Rev. sci. tech. Off. int. Epiz., 2013, **32** (3), ... - ...

Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia

This paper (No. 28052013-00001-EN) has been peer-reviewed, accepted, edited, and corrected by authors. It has no yet been formatted for printing. It will be published in December 2013 in issue 32-3 of the *Scientific and Technical Review*.

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Summary

Bovine brucellosis, an important bacterial zoonosis, is usually associated with intensive systems of production. A cross-sectional study was conducted in western Ethiopia to determine the seroprevalence of bovine brucellosis in cattle under traditional extensive husbandry. Sera collected from 1,152 cattle originating from 164 herds were screened, using the Rose Bengal test, and all positive sera were then examined, using complement fixation as a confirmatory test. Based on the results of two-step testing, the apparent seroprevalences were 1% (95% confidence interval [CI]: 0.5%, 1.7%) at the animal level and 4.9% (95% CI: 1.6%, 8.2%) at the herd level. A random-effects binary logistic regression model was used to examine potential risk factors, using 'herd' as a random effect. Herd size (p = 0.009) and abortion (p = 0.015) were significant risk factors for animal-level seropositivity, after controlling for other factors. Although bovine brucellosis was found at a low prevalence in the indigenous cattle population, the disease should be considered in

any future expansion of dairy cattle production involving improved breeds.

Keywords

Bovine – Brucellosis – Complement fixation test – Ethiopia – Rose Bengal test – Seroprevalence – Western Ethiopia.

Introduction

Ethiopia has one of the largest livestock resources in Africa, with a total cattle population of 47.6 million (1). Livestock contributes more than 30% of the agricultural gross domestic product and 19% in export earnings. Oxen provide draught power to cultivate grain crops in rural agriculture, which is the backbone of the economy. The comparatively huge livestock resources of the country and the economic return gained from this subsector do not coincide, because of prevalent infectious diseases, among other factors. Bovine brucellosis is one of these infectious diseases and has been reported from several parts of the country (2, 3, 4, 5, 6, 7).

Brucellosis is one of the oldest and most widespread zoonotic diseases, affecting food production in the tropics and subtropics (8). It is an economically important disease of livestock causing reproductive wastage through infertility, delayed heat, loss of calves, reduced meat and milk production, culling and economic losses from international trade bans (9).

Bovine brucellosis is mainly caused by *Brucella abortus*; to a lesser extent by *B. melitensis*, and occasionally by *B. suis*. Clinically, it is characterised by abortion and retained fetal membrane (RFM) in cows and orchitis and epididymitis in bulls (10). Sources of infection include aborted fetuses, fetal membranes, vaginal discharges and milk from infected cows. The most common route of transmission in cattle is through direct contact with an aborting cow and the aborted fetus or by indirect contact with contaminated fomites. Ingestion of contaminated pasture, feed, fodder and water may also play a secondary role (11). Although brucellosis has been almost eradicated

from most of the developed countries, it is still a major public and animal health problem in many developing countries, where livestock are a major source of food and income (12). The incidence of human brucellosis is correlated with the level of incidence in domestic animals (11). Human cases occur after ingesting raw milk and milk products and coming into close contact with infected animals. Human brucellosis can be a very debilitating disease, although the case fatality rate is generally low (12).

Most studies on bovine brucellosis in Ethiopia have been carried out on state dairy farms in the central parts of the country, where dairy cattle production, using mainly exotic breeds, is intensive. The disease has not previously been reported in the Benishangul Gumuz region in the north-west of the country. The findings of a cross-sectional seroepidemiological study to determine the seroprevalence of bovine brucellosis and its associated risk factors in indigenous cattle under traditional extensive husbandry in the Metekele zone of Benishangul are reported here.

