Prevalence and Factors Associated with Cattle Brucellosis in Animal Herds of the Namibe Province in Angola

Franco Mufinda
Ministry of Health, Angola

Carla Nunes
Universidade NOVA de Lisboa

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Franco C. Mufinda [1, 2,*], Fernando S. Boinas[3], Carla S. Nunes[4]
[1]Public Health (Epidemiology) at Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Portugal
[2]Departamento Provincial de Saúde Pública e Controlo de Endemias do Namibe-Angola.
Rua Padre Carlos Esterman, Namibe, Angola
[3]Faculdade de Medicina Veterinária, Universidade de Lisboa, Portugal
[4]Escola Nacional de Saúde Pública (ENSP), Universidade Nova de Lisboa, Portugal

Key words
Bovine Brucellosis; Prevalence; Risk factors, Namibe, Angola

ABSTRACT:

Brucellosis is considered to be one of the most neglected zoonosis worldwide. In Angola data about brucellosis has not been consistently reported to the World Organization for Animal Health. Therefore, the aim of this study was to estimate the seroprevalence of bovine brucellosis and to identify the risk factors associated with this disease. A seroepidemiological survey was undertaken on 1344 cattle from 192 herds between February and December 2012. The testing for brucellosis was performed using the Rose Bengal test (RBT) for serum analysis and a questionnaire was administered to local farmers. After a descriptive approach, Chi-Square Tests of Independence and Spearman correlations were used, followed by logistic regressions tests in order to determine the odds ratio and confidence interval of risk factors associated with brucellosis. Overall, the seroprevalence of brucellosis in animals and herds was 14.96% (CI95%: 4.20-25.71) and 40.10% (CI95%: 29.14-51.06), respectively. A positive correlation was found between the proportion of animals infected per herd and the average number of abortions in those facilities ($r_{(192)} = 0.531$, $p < 0.001$). The risk factors associated with brucellosis seroprevalence were: inadequate facilities for isolation of infected animals (OR = 6.66; CI95%: 1.16-38.34); purpose of production other activity (social representation and economic power) than meat (OR = 3.13; CI95%: 1.07-9.19); abortions (OR = 3.98; CI95%: 1.92-8.28); female infertility and neonatal mortality (OR = 3.07; CI95%: 1.42-6.65). Our data shows that brucellosis prevails in the Namibe province as 40.10 % of farms are infected. The number of abortions is clearly correlated with the number of herds infected. Local veterinary authorities have a fundamental role in what concerns not only the disease control and surveillance but also in the development and implementation of farmers' educational programmes.

*Corresponding Author : **Franco C. Mufinda**: mufinda@portugalmail.com

1. INTRODUCTION

Brucellosis is an anthropozoonosis caused by *Brucella*, a gram negative coccobacillus measuring 0.5-0.7 by 0.6-1.5 µm (Elberg, 1981; OIE, 2009 ; Nielsen and Yu, 2010). *Brucella mellitensis*, first identified in Malta in 1887 by David Bruce, is a species that infects man and has goats and sheep as the main hosts (Falagas and Bliziotis, 2006). *B. abortus*, with cattle as principal host, was first identified by Bang in 1897 and *B. suis* was identified by Traum in pigs in 1914. In 1914, Alice Evans showed a taxonomic relation between *B. abortus* and *B. mellitensis* and identified the first *Brucella* in humans in the USA. After that, many other species have been identified namely *B. ovis, B. canis, B. neotomae, B. microti, B. ceti, B. pinnipedialis and inopinata* (Carter, 1988; OIE, 2009; Nielsen and Yu, 2010; Scholz et al., 2010).

In the world the most serovars reported in the literature for each species are nine for *B. abortus* (Priyantha, 2011), three for *B. melitensis*, and five for *B. suis* (Corbel, 1997; World Health Organization, 2005; OIE, 2009). *Brucella biovars* are known to be regionally restricted in their distribution (Valdezate et al., 2010).

The diagnosis of brucellosis is carried out by detection of the agent or by serology to confirm the clinical symptoms and the evaluation of risk factors by anamnesis (Carvalho et al., 2014). In animals, FAO established as laboratory criteria (local or regional level), the first screening (sero-epidemiology) of animals should be based the Rose Bengal Test (RBT) (Elshemey and Abd-Elrahman, 2014) or Immunoenzymatic Test (ELISA), the sensitivity and then sera should be confirmed by more specific tests such as the Complement Fixation Test (Corner, 1993; FAO, 2002).

