Se (iELISA)=96.1% (95% Credibility Interval (Cr.I.): 92.7, 99.8), Sp (iELISA)=95.0% (95% Cr.I.: 91.2, 99.5), Se (RBT)=54.9% (95% Cr.I.: 23.5, 95.1), Sp (RBT)=97.7% (95% Cr.I.: 95.4; 99.4) in our context (Sanogo et al., unpublished data), the combined diagnostic sensitivity and specificity for RBT and iELISA interpreted in parallel were estimated to be, respectively 98% and 93% (Thrusfield, 2005).

### 2.3. Statistical analysis

Serological results (seronegative=0/seropositive=1) and information on locality of origin, herd size (<50, 50–100, >100), breed (*B. taurus*, *B. indicus*, cross-bred), age (<3 years, 3–5 years, ≥5 years) and sex (cows or bulls) were recorded for all cattle considered for the study.

**Table 1**

Serological detection of antibodies against *Brucella* among 907 unvaccinated cattle from the Soudano-Guinean areas of Ivory Coast using Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA).

Serological results	Number of cases (%)
RBT (+)	44(4.9%)
RBT (–)	863(95.1%)
iELISA (+)	84(9.3%)
iELISA (–)	823(90.7%)
RBT (+) iELISA (+)	35(3.9%)
RBT (+) iELISA (–)	9(1.0%)
RBT (–) iELISA (+)	49(5.4%)
RBT (–) iELISA (–)	814(89.7%)
RBT or iELISA (+)	93(10.3%)
RBT or iELISA (–)	814(89.7%)

RBT: Rose Bengal test; iELISA: indirect enzyme linked immunosorbent assay; (+): positive; (–): negative.

Unconditional and conditional logistic regression models were used to assess the association between brucellosis seropositivity and risk factors by setting herds as primary sampling unit and by including weights derived from the sampling fraction in each selected herd. A single stratum was considered. The analysis was conducted in two steps. Firstly, unconditional regression models were used to investigate the association between each risk factor and brucellosis seropositivity. Based on the unconditional analysis, only variables with  $p \leq 0.25$  were considered for further analysis. The second stage of the analysis consisted in building a conditional logistic regression model based the potential risk factors identified from the unconditional models. The most appropriate model was selected using the backward stepwise selection approach. The effects of confounding were investigated by observing the changes in the estimated odds ratios of the variables that remained in the model once a non-significant variable was included. When the addition of a variable led to a change of more than 25% in the estimated regression coefficients or odds ratios, that variable was considered as a confounder and was not removed from the model. All pairwise interactions between the variables in the final model were examined for significance.

Goodness of fit of the final model was assessed using the *F*-adjusted mean residual test (Archer and Lemeshow, 2006; Archer et al., 2007). All statistical analyses were performed using STATA, version 12, software (Stata Corp., College Station, Texas). Due to differences in sampling period and strategy across the surveys, the two datasets were analysed separately and the results were compared.

### 3. Results

#### 3.1. Serological results

A total of 907 out of 1036 blood samples collected gave serological results to both RBT and iELISA and were considered for further analysis. Both combined and separate serological results for RBT and iELISA are presented in Table 1. Together, the two tests identified 93/907 (10.3%) sera as serologically positive. When a single test was

used, only 84/93 (90.3%) and 44/93 (47.3%) were, respectively classified as serologically positive by iELISA and RBT. Indirect ELISA identified almost twice more sera (9.3%) as positive compared to RBT (4.9%). Serological results adjusted for the survey design effect for dataset 1 indicated that 11.2% (95% CI: 5.4, 17.1) of the sera tested ( $n=614$ ) had antibodies against *Brucella* using a parallel interpretation scheme with the two tests. Highest seropositive results were registered among cattle above 5 years of age (17.6%), among cattle sampled from herds with more than 100 heads (14.1%) and among cattle of *B. taurus* type (16.4%). Seropositivity was apparently quite the same among *B. indicus* and cross-bred types, ranging between 3.5 and 13.1% in our study area. Cows represented more than 80% of the study population and showed apparently almost the same proportion of seropositivity cases compared to bulls. Twenty-seven herds out of 44 sampled (61.4%; 95% CI: 45.5, 75.6) had at least one seropositive cattle for brucellosis in our study area.

For the convenient sample ( $n=293$ ), the seroprevalence of antibodies against brucellosis was estimated to be 15.5% (95% CI: 0.0, 30.6). The number of serologically positive sera among different factors showed the same trends as for dataset 1 except for breeds where cross-bred (21.9%, 95% CI: 0.0, 43.4) had twice more seropositive sera than *B. indicus* (10.5%, 95% CI: 0.1, 11.8). In this sample, four herds out of 6 gave at least one cattle positive to both RBT and iELISA. Serological results per variable are summarized in Table 2 for both datasets.