Materials and methods

Study area and animals

The study was conducted between October 2007 and March 2008, in Dibate and Wembera districts (out of seven districts) of the Metekele zone, in the Benishangul Gumuz region of north-western Ethiopia. The altitude of the zone ranges from 580 m to 2,731 m above sea level. The zone has a uni-modal rainfall pattern, with high rainfall from May to October and an average annual rainfall of 1,000 mm. The mean minimum and maximum annual temperatures range from 17°C to 32°C. The main economic activity in the zone is mixed crop and livestock production. The dominant animal species is cattle, followed by goats. Cattle are the mainstay of the household economy as they provide draught power for tillage for crop production, are the main sources of meat and milk, and provide income through the live animal market. The Dibate and Wembera districts were selected for this study because of their high cattle population in relation to other districts.

According to data from the Central Statistical Agency (1), the total number of cattle in Metekele is 193,914, of which 100,589 are found in the Dibate and Wembera districts. Twelve (35.3%) peasant associations (the lowest administrative unit in Ethiopia) were randomly selected for the study from a total of 34 in these two districts. The 12 associations had a total cattle population of 36,000 (1), representing about 37% of the cattle population from the two study districts. A total of 164 herds were selected (an average of 14 herds from each peasant association), including cattle of both sexes and at least one year of age. A total of 1,152 cattle (596 from Dibate, 556 from Wembera) were sampled, representing 3.2% of the total cattle population of the selected associations. The sample size was calculated using the formula recommended for multistage sampling (13), where each herd was a primary sampling unit and individual animals were considered as a unit of concern. The animals in the study were not vaccinated against bovine brucellosis and had been reared in a traditional extensive animal production system. All were local indigenous zebu-type cattle.

Study design and sampling

Seropositivity to brucellosis was determined by using two serological tests in a cross-sectional study. Information on the reproductive status and problems of female animals and the ages of all study cattle were obtained during blood collection. Serum samples were kept at –20°C until they were tested.

Serological testing

All serum samples were screened for bovine brucellosis antibodies, using the Rose Bengal test (RBT) and according to the World Organisation for Animal Health (OIE) protocol (10). To confirm the diagnosis, all RBT-positive sera were further tested in a complement fixation test (CFT). A screening test with high sensitivity was used to increase the likelihood of detecting a seropositive animal. For practical and cost reasons, the CFT was used to confirm RBT-positive samples only. The RBT antigen, comprising a suspension of *B. abortus*, pre-standardised according to OIE requirements, was

obtained from the Institut Pourquier, France. Complement fixation tests were undertaken at the Immunology Laboratory, the National Veterinary Institute, Debre Zeit, Ethiopia, according to OIE guidelines (10). The OIE standard *B. abortus* antigen S99 used for CFT was obtained from the Veterinary Laboratories Agency, United Kingdom. The control sera and complement were obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany. Sera showing 100% fixation of the complement (4+) at a dilution of 1:5; 75% fixation (3+) at a dilution of 1:10; 50% fixation (2+) at a dilution of 1:20; and 25% fixation at a dilution of 1:40 (1+), were classified as positive.

Data analysis

Categorical variables were summarised as frequency and percentages; continuous variables were summarised as mean ± standard deviation (SD). An animal was considered seropositive if it tested positive to both RBT and CFT. A herd, defined as the total number of cattle belonging to the same household, was considered seropositive if it included at least one seropositive animal. Animal and herd apparent seroprevalences were calculated by dividing the number of positive test results by the total number of animals and herds sampled, respectively. The within-herd seroprevalence was calculated by dividing the number of seropositive animals in the herd by the total number of animals tested in that herd.

The association between seropositivity and categorical risk factors was assessed using Fisher's exact test and contingency table analysis wherever appropriate. For variable selection, univariable random-effects (RE) logistic regression with 'herd' as a random effect was fitted to assess individual associations of predictors with the dependent variable (seropositivity). Independent variables that were significantly associated with seropositivity in the univariable analysis ($p < 0.25$) were included in the multivariable RE binary logistic regression model. In the multivariable RE logistic regression analysis, the full model was first fitted with the variables that were significant at univariable analysis. Variables were then removed, one at a time,