The control of brucellosis in cattle is potentially based on vaccination and serological testing of adult animals. The S19 vaccine may confuse the serological diagnosis affecting the interpretation of the test result (Center for Food Security & Public Health, 2009). The association of vaccination and elimination of infected animals increases the likelihood of elimination of brucellosis (Siegmund and Fraser, 1981; Costa, 1998; Center for Food Security & Public Health, 2009).

In Angola, brucellosis is a major concern for local sanitary authorities since the 1950's. Indeed, it was in 1955 in the Huambo province (ex-Nova Lisboa) that the first studies concerning animal screening were performed and isolated B. *abortus* (Baptista, 1991). Although this disease has been recognized for decades, the associated data has not been consistently reported to international organizations such as the World Organization for Animal Health and the vaccination programme is absent. Animals are not vaccinated against brucellosis (Baptista, 1991; Corbel, 1997; OIE, 2009).

The aim was to determine the seroprevalence of cattle brucellosis and to characterize the risk factors associated with this disease in cattle farms of the Namibe province in Angola.

2. MATERIALS AND METHODS

2.1. The study area

The Namibe is a coastal province in the South of Angola with a total area of 57.097 sqKm. It includes five municipalities and had an estimated population of 1.195.779 habitants in 2012 (Figure 1). In terms of animal production, official data reports the existence of 500.500 cattle, 36.800 pigs, 1.250.000 goats and 570.000 sheep (Angola, 2013). In this province, the animals are usually bred in close proximity to the farmers and their families. Farms are often composed of a central area where the animals are kept in an enclosure surrounded by a larger area where the houses, cooking areas and dairy production facilities are located. The human population of this ecosystem is split into settlement (Kimbos, onganda), where they can cohabit next to the owner, family members, or other individuals connected by kinship degree, as shown in Figure 2 (Kuval village).This province were selected for this study because (i) Brucella serorevelance using RBT were published in 2001 for most of the provinces; (ii) were not vaccinating their cattle against brucellosis using *B. abortus* S19 or any other approved vaccine. In the Namibe province, previous studies using the RBT indicated a 5% seroprevalence for human brucellosis in the Bibala and Kamucuio municipalities (Médicos Sem Fronteiras, 2001), and 27.7% and 9.7% seroprevalence of bovine brucellosis in the Virei and Bilaba municipalities, respectively (Ministério da Agricultura e Desenvolvimento Rural de Angola, 2005; Ministério da Agricultura e Desenvolvimento Rural de Angola, 2010).

Bibala municipality is located in Namibe province, with territorial extension of 7, 674 sqKm, between the 13º-21º coordinates of latitude and 14º and 46º latitude. There are four communes: Bibala, Kapagombe, Caitou and Lola. Its activities are essentially agriculture and Livestock. Is confined to the north with the Camucuio municipality, to the south by Huila Province, to the west by the Namibe Municipality. The livestock sector has a production system, associate to the traditional practice of grazing passerby, extremely dependent on watering points and the availability of grazing.

Kamucuio municipality has 7,452 sq Km of the land area. There are three communes Kamucuio, Mamue and Chingo. It is located north of Namibe and borders the northern province with Baia Farta municipality of Benguela province, south with Bibala municipality, to the east with Quilengues municipality of Huila Province and west by the Namibe municipality. Livestock farming is the main economic activity of the population, predominantly for cattle and goats. Due to drought, we witness cattle to grazing in areas near rivers and grazing, thus causing the existence of many nomadic populations.

Namibe municipality is limited to the north by the province of Benguela (Dombe Grande municipality), the Northeast with Kamucuio municipality, to the east with the Tômbwa municipality and to the west by the Atlantic Ocean. It has a land area of 8,916 sq Km. The livestock sector has two production systems: the traditional approach the practice of grazing passerby, extremely dependent on watering points and pasture availability, and business type.

Tombwa municipality is limited to the north with the Namibe municipality, the South with the Republic of Namibia, the Northeast with the Cunene Province and to the West by the Atlantic Ocean. Their extreme coordinates are: 15º 37 'South latitude North 17 10' - Latitude South, 12 30 '- Longitude East 11º 46' - West Longitude. The land area is 18,019 sq Km. the livestock sector has a production system, associate to the traditional practice of grazing passerby, extremely dependent on watering points and the availability of grazing.

