Veterinary Medicine: Research and Reports

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

A Seroprevalence Study of Brucellosis in Boran (Zebu) Breeds of Pastoral Area

Alebachew Tilahun[1], Silto Kegno[2], Takele Adugna[1], Dinberu Mamuye[3]

[1]University of Gondar, College of Veterinary Medicine and Animal Science, Department of Veterinary Clinical Medicine, Gondar, Ethiopia; [2]Wolaita Sodo University, School of Veterinary Medicine, Sodo, Ethiopia; [3]Dilla University, Dilla, Ethiopia

Correspondence: Alebachew Tilahun, Tel +251-949-0220-68, Email alebmen19@gmail.com

Purpose: The economic and health implications of brucellosis are of particular concern in developing countries, primarily in the vulnerable sector of rural herders. A cross-sectional study was done in Boran breeds to estimate the seroprevalence of bovine brucellosis, identify risk variables and assess public health implications in Borena zone, Oromia region, Ethiopia in the questionnaire survey.

Methods: The sampling animals were chosen from smallholders using a simple random sampling procedure. The study involved a total of 788 animals. Animals of both sex and different age groups with the age of 6 months or above found during the study interval were included. The Rose Bengal plate test was used to screen sera, and positive samples were subsequently retested using a Direct Enzyme-Linked Immunosorbent Assay for confirmation.

Results: The overall seroprevalence of bovine brucellosis was 7.6% (60/788) in the Direct Enzyme-Linked Immunosorbent Assay test. Herd size, age, history of abortion, testicular hygroma, and retained fetal membrane were statistical significance for the *Brucella* seropositivity ($P<0.05$). Whereas, district, sex, body condition score, and management did not influence the disease occurrence ($P>0.05$). The majority of the participants, 91.7% (55/60) did not aware of the zoonotic implications of brucellosis. Only 10% (6/60) of interviewed respondents disposed of aborted fetuses and retained fetal membrane properly and the rest 90% (54/60) left in the environment. Ninety percent and 83.3% of the respondents revealed that they consumed raw milk and meat, respectively.

Conclusion: The presence of *Brucella* infection is highly correlated with age, history of abortion, and testicular hygroma. According to the collected data: sex, body condition score, district, and management had no statistically significant effect on *Brucella* occurrence. The majority of respondents were unaware of the disease's zoonotic consequences. Finally, creating community awareness about its transmission, zoonotic significance, and hygienic practices were recommended.

Keywords: Boran breed, bovine brucellosis, D-ELISA, seroprevalence, Rose Bengal plate test

Introduction

Brucellosis is a globally important and widespread zoonotic illness caused by bacteria belonging to the genus *Brucella* that infect specific animal species.[1] *Brucella* is a gram-negative bacterium that causes disease in animals. *B. abortus, B. melitensis*, and *B. suis* are species that have a significant impact on domestic animal productivity as well as human health.[2] In cattle, the infection is predominantly caused by *B. abortus*, less frequently by *B. melitensis*, and occasionally by *B. suis*.[3] Brucellosis in humans occurs mainly through the consumption of unpasteurized milk and milk products, and occasionally by inhalation of aerosols and contamination with infected excreta.[3]

In Bovine, *B. abortus* causes abortions, retained fetal membranes, stillbirths, and weak calves; abortions mainly happen in the second half of pregnancy. Furthermore, the placenta may be retained, and milk production may also be reduced. Though abortion occurs only in the first parity, the infected animals can shed milk and uterine discharge the organism in subsequent pregnancies. In bulls, the disease causes epididymitis, seminal vesiculitis, orchitis, and testicular abscesses.[4]

The economic and health implications of brucellosis are of particular concern in developing countries, primarily in the vulnerable sector of rural herders. Risks are considered high in the pastoral societies where close and frequent contact

Received: 11 February 2022
Accepted: 30 April 2022
Published: 13 May 2022

between humans and animals is an unavoidable part of ecology. The occurrence of bovine brucellosis has been investigated in the intensive and semi-intensive farming system of Ethiopia, especially in central Ethiopia.[5–8] Despite the huge potential of the Borena Zone with regard to its livestock resources, which enables the earnings of foreign currency that could play a great role in food self-sufficiency of the country. There has been little attempt in the past to assess the perception of pastoralists towards the disease, identify potential risk factors, and determine the prevalence of bovine brucellosis in indigenous (Boran) cattle kept in extensive management regimes. As a result, the current study aimed to estimate sero-prevalence, investigate main possible risk factors for bovine brucellosis, and assess pastoralists' understanding for the zoonotic implications of brucellosis.