and their significance assessed using the Wald statistic. The newly fitted models at each step were compared with the previous larger model, using likelihood ratio tests. Interaction terms were assessed for variables in the final main-effects model. Since sex and reproductive status had one or more empty cells, they were not included in the RE logistic regression and Fisher's exact test was used to assess their association with prevalence at the animal level. Because of the problem of zero cells, it was not possible to assess the linearity assumption of the continuous variables (age, herd size, parity number) and therefore they were analysed as ordinal continuous variables. Results were presented as crude and adjusted coefficients, together with their corresponding standard errors and 95% confidence intervals (CIs). STATA version 12 for Windows (STATA Corporation, College Station, Texas) was used for all statistical analyses.

Results

Descriptive statistics

Tables I and IV present the descriptive characteristics of the cattle and herds in the study. The average age of the cattle was 5.7 years (standard deviation [SD] ± 3.2; range 1–14) and the average number of cattle per herd was 7 (SD ± 3.1; range 2–21). Among the female animals studied, 38% were lactating and the mean parity number was 1.7 (SD ± 1.9; range 0–8). Abortion and RFM were observed in 4.7% and 2.1% of the cows, respectively.

Animal seroprevalence

Among the 1,152 cattle screened for *B. abortus* antibodies, 14 (1.2%) tested positive by RBT. Of these, 11 animals (79%; 95% CI: 0.5, 1.7) were confirmed positive by CFT, giving an apparent seroprevalence of 1.0% in the study area (Table I). The intra-class (within herd) correlation coefficient (ICC) for seropositivity was 0.31 (95% CI: 0.07, 0.73). All seropositive animals were females and were either pregnant or lactating. The seroprevalences among aborting cows and cows with a history of RFM were 8.1% and 11.8%, respectively (Table I).

Using Fisher's exact test, the prevalence of bovine brucellosis was significantly higher in females than in males (p = 0.022), and did not significantly differ according to the reproductive status of the females (p = 0.056). Except for the parity number, which was also collinear with age, other variables were independently associated with animal-level seropositivity in univariable analysis at a liberal p value of 0.25 (Table II). Parity number and RFM were not included in the multivariable regression because of their multicollinearity with age and abortion, respectively. In the multivariable analysis, only herd size and abortion remained to be independently associated with brucellosis seropositivity (Table III). The odds of an animal being seropositive increased by 1.4 fold (95% CI: 1.1, 1.7) with a unit increase in the herd size adjusted for the herd clustering effect. Similarly, the odds of seropositivity were 6.7 times (95% CI: 1.5, 30.9) higher in a cow with a history of abortion, compared to a cow with no such history.

Herd prevalence

The mean number of lactating cows per herd was 1.8 (SD ± 1.6). Among the 164 herds, eight had at least one CFT seropositive cow, giving an apparent herd seroprevalence of bovine brucellosis of 4.9% (95% CI: 1.5, 8.2) in the study area. Among herds that tested seropositive, the within-herd seroprevalence ranged from 5% to 15%. The mean herd-level prevalence among all herds was 0.46% and did not significantly vary between the study districts (p = 0.160).

The mean apparent prevalence among animals in the seropositive herds was 9% (n = 121; 95% CI: 5, 16) but was significantly higher in herds with aborting cows (p = 0.012) and RFM (p = 0.038) (Table IV). With regard to herd seropositivity, the mean herd age, herd size and numbers of pregnant, lactating and dry cows were significant at p < 0.25 in the univariable analysis. In multivariable logistic regression, however, none of the risk factors remained significant (p > 0.05) (Table V).

Discussion

Animal seroprevalence

The apparent seroprevalence of bovine brucellosis at the animal level, adjusted for the herd effect, was 1.0%. This relatively low prevalence might be attributable to extensive grazing conditions; these could reduce both animal-to-animal contact and the contamination of pastures under dry climatic conditions (14). Another explanation could be that, in the area studied, most of the farmers replace their animals from their own stock instead of buying animals from markets. Thus, the mixing of cattle from many herds, especially at watering points, is less marked than in the pastoral areas of the country. The average herd size (seven animals) in this study is generally smaller than herds under pastoral and intensive production systems.