Virei municipality has turned a geographic area of 15.092 sqKm, bordered to the north with the Bibala municipality of Namibe Province, to the south by the Kuroka municipality of Cunene Province, the east with the Humpata municipality of Huila province, southeast with Chibia and Ngambos municipalities of the Huila province, southwest to the Tômbwa municipality. It has two communes: Cainde and Virei. The livestock sector has a production system, associate to the traditional practice of grazing passerby, extremely dependent on watering points and the availability of grazing.

2.2. Study design

A cross-sectional study was performed to determine the prevalence of brucellosis in only sexually matures animals and herds and to identify and characterize the risk factors associated with this disease in September to December 2012.

2.3. Sampling

Herds were officially represented by farmers that were selected through a stratified random sampling (at municipally level) and taking into account the number of herds per municipality. Starting with a total of 1204 farmers registered for the Namibe province at the Institute of Veterinary Services, a minimum sample size of 192 farmers was calculated taking into account 5% human prevalence rate of brucellosis (Médicos Sem Fronteiras, 2001), assuming a margin of error of 3% and a confidence level of 95%. More 10% were done to compensate possible sample problems related with non/badly answered questionnaires. This number was them proportionally distributed by municipally. Due to time, financial or biological (only sexual mature animals could be included) limitations only seven female cattle aged one year or older were blood random sampled per herd resulting in a total of 1344 animals sampled. The average cattle herd size was 89.14 animals. The sensitivity and specificity for RBT considered are 98.1% (CI95% 96.8-99.1%) and 99.8% (CI95% 99.7-99.8%) (European Food Safety Authority, 2006).

2.4. Blood sampling and testing

Blood was collected by jugular vein puncture with a sterile disposable needle into a pre-identified vacuum tube. Sera samples were separate by centrifugation and frozen at -20°C until further analysis. Screening for brucellosis, using the RBT, was performed by a certified veterinary technician from the Veterinary Research Institute of Lubango in Huila as described by Alton et al. (1976). The study took into account the recommendations of the OIE, FAO and WHO on how to conduct RBT serological tests for brucellosis screening (Corner, 1993; European Food Safety Authority, 2006; OIE, 2009; Nielsen and Yu, 2010). After thorough mixing of the serum and antigen, agglutination must be visible be within in 4 minutes (Nielsen and Yu, 2010).

2.5. Epidemiological data collection

The questionnaire was developed based on a previous study from the General Veterinary Services – Ministry of Agriculture, Development and Fisheries of the Portuguese Republic (Ministério da Agricultura, do Desenvolvimento e das Pescas, 2011) which aimed at characterizing cattle herds and livestock management (Table 2). After the questionnaire was translated into the local dialect (Nhaneca-Umbi) by a certified translator with a background in health sciences under the supervision of previously trained local health officials fluent in the local dialect. This training included an overview of the disease and of the questionnaire's content, highlighting the need to avoid influencing the respondent.

The chosen model took into account the factors related with the disease prevalence and the variable "herd" corresponds to the identification of the farm through the name of the farmer, as identified in the Namibe Provincial Veterinarian Services. The farm size depend the number of animals and extension of the settlement.

Information was collected that included occurrence of abortions, fertility problems of breeding females and neonatal mortality, direct contact with cattle from neighbouring herds, sharing of equipment and tools with neighbouring herds, cases of brucellosis in the last five years, exit method of animals from the herd, transhumance practice/common grazing, history of human brucellosis, veterinary assistance, introduction of new animals in herds and/or in the area, time of the abortion, end product type (namely meat for self-consumption or other representing milk and other products), purpose of breeding considering reproduction, production for meat and milk and other for social status (increase richness or for traction in farm work), isolation facilities relating to the animal and/or village enclosure, and hygiene related with the amount of manure present, number of breeding females, number of breeding males and the number of abortions was provided by the farmers (not observed) (Table 2).

In this study the herds were considered infected if at least one of the animals sampled tested positive in the RBT.

This study considers as risk factors the farm features that are associated with increased infection likelihood and with the spreading of the brucellosis to surrounding farms. Because this is a transversal study, cause-effect relationships were not analysed and could be considered a study limitation. Additionally, as part of this study (questionnaire) is based on self-reported information and on the understanding of the content of the questionnaire by farmers, some bias could occur.

2.6. Ethical considerations

For ethical purposes official authorizations were obtained from the National Institute of Public Health – Ministry of Health of Angola – and from the Regional Veterinary Services of Namibe. After the data sampling (blood and questionnaires) farmers were informed about the risks and consequences of having infected animals and about the sanitary measures they needed to follow in case they had infected animals since, in Angola, the slaughter of infected animals is not compensated by the Government.