#### 3.2. Logistic regression analysis

Based on the unconditional regression model, factors as age, herd size, breed and locality showed a  $p$  value  $< 0.25$  and were considered as potential risk factors in the conditional logistic regression model. Out of these four potential risk factors, only age and herd size were included in the final model. None of the two-way interaction terms were statistically significant ( $p > 0.05$ ). In addition, there were no confounding factors. The estimated odds ratios and their 95% confidence intervals are presented in Table 3. The *F*-adjusted mean residual goodness of fit test suggested no evidence of lack of fit of the final model using dataset 1 (*F*-adjusted test statistic = 1.08,  $p=0.4$ ).

These results suggest that cattle above 5 years of age had significantly higher odds of brucellosis seropositivity compared to those younger than 3 years old (OR = 2.8; 95% CI: 1.3, 6.3). Similarly, the odds of brucellosis seropositivity for herds with more than 100 cattle were 3.3 (95% CI: 1.2, 8.9) times higher compared to those with less than 50 cattle (Table 3).

Logistic regression analysis of the convenient sample (dataset 2) led to almost the same conclusion where only age was found to be independently associated with the seropositivity of brucellosis with quite similar odds (OR = 2.5; 95% CI: 1.5, 4.2). In the latter case, herd size was not proven to be a significant risk factor for brucellosis seropositivity.

**Table 2**

Potential risk factors associated with brucellosis seropositivity among cattle from Soudano-Guinean zone of Ivory Coast using parallel interpretation of Rose Bengal test (RBT) and indirect enzyme linked immunosorbent assay (iELISA). The results are based on data from a simple random sample collected in 2005 and a convenient sample collected in 2009.

Variables	Category	Dataset 1 (2005)		Dataset 2 (2009)	
		<i>n</i>	Seropositivity (95% CI)	<i>n</i>	Seropositivity (95% CI)
Locality <sup>a,b</sup>	Bongouanou	210	9.5(2.5,16.6)	–	–
	Dimbokro	177	17.1(7.0,27.1)	104	19.6(0.0,43.7)
	Tiebissou	90	5.5(1.8,9.2)	–	–
	Toumodi	66	6.2(3.3,9.2)	25	0.0(0.0,11.3)
	Yamoussoukro	71	3.8(0.0,8.6)	164	14.9(0.0,34.7)
Herd size <sup>a</sup>	<50	256	6.4(3.3,9.4)	51	0.0(0.0,5.7)
	50–100	282	10.8(4.9,16.7)	–	–
	>100	76	14.1(1.6,26.7)	242	17.7(1.5,33.9)
Breed <sup>a,b</sup>	<i>B. indicus</i>	44	8.3(3.5,13.1)	38	10.5(9.1,11.8)
	Cross-bred	354	7.9(3.6,12.2)	117	21.9(0.3,43.4)
	<i>B. taurus</i>	216	16.4(5.4,27.4)	138	12.2(0.0,32.0)
Sex	Bulls	134	10.9(3.8,18.0)	27	13.4(0.0,30.3)
	Cows	480	11.3(5.5,17.1)	266	15.7(0.6,30.8)
Age <sup>a,b</sup>	<3	203	8.8(0.6,16.9)	63	9.7(0.0,24.9)
	3–5	201	8.5(2.5,14.5)	148	14.8(3.8,25.8)
	≥5	210	17.6(10.4,24.7)	82	21.5(0.0,48.0)

CI: confidence interval; *n*: number of sera tested.

<sup>a</sup> Variables with *p* < 0.25 in dataset 1 (2005).

<sup>b</sup> Variables with *p* < 0.25 in dataset 2 (2009).

#### 4. Discussion

The main objective of this study was to investigate the potential risk factors associated with brucellosis seropositivity among cattle reared in Ivory Coast. Thus, serological results obtained from two sero-epidemiological surveys undertaken in Soudano-Guinean savannah-forest region with 4 years interval were used for this purpose. The data came from the same non-vaccinated target cattle population, managed sedentarily in absence of any official control programme.

Without an appropriate gold standard test useful to detect infected animals with certainty, results of RBT and iELISA were used as outcome variable for this risk factor analysis. The two tests are convenient and suitable serological tests for screening of brucellosis antibodies (Saegerman et al., 2004; Corbel, 2006). They are regarded as very sensitive tests but may demonstrate some limitations as other serological tests for brucellosis when used alone for individual testing (OIE, 2009). No single serological test is appropriate for all epidemiological situations.

When applied individually in some contexts, RBT or iELISA may suffer from possible false positive reactions due to gram negative bacteria closely related to *Brucella* such as *Yersinia enterocolitica* O:9, *Escherichia coli* O157:H7, *Xanthomonas maltophilia* and *Salmonella urbana* (Saegerman et al., 2004). Then, RBT was combined with iELISA in a parallel interpretation scheme for this study. This combination is expected to reduce the occurrence of misclassification and increase the chance to detect antibodies against brucellosis when present for a given sera. Even if RBT and iELISA are not absolutely immunologically independent (Nielsen, 2002), their association is also expected to detect antibodies of both acute and chronic cases, improving the testing sensitivity. RBT is known to detect IgG<sub>1</sub> and IgM produced during acute cases of brucellosis while iELISA is more appropriate for detecting IgG dominant in chronic cases (Nielsen, 2002; Saegerman et al., 2004). In our context, iELISA classified twice more sera as serologically positive cases suggesting a possible predominance of IgG, indicating a chronic infection context. Results also indicated that about 10–50% of sera showing antibodies against

**Table 3**

Conditional logistic regression analysis of risk factors associated with brucellosis seropositivity among cattle from the Soudano-Guinean areas of Ivory Coast.