Similar low prevalences of bovine brucellosis, based on RBT and CFT, have been reported in other studies on indigenous cattle under extensive production systems (3, 7, 15, 16, 17, 18, 19, 20). Based on the same diagnostic tests, a higher prevalence has been reported from the highland areas of Ethiopia among cattle in intensive production systems (3, 4, 5, 19, 21, 22). This variation is merely due to differences in cattle production systems (23). Based on RBT only, a higher prevalence was also reported in pastoral areas, compared with an extensive cattle production system (17).

The absence of any significant difference in cattle seroprevalence between the Dibate and Wembera districts could be due to the similarity of management practices in the two districts. As in previous reports, all seropositive cattle were females (5, 7, 17, 21). The apparent seroprevalence in the females (1.4%) was 40% higher than the overall seroprevalence in the study cattle population of 1,152 males and females combined (1.0%). The explanation for this finding could be that male animals are kept for a shorter time than females and thus the chance of exposure is lower for males (8).

Reproductive status was not found to be a significant determinant of seroprevalence. Seropositivity was observed only in pregnant and

lactating cows, with no significant difference between the two. This scenario has also been reported in other studies (7, 24). Sexually mature and pregnant cows are more susceptible to infection with brucellae than sexually immature cattle of either sex. This has been attributed to the affinity of these bacteria to the pregnant uterus and to erythritol in fetal tissue, possibly also to steroid hormones (25).

A previous history of abortion and RFM was, as expected, significantly associated with seropositivity. Other studies have also shown a significant association between seropositivity and abortion and RFM (7, 16, 18). Because of its collinearity with abortion, the effect of RFM was not studied in the multivariable analysis. Abortion was chosen for inclusion in the multivariable model.

Parity number was not found to be significantly associated with seropositivity in the univariable analysis. Eight of the 11 seropositive cows were in their second (36%) or third (36%) parity. Furthermore, the parity number of 72% of the female animals was between zero and three, with the frequency of animals diminishing as parity number increased (data skewed to the right). This could impair the power to show statistical difference, as has been reported in other studies (7, 16, 17). The effect of parity could also be confounded by age but this was not controlled for; parity number was excluded from the multiple mixed effects logistic regression because of multicollinearity with age.

After controlling for other risk factors, the animal-level seroprevalence of bovine brucellosis was not significantly associated with the age of the cattle, possibly reflecting the generally low mean age of cattle in the study (5.7 years, range 1–14). However, seropositive cows were significantly older (p = 0.019) than seronegative cattle (males included): the mean (± SD) age of seropositive cows was 7.9 ± 1.4 years (range 5–10) while that of the seronegative overall population was 5.7 ± 3.2 years (range 1–14). Similar studies (2, 3, 4, 17, 19, 24) could not show any significant effect of age, although the prevalence was higher in animals older than two years. In the present study, seropositivity occurred only in animals older than five years. Similarly, higher seropositivity has been

reported in other studies in animals older than five years, when compared with younger animals (16, 17).

In the present study, herd size remained independently and significantly associated with the seroprevalence of bovine brucellosis. The authors' finding is in accord with other reports from Ethiopia (2, 3, 7, 18, 20). An increase in herd size is usually accompanied by an increase in stocking density, as well as an increase in the risk of exposure to infection, especially after abortion. Stocking density is an important determinant of the potential for transmission between susceptible and infected animals (14, 24). The number of animals per herd was generally low in this study, with a maximum herd size of 21 animals, which is typical of mixed livestock and crop production. However, the mean herd size in cases where seropositive animals were detected was 15.6 (SD ± 3.8), and all seropositive animals were in herds with ten animals or more. This would suggest that the risk of animal brucellosis increases with herd size and that bovine brucellosis should be considered in relation to the future expansion of cattle production in Ethiopia. Similarly, the increased odds of herd seropositivity with increasing herd size has also been reported in Zimbabwe (26).