2.7. Statistical analysis

Statistical analysis was performed using the SPSS version 18.0 (SPSS, 2010) software. Statistical differences were considered significant if p<0.05. After an initial overview of the data through the use of descriptive statistics, the relationship between qualitative variables was tested using the Chi-square tests (with or without Monte Carlo simulation) or Fisher Exact tests. The study of correlations between quantitative variables was performed using the Spearman correlation test. To evaluate and characterize the significance of herd factors associated with cattle brucellosis logistic regressions were performed using the Enter and Forward method (Marôco, 2010). In addition, the determination of the probability of brucellosis occurring in a herd in the presence of certain variables was assessed using the following formula where α and β_i the unknown parameters estimated through the Forward: LR method (Marôco, 2010).

$$\hat{\pi} = \frac{1}{1 + e^{-(\alpha + \sum \beta_i X_i)}}$$

2.8. Prevalence assessment

Prevalence of brucellosis in animals ($P_a A$) and farms ($P_a E$) were assessed using these formulas:

$$P_a\,A = \frac{aA}{nA} \quad \text{and} \quad P_a\,E = \frac{aE}{nE}$$

where: aA= number of animals that tested *Brucella* seropositive in the RBT, nA= total number of animals tested, aE= number of herds flagged as *Brucella* seropositive, nE= total number of herds tested.

3. RESULTS

Table 1 Seroprevalence of brucellosis in animals and herds per municipality in Namibe province in Angola using Rose Bengal Test (RBT).

Municipality	NEt	NE	nA	Einf	Ap	$P_a E$(%) 95% CI	$P_a A$(%) 95% CI
Namibe	51	9	63	4	20	44.40 (41.32-47.47)	31.75 (26.71-36.79)
Tombwa	11	7	49	4	15	57.10 (53.40-60.79)	30.61 (26.30-34.92)
Bibala	748	113	791	48	113	42.50 (33.55-51.45)	14.28 (6.39-22.17)
Kamucuio	276	44	308	14	39	31.80 (27.54-36.06)	12.66 (8.29-17.03)
Virei	118	19	133	7	14	36.80 (33.43-40.17)	10.53 (8.13-12.93)
Total	**1204**	**192**	**1344**	**77**	**201**	40.10 (29.14-51.06)	14.96 (4.20-25.71)

NEt: Number of herds represented by farmers, NE: Number of herds tested, nA: Animal samples, Einf: infected cattle herds, Ap: *Brucella* seropositive animalsPositive Animals, P_aE: Prevalence of disease in cattle herds based on RBT (herd prevalence), P_aA: Prevalence of disease in animals based on RBT (individual prevalence).

Overall, the prevalence of brucellosis was 15.0 % (95% CI: 13.15-16.96) in individual animals and 40.1% (95% CI: 33.43-47.17) in herds in the Namibe province in Angola in 2012 (Table 1). The Namibe municipality had the highest rate of infected animals (31.8%) while the Virei municipality presented the lowest rate (10.5%). Concerning the herds infection rates, Tombwa municipality showed the highest value (57.1%) and Kamucuio municipality presented the lowest one (31.8%).

The correlations between the variables analyzed and the presence of brucellosis in herds are presented in Table 2. Additionally, Table 3 shows the odds ratio (OR) of the significant variables showed in Table 2.
In infected herds we verified that 78.0% (60 out of 77 herds) had no veterinary assistance although this variable was not significant when compared with herds without brucellosis cases (p= 0.289). Three quarters (75.3%) of the herds reported the occurrence of abortions (58 out of 77 herds; OR=4.26, 95% CI: 2.25-8.06; p< 0.001) and 80.5% (62 out of 77 herds; OR=3.92, 95% CI: 2.00-7.68; p< 0.001) reported either fertility problems in breeding females or neonatal mortality. Except for one herd, all infected animals were in direct contact with cattle from other farmers (98.7% or 76 out of 77 farms; p= 0.065). Additionally, it was found that it was common for farmers to introduce animals from other herds (93.5% or 72 out of 77 herds; p=0.900). Over half of the farmers (51.9%; 40 out of 77 farms) report sharing equipment and tools with neighbors (OR=2.19;95% CI: 1.21-3.96; p= 0.009). Only one herd reported having cases of brucellosis (history) in the last five years (p= 0.809) and all farmers reported having sold animals. The greatest number of infected animals was found in the Bibala municipality (p= 0.640). Finally, farmers reported that 50% of abortions occurred in the second trimester and only 10.3% in the third trimester (p>0.99).