Materials and Methods

Study Area

The research was conducted in the Borena zone of the Oromia Regional State in Southern Ethiopia. The Borena zone is around 570 kilometers south of Addis Ababa, capital city (Figure 1). The districts comprise about 20 Peasant Associations (PA), in which 9 Peasant Associations practiced pastoralism and the remaining 11 PAs practiced in Agro-pastoralism activities.[9] For interpretation, pastoralism means rearing livestock to derive income, whereas, agro-pastoralists imply people rearing livestock and cultivating crops to generate income. Herd contact slightly restricted in agro-pastoral than pastoral which again reduce the chance of disease incidence. The district is situated at an elevation of 1000–1500 meters above sea level and is located at a latitude of around 5°23′49 N and a longitude of approximately 39° 31′52 E. The average lowest and maximum temperature for the year is 24 and 29°C, respectively. The location is semi-arid, with an average rainfall of 300mm in the South and greater than 700 in the Northern Hemisphere.

Study Population and Management

The study included indigenous Zebu cattle (Boran) breeds with different sex, body condition, and age category, which are kept under extensive management system. According to,[10] cattle with the age of 6 months or above with no previous history of vaccination were considered as the study animals; the age further grouped into young (≤2) and adult (>2) years.

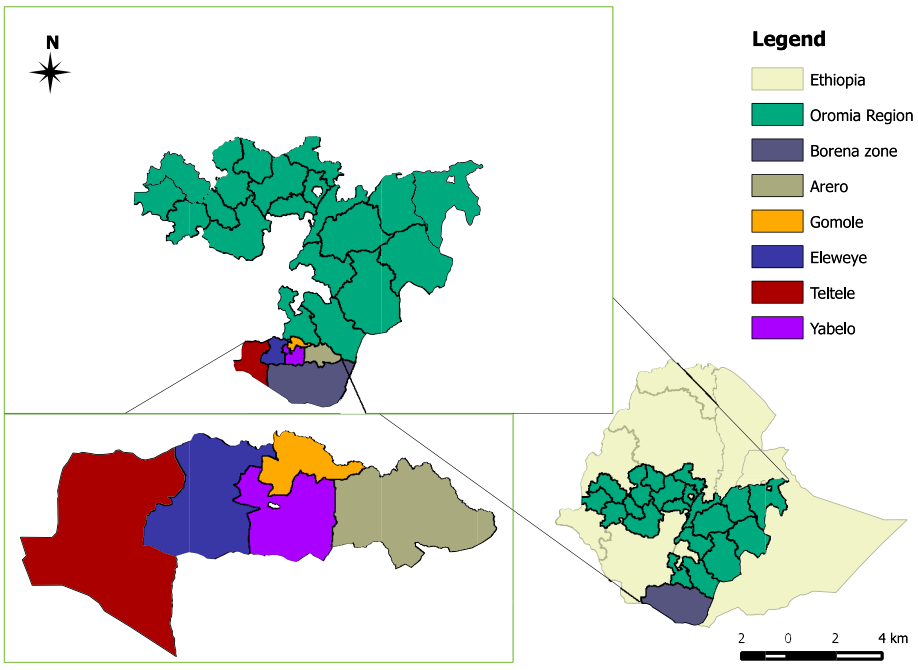

Figure 1 Map of the study location.

Body condition scores, on the other hand, were divided into: poor, medium, and good based on Bayemi et al.[11] The herd sizes were classified as 25, 25–40, and >40.[9]

The management system consisted of a few semi-intensives scattered throughout the city, with the majority being extensive. Humans were pastoralists and agro-pastoralists, while animals were free to graze. The drinking and feeding ranges are where the cattle congregate. The study included animals who had never been vaccinated before. The total cattle populations of selected pastoral districts were 300,138, from which proportional samples were drawn (Table 1).

Inclusion and Exclusion Criteria

Animals of both sex and different age groups with the age of 6 months or above found during the study interval were included. However, animals/herds with inadequate information were excluded from the study. Respondents who were refused to be interviewed for questionnaire survey and complained information for their herds were also excluded.

Study Design

In selected district of the Borena zone, a cross-sectional study was conducted to quantify the occurrence of brucellosis and identify potential risk variables. Semi-structured questionnaires were used to assess the human perception about ways of transmission and zoonotic effects of the disease.

Sample Size and Sampling Methods

The Borena zone was purposefully chosen, and the study animals were selected from smallholders using simple random sampling methods. The sample size was figure out according to Thrusfield.[12] With 50% estimated prevalence and 95% confidence interval. Accordingly, the total sample was 384; however, 788 samples were included to reduce uncertainty and to increase the power for differentiating the differences among groups.

Data Collection

Relevant data on the animals, such as sex, body condition score, age, and other potential risk factors associated with brucellosis were recorded during each sample collection. Furthermore, a semi-structured questionnaire format was created in local language and administered to 60 (28 from agro-pastoral and 32 from pastoral) selected respondents. A systematically random sampling technique was used to select respondents within the PA's of study settings to collect information regarding history and period of abortion, retention of fetal membranes, herd size, knowledge of zoonosis, and presence of testicular and joint swellings. Moreover, the survey emphasized milk consumption practices, meat sources, and local meat consumption practices were taken into account.

Table 1 Total Study Population and Production Systems