In this study, the ICC (correlation of observations within an individual herd) for bovine brucellosis seropositivity has been calculated in Ethiopian cattle for the first time. The ICC value of 0.31 is similar to that of a previous report (ICC 0.46) (27). The authors believe that the ICC is an important parameter that could be used for sample size calculations in future studies in Ethiopia (28).

Herd seroprevalence

The overall herd seroprevalence of bovine brucellosis was 4.9%, which is similar to a value of 3.3% reported in another study of Ethiopian herds under extensive systems (20). Nevertheless, higher herd-level seroprevalences have been reported in other parts of Ethiopia in herds under extensive production systems (3, 5, 7, 16, 17, 18, 19). This discrepancy could be attributed to the presence of cross-bred animals in herds kept close to the main cities, where milk

production is more market oriented than in this study area. Furthermore, the discrepancy could be due to relatively larger herd sizes compared with herds in the present study; herd size was found to be a significant factor in relation to herd seropositivity. Higher herd-level prevalence has also been reported in dairy cattle in other African countries (24, 26).

When the eight seropositive herds alone were considered, the within-herd apparent seroprevalence ranged from 5% to 15%, with a mean apparent prevalence of 9%, similar to that reported from northern Ethiopia (16). One or two animals tested seropositive in each of the eight seropositive herds. The variation in seroprevalence within the herds, coupled with the overall low herd prevalence, may indicate clustering and that more transmission occurs within herds than between herds. This indicates that the spread of bovine brucellosis between herds can be controlled through the elimination of seroconverters in the infected herds.

The presence of a cow with a history of abortion and/or RFM did not significantly affect herd seropositivity in the multivariable regression. This might be due to the presence of non-specific causes of abortion and/or RFM, or possibly to information bias from a lack of record-keeping by the herd owners. This finding is consistent with a previous report (5). Nevertheless, the herd seroprevalence of brucellosis was higher in herds that had a history of abortion (14.3%) and RFM (17.6%), compared with herds with no history of abortion (2.3%) or RFM (3.4%).

The number of animals of differing reproductive status did not affect herd seropositivity under multivariable RE logistic regression; however, all seropositive animals were either pregnant or lactating. This finding has a great implication for public health, especially in rural parts of Ethiopia where milk is usually consumed raw, thus exposing people to a greater risk of milk-borne zoonoses, such as brucellosis.

In conclusion, bovine brucellosis persists at a low prevalence in the area of western Ethiopia studied by the authors. The infection is

strongly associated with large herds and is manifested by abortion in infected cows. Bovine brucellosis should be taken into consideration when planning for the future expansion of intensified dairy cattle production in Ethiopia.

Acknowledgements

This study was financed by the Graduate School of Addis Ababa University. The authors thank Dr Anteneh Yenehun, Dr Tadese Kebede and Mrs Almaz Sharew for their support.

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Table I

Overall cattle- and animal-level prevalences of bovine brucellosis in the Dibate and Wembera districts in western Ethiopia, by complement fixation testing

Variable	Number of animals studied (%)		Number testing positive	Prevalence % (95% CI) [(a)]	*p* value [(b)]
All animals	1,152	(100)	11	1 (0.5, 1.7)	
Study districts					
Dibate	596	(51.7)	8	1.3 (0.1, 2.6)	0.227
Wembera	556	(48.3)	3	0.5 (0, 1.6)	
Sex					
Female	794	(68.9)	11	1.4 (0.1, 2.5)	0.022
Male	358	(31.1)	0	0 (0, 0.83)	
Reproductive status					
Heifer	251	(31.6)	0	0 (0, 1.5)	
Pregnant	205	(25.8)	5	2.4 (1.1, 5.6)	0.056
Lactating	302	(38)	6	2 (0.9, 4.3)	
Dry	36	(4.5)	0	0 (0.1, 9.5)	
Abortion					
Present	37	(4.7)	3	8.1 (2.9, 21.4)	0.012
Absent	757	(95.3)	8	1.1 (0.5, 2.1)	
Retained fetal membrane					
Present	17	(2.1)	2	11.8 (3.6, 34.7)	0.021
Absent	777	(97.9)	9	1.2 (0.6, 2.2)	