The purpose of most farms (92.2%) was to sell products such as milk (OR=4.18%; 95% CI: 1.64; 10.60; p= 0.003) compared with those that use products for self-consumption. Nearly half of the farms (48.1%, 37/77) use their animals for production (OR=0.21 95% CI: 0.09; 0.46; p< 0.001) compared with “reproduction” purpose. All herds used common pasture. Only 5 farms (6.5%) consider having bad facilities for infected animal isolation (OR= 3.23, 95% CI: 0.80; 12.93; p= 0.097); 81.8% (63/77) had average hygiene (p=0.198) and 7.8% had history of human brucellosis.

The average number of abortions per herd was 0.07±0.09 with a minimum of no abortions and a maximum of 0.42. In addition, the number of *Brucella* seropositive animals per herd showed a moderate positive correlation with the average number of abortions ($\mathbf{r}_{(192)}$= 0.531, p< 0.001) (Table 4). We also found significant differences in prevalence rates of *Brucella* seropositive animals between municipalities (p <0.001) (Table 5).

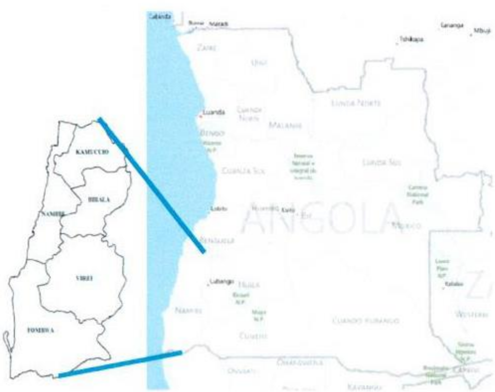

Figure 1 Map of the Namibe province in Angola

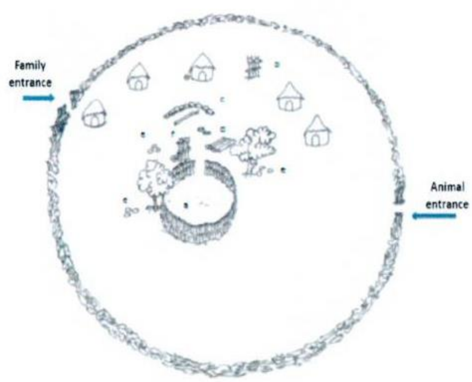

Figure 2 Schematic representation of the layout of most villages in Namibe province in Angola using the Kuval village as example; a – Animal enclosure. b – Sacred altar for religious celebrations. c – Sacred fire. d – Meat storage. e – Cooking sites. f – Site for the preparation of dairy products (adapted from Morais, 1974)

Table 2 Evaluation of risk factors associated with brucellosis infection in cattle herds of the Namibe.