Variables	Category	Dataset 1 (2005)				Dataset 2 (2009)			
		OR	95% CI	S.E.	<i>p</i>	OR	95% CI	S.E.	<i>p</i>
Age	<3	1.0	–	–	–	–	–	–	–
	3–5	1.1	(0.6, 1.8)	0.285	0.824	1.6	(0.6, 4.1)	0.580	0.241
	≥5	2.8	(1.3, 6.4)	1.138	0.013	2.5	(1.5, 4.2)	0.496	0.005
Herd size	<50	1.0	–	–	–	–	–	–	–
	50–100	1.9	(0.8, 4.3)	0.770	0.140	–	–	–	–
	>100	3.3	(1.2, 8.9)	1.628	0.023	–	–	–	–

OR: odd ratio; CI: confidence interval; S.E.: linearized standards error; *p*: *p*-value.



brucellosis would have been classified as serologically negative in a single testing approach by iELISA or RBT, underestimating the seroprevalence of brucellosis. Thus, since different status of the disease might coexist, a combination of tests could ensure a more effective diagnosis and control of brucellosis.

The regression analysis identified age of cattle and herd size as risk factors associated with brucellosis seropositivity in our study population. The separate regression built with the two datasets resulted in quite similar odds of brucellosis seropositivity for age. However, the analysis failed to confirm herd size as a risk factor with the convenient sample (dataset 2). This could be related to the difference in sampling strategy. The six cattle herds conveniently chosen during the second survey were less representative of the cattle population of the savannah-forest area than 44 randomly herds selected with a comparatively structured sampling strategy.

Brucellosis seropositivity was found to be higher in herds with more than 100 cattle and among cattle above 5 years of age. These results are in agreement with the findings of Akakpo (1987). A similar observation was made by Kadohira et al. (1997) for whom cows kept over 4 years old, on a large farm and grazing on a community pasture had more chance to be seropositive than younger cows (Kadohira et al., 1997). Association of herd size with the risk of exposure to *Brucella* infection could be related to a high cattle density on pasture and consequently to an increased chance of contact with infection when present (Camus, 1980a; Akakpo, 1987). Since the Soudano-Guinean zone offers more space for livestock breeding and for grazing compared to the south, it has concentrated herds from different regions of the country during the last years subsequent to the socio-political crisis. According to McDermott and Arimi (2002), the incidence of brucellosis decreases when the herd size decreases in pastoral production systems. That statement is in agreement with our findings where small herds count less seropositive cattle. The effect of herd size was also mentioned in different contexts across Africa (Nicoletti, 1984; Muma et al., 2007; Jergefa et al., 2009). In our study area, brucellosis seropositivity was also found to be associated with the age of animals sampled. The influence of age on seroprevalence has already been mentioned in some previous brucellosis studies (Kadohira et al., 1997; Kubuafor et al., 2000; Faye et al., 2005; Muma et al., 2006; Chimana et al., 2010) but conversely in case of false positive serological reaction for which seropositivity is not linked with age (Saegerman et al., 1997; Pouillot et al., 1998). Age is known as one of the intrinsic factors influencing brucellosis seropositivity (Megersa et al., 2011). This influence could be explained by the fact that the older an animal, the higher the likelihood of contact with infected animals and therefore an increased accumulated chance of becoming seropositive. In Ivory Coast, screening and stamping out are not practised. Consequently, animals with infection could be kept in herds for long time periods without being screened for the presence of brucellosis. Absence of culling strategies and the fact of keeping old cattle in herds could result in large herd sizes and might explained the relationship observed between age and herd size with regard to seropositivity in our context.

No statistically significant effect was demonstrated for breed type with our data. Nevertheless, the seroprevalence trends observed are in agreement with some previous studies (Chantal and Thomas, 1976; Akakpo, 1987) where it was argued that *B. taurus* and *B. indicus* crossbred were more susceptible to *Brucella* infection than *B. indicus*. Moreover, as previously observed by Ocholi et al. (1996), our data did not show any significant difference between bulls and cows regarding the seropositivity. It should be noticed that more than 80% of the samples came from cows, limiting comparison with bulls in our context.

## 5. Conclusion

In this paper, we investigated risk factors for bovine brucellosis seropositivity in the savannah-forest area of Ivory Coast. This investigation revealed that the age of animal tested and herd size were independently associated with seropositive cases of brucellosis, with highest risk of seropositivity among larger herds and among cattle above 5 years of age. Although the presence of antibodies does not necessarily mean cattle are infected, these preliminary results indicate the presence of brucellosis in the country. Further information on risk factors at individual level and also at herd level would be helpful for setting an efficient control programme. However this study should be considered as a contribution to the epidemiology of bovine brucellosis in Ivory Coast.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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