(a) Confidence interval
(b) Based on Fisher's exact test

Table II
Association of bovine brucellosis seropositivity with animal-level risk factors, adjusted for the herd effect

Variable	Crude estimates				Adjusted estimates			
	Coefficient	95% CI [a]	Standard error	*p* value	Coefficient	95% CI [a]	SE [a]	*p* value
Age	0.21	0.02, 0.41	0.10	0.030	0.14	–0.07, 0.35	0.12	0.193
Herd size	0.30	0.19, 0.42	0.06	<0.001	0.34	0.08, 0.61	0.13	0.011
Parity number	0.07	–0.25, 0.38	0.16	0.686	–	–	–	–
Abortion	2.23	0.72, 3.78	0.77	0.004	1.69	0.12, 3.26	0.80	0.035
RFM [b]	2.73	0.81, 4.66	0.98	0.005	–	–	–	–
District	–0.91	–2.40, 0.57	0.76	0.227	0.78	–1.15, 2.71	1.0	0.429

(a) Confidence interval
(b) Retained fetal membrane

Table III

Final model for risk factors of bovine brucellosis seropositivity at the animal level, adjusted for the herd clustering effect

Variable	Coefficient	95% CI [(a)]	Standard error	*p* value
Herd size	0.31	0.08, 0.55	0.12	0.009
Abortion	1.90	0.37, 3.43	0.78	0.015

(a) Confidence interval

Table IV

Seroprevalence of bovine brucellosis in the study herds and at herd level in the Dibate and Wembera districts of western Ethiopia, by complement fixation testing

Variable	Number of herds (%)		Number testing positive	Prevalence % (95% CI) [a]	*p* value [b]
All herds	164	(100)	8	4.9 (1.6, 8.2)	
Study districts					
Dibate	80	(48.8)	6	7.5 (1.6, 13.4)	0.160
Wembera	84	(51.2)	2	2.4 (0.9, 5.7)	
Abortion					
Present	35	(21.3)	5	14.3 (2.4, 26.1)	0.012
Absent	129	(78.7)	3	2.3 (0.3, 5.0)	
Retained fetal membrane					
Present	17	(10.4)	3	17.6 (0, 36.6)	0.038
Absent	147	(89.6)	5	3.4 (0.4, 6.3)	

(a) Confidence interval
(b) Based on Fisher's exact test

Table V
Association of herd seroprevalence of bovine brucellosis with herd-level risk factors

Variable	Crude coefficients				Adjusted coefficients			
	Estimate	95% CI [a]	Standard error	*p* value	Estimate	95% CI [a]	SE [b]	*p* value
Mean herd age	1.5	–0.3, 3.2	0.89	0.100	1.8	–3.0, 6.6	2.47	0.464
Herd size	2.4	–1.3, 6.1	1.88	0.204	3.3	–1.6, 8.3	2.54	0.189
Mean herd parity number	1.3	–2.0, 4.7	1.72	0.442	–	–	–	–
Abortion	2.0	–73.2, 77.1	38.34	0.959	–	–	–	–
RFM [b]	1.8	–185.3, 188.9	95.46	0.985	–	–	–	–
Study district	–1.2	–57.6, 55.2	28.76	0.967	–	–	–	–
Reproductive status								
Heifer	0.9	–1.7, 3.6	1.35	0.493	–	–	–	–
Pregnant	0.8	0.3, 1.4	0.27	0.002	–0.9	–4.1, 2.4	1.64	0.597
Lactating	1.1	0.4, 1.9	0.38	0.003	–1.4	–5.2, 2.5	1.95	0.483
Dry	1.9	–0.5, 4.2	1.20	0.115	–1.5	–7.8, 4.8	3.22	0.641

(a) Confidence interval

(b) Retained fetal membrane