Variable	Level	Total n(%)	Brucella seropositive herds using Rose Bengal TestHerds		*p*
			Positive n (%)	Negative n (%)	
Veterinary care	Yes Seldom No	14(7.3) 19(9.9) 159(82.8)	8(10.4) 9(11.6) 60(78.0)	6(5.2) 10(8.7) 99(86.1)	0.289*
Occurrence of abortions	Yes No	106(55.2) 86(44.8)	58(75.3) 19(24.7)	48(41.7) 67(58.3)	<0.001*
Low fertility of breeding females or neonatal mortality	Yes No	121(63.1) 71(36.9)	62(80.5) 15(19.5)	59(51.3) 56(48.7)	<0.001*
Direct contact of animals with cattle from neighbors	Yes No	183(95.3) 9(4.7)	76(98.7) 1(1.3)	107(93.0) 8(7.0)	0.065*
Number of animals introduced	Cattle farms Assembly centers	179(93.2) 13(6.8)	72(93.5) 5(6.5)	107(93.1) 8(6.9)	0.900*
Sharing equipment/tools with other cattle farms	Yes No	78(40.6) 114(59.4)	40(51.9) 37(48.1)	38(33.1) 77(66.9)	0.009**
Cases of brucellosis in the last five years	Yes No	3(1.6) 189(98.4)	1(1.3) 76(98.7)	2(1.7) 113(98.3)	0.809**
Sells animals	Yes No	192(100.0)	77(100.0)	115(100.0)	NA
Trimester of abortions	1st Trimester 2nd Trimester 3rd Trimester	42(39.6) 53(50.0) 11(10.4)	23(39.7) 29(50.0) 6(10.3)	19(39.6) 24(50.0) 5(10.4)	1*
Cattle herds per municipality	Namibe Tombwa Bibala Kamucuio Virei	9(4.7) 7(3.7) 113(58.8) 44(22.9) 19(9.9)	4(5.2) 4(5.2) 48(62.3) 14(18.2) 7(9.1)	5(4.3) 3(2.6) 65(56.5) 30(26.1) 12(10.5)	0.640***
End-product	Meat Other (social representation, economic power and sale)	36(18.7) 156(81.3)	6(7.8) 71(92.2)	30(26.1) 85(73.9)	0.001**
Purpose of breeding	Reproduction Production Other	38(19.7) 128(66.8) 26(13.5)	25(32.5) 37(48.1) 15(19.4)	13(13.3) 91(79.1) 11(9.6)	<0.001*
Practice of transhumance/common grazing	Yes No	192(100) 0 (0,0)	77(100.0) 0(0.0)	115(100.0) 0(0.0)	NA
Isolation facilities	Good Average Poor	55(28.7) 127(66.1) 10(5.2)	13(16.9) 59(76.6) 5(6.5)	42(36.5) 68(59.1) 5(4.4)	0.013*
Hygiene (appreciation of the hygienic conditions of cattle farms)	Good Average Poor	10(5.2) 154(80.2) 28(14.6)	6(7.8) 63(81.8) 8(10.4)	4(13.5) 91(59.1) 20(17.4)	0.198*
History of Human brucellosis (farmer)	Yes No	10(5.2) 182(94.8)	6(7.8) 71(92.2)	4(3.5) 111(96.5)	0.187**

*Chi-square test, **Fisher test, ***Chi-square test with Monte Carlo simulation, NA – not Applicable

Table 3. Odds ratio and confidence interval of risk factors associated with Brucella seropositivity using Rose Bangal Test in cattle herds in Namibe province in Angola (2012)

Variable	**level**	***p***	**OR 95% CI**	***p***	**OR Adjusted** 95% CI**	α, β_i***
Occurrence of abortions	No*					
	Yes	<0.001	4.26 (2.25- 8.06)	<0.001	3.98 (1.92-8.28)	1.382
Problems related to female fertility or neonatal mortality	No*					
	Yes	<0.001	3.92(2.00-7.68)	0.005	3.07(1.42-6.65)	1.121
Shared equipment/tools with other cattle farms	No*					
	Yes	0.009	2.19 (1.21-3.96)			
End-product	Meat*					
	Other	0.003	4.18(1.64-10.60)	0.038	3.13(1.07-9.19)	1.140
Purpose of breeding	Reproduction*					
	Production	<0.001	0.21 (0.09-0.46)	0.020	0.34(0.14- 0.84)	- 1.065
	Other	0.512	0.71(0.25-1.98)	0.835	1.14(0.34-3.78)	0.128
Isolation facilities	Good*					
	Average	0.005	2.80 (1.37- 5.72)	0.020	2.71 (1.17- 6.28)	0.997
	Poor	0.097	3.23 (0.80-12.93)	0.034	6.66 (1.16- 38.34)	1.896
Constant						-3.051

*Reference class; **ORs Final model (Forward method); *** Coefficients of final model

The results of the logistic regression, in a multivariate context and through the Forward: LR method, between the presence of brucellosis in cattle (final model presented in Table 3) and the variables previously identified as statistically significant revealed that the occurrence of abortions ($b_{ocorrabo}$= 1.382, X^2_{wald}= 13.688, p< 0.001, OR= 3.98), female fertility problems or neonatal mortality ($b_{proferf}(1)$= 1.121, $X^2_{wald}(1)$= 8.068, p= 0.005, OR= 3.07), the end-product ($b_{aptprod}(1) =$ 1.140, $X^2_{wald}(1)$= 4.300, p= 0.038, OR= 3.13 for the production of meat), purpose of production (production ($b_{finapraa}(1) =$ - 1.065, $X^2_{wald}(1)$= 5.402, p= 0.020, OR= 0.34), and for other purposes (sales, consumption) ($b_{finapraa}(1) =$ 0.128, $X^2_{wald}(1)$= 0.043, p= 0.835, OR= 1.14) in relation to reproduction), and the average and poor isolation facilities in relation to good ones ($b_{condisoa}(1) =$ 0.997, $X^2_{wald}(1)$= 5.422, p= 0.020, OR= 2.71; $b_{condisoa}(2) =$ 1.896, $X^2_{wald}(1)$= 4.504, p= 0.034, OR= 6.66) are risk factors for cattle brucellosis infection (Table 3).

The probability model indicated that probability of finding infected animals in the presence of abortions and fertility problems or neonatal mortality, and considering the productive capacity for "other activity", for end-purpose "production and other" and for average or poor conditions for the isolation of infected animals, was 0.9274, based on :

$$\hat{\pi} = \frac{1}{1+e^{-[-3.051+1.382ocorrabo(1)+1.121proferf(1)+1.140aptprod-1.065finalpro(1)+0.128finalpro(2)+0.997condisoa(1)+1.896condisoa(2)]}}$$

This model has 74% of correct predicted probability. The sensitivity of the model was 77.5% and the specificity 71.9% with a classification cut-off probability of 0.31. The discrimination accuracy was considered good (ROC c = 0.811, p <0.001).

4. DISCUSSION

This study aimed to determine the prevalence of bovine brucellosis and to characterize the risk factors associated with the disease in cattle farms in the province of Namibe, Angola in 2012. Research data involved 192 herds and 1344 animals.

Apparent brucellosis prevalence for animals (individual) and farms were 14.96% and 40.1%, respectively. In terms of *Brucella* seropositive animal prevalence, this research found 10.5% in Virei and 14.3% in Bibala municipalities.

Previous *Brucella* seropositive prevalence study done by Leal (1980) in Angola, involved 43780 cattle with brucellosis prevalence of only 0.9% (based on Elisa test) and another study from 1991 during the dry season in the province of Huila, a location of choice for the transhumance pasture of cattle herds, reported a individual prevalence of 6.4% using ELISA testing (Baptista, 1991). The essay of this studies (ELISA) and the present (RBT) are different. RBT can increase the prevalence, false positives, due to S19 vaccination (OIE, 2009), but in Namibe province cattle and others animals are not vaccinated since 1970 (Baptista, 1991). According to the Scientific Opinion of European Food Safety Authority for testing Brucellosis in bovines: RBT, CFT and ELISA are suitable for remaining as standard tests because their sensibility was not uniformly lower when compared to each other. It may be noted that these tests were also equivalent with regard to specificity (European Food Safety Authority, 2006).

Based in meta-analysis study realize by FAO (2002) in cattle population in Sub-Saharan Africa, using the RBT test, the seroprevalence of brucellosis was 16.2%. The value (prevalence) of the present study is lower. The study of FAO (2002) refer that RBT is the test useful to estimate the seroprevalence of Brucellosis in Sub-Saharan Africa. Comparing the presents results in animals individuals to others based in ELISA test and assuming the difference of this two essays, is interestingly to observe the similarity to those reported in Tanzania (4.3-15.8%; Jiwa et al., 1996), Kenya (5.45-17.5%; Kunda et al., 2005) and Guinea Bissau (15.1%; Unger et al., 2003). It is possible that this similarity is associated with traditional grazing practices that farmers from Namibe have in common with farmers from other East Africa countries. About the herd prevalence the present result is high than reported in Ethiopia (4.1-15.2%, Dinka and Chala, 2009) and low than presented in Sudan (90%, Angara et al., 2007).

The most recent study in northern Nigeria, using RBT and c-ELISA (Competitive Enzyme-Linked Immunosorbent Assay) as serological tests for brucellosis screening in farm animals, reported higher prevalence rates in animals (26.3%) and herds (77.5%) (Mai et al., 2012) when compared to our results. In 2011, other studies conducted in the urban and peri-urban areas of Kampala, Uganda, based on c-ELISA serological test, found 6.5% of herds infected and 5.0% of animals (Makita et al., 2011). Individual bovine brucellosis seroprevalence of 15.5% was found in an endemic area of Egypt (Holt et al., 2011) and in Ethiopia, 3.5% and 26.1% of animals and herds, respectively, were positive (Megersa et al., 2011).

Table 4. Relationship between the number of infected *Brucella seropositive* animals and the total number of animals in Namibe province in Angola

	Number of infected animals		
Data from the cattle herds	n	$r_{(192)}$	*p**
Number of abortions (mean)	192	0.531*	< 0.001
Trimester in which abortion occur	192	-0.001*	0.992
Number of reproductive males	192	-0.173	0.077
Number of mature females	192	-0.063	0.387

*Significance level using Spearman correlation

Table 5. Relationship between animal seroprevalence results and the number of breeding females infected per municipality

Brucella seropositive female animals using Rose Bengal Test	n(%)	Namibe n(%)	Tombwa n(%)	Bibala n(%)	Kamucuio n(%)	Virei n(%)	*p*
Positive	201(15.0)	20(31.7)	15(30.6)	113(14.3)	39(12.7)	14(10.5)	< 0.001
Negative	1143(85.0)	43(68.3)	34(69.4)	678(85.7)	268(87.3)	119(89.5)	

In the multivariate analysis, the factor positively associated with brucellosis prevalence in cattle herds was the occurrence of abortions (OR = 3.98, 95% CI: 1.92-8.28) as 75.3% of the infected herds had a history of abortions. Surprisingly, the trimester in which most abortions occur is not significantly correlated with the presence of disease in herds as only 10.3% of abortions occurred in the third trimester of gestation since. These results were again lower than the values reported by Dhand et al. (2005) in a study conducted in some India provinces, where 25.8% of abortions in the last trimester were significantly correlated with the prevalence of brucellosis in cattle herds and with history of abortions. *Brucella* abortions are frequent from the 5^{th} to 8^{th} month of gestation (Center for Food Security & Public Health, 2009; Megid et al., 2010). Nonetheless, third trimester abortions are considered by many researchers a clear indicator of brucellosis (Putt et al., 1988; Corbel, 1997; FAO, 2002; Dhand et al., 2005; World Health Organization, 2005; Lopes et al., 2010; Karadzinska-Bislimovska et al., 2010). In accordance with other studies, the infertility of breeding females and the neonatal mortality was positively correlated with brucella antibody presence (OR = 3.07, 95% CI: 1.42-6.65) (Putt et al., 1988; Dhand et al., 2005; Lopes et al., 2010; Sahilu et al., 2011).

The association positive of the factors: the end-product (other than meat), the breeding purpose, the average , and poor conditions in the isolation facilities for sick animals help a probability (92.74%) for the prevalence of brucellosis in cattle farms in Namibe province. In the Namibe province it is a common practice among farmers to exchange animals without previously assessing if they are carriers of any infection. Considering the above mentioned, it is clear that specific measures should be carried out in order to control/restrict the exchange of animals amongst herds (Putt et al., 1988; Sahilu et al., 2011). Unfortunately, Namibe is a semi-desert area with cyclic episodes of drought, a condition that enhances the transhumance of humans and animals, impairing sanitary management. In addition, the frequent use of common pastures and the grouping of animals for drinking increase the contact between herds further promoting the risk of infection (Mufinda, 2010).

In this study nearly 83.1% of the Namibe infected herds reported poor to average conditions to isolate infected animals. In the rural areas of Angola, animals are kept at night in enclosures at the center of the farm where they share the same environment as humans. Animals leave the holdings under the surveillance of farmers but are free to feed in the same grounds as animals from neighboring herds (Mufinda, 2010; Mufinda and Klein, 2011). In fact, although manure is often used to coat the farm houses, it is custom for farmers and family to abandon the settlements when the amount of manure in the holding makes the location uninhabitable (Carvalho, 1997).

In Angola, animals that test positive for brucellosis are not sacrificed and their owners are not compensated hence there is a high risk for disease spreading. This study is the first in Angola and in Namibe province to evaluate the factors associated with the prevalence of brucellosis in cattle farms.

5. CONCLUSION

In this study we demonstrated that animal brucellosis is widespread in the Namibe province as 40.10% of herds are infected, with Namibe and Tombwa municipalities showing the highest prevalence of infected animals.

The factors that are positively correlated with the prevalence of bovine brucellosis and that could be considered a predictor of possible brucellosis infection include the poor hygiene conditions for isolating infected animals, the end-product, the purpose of breeding, the occurrence of abortions, fertility problems in females and neonatal mortality.

This study contributes towards the understanding of the prevalence of cattle brucellosis in Angola. Additionally, the identification of risk factors associated to brucellosis provides an important tool for the development of Public Health measures that are essential for its control and favours additional epidemiological studies. Finally, our work highlights the need not only to educate and inform farmers about brucellosis but also for provincial veterinary services to implement awareness surveillance campaigns aiming at animal brucellosis control and eradication.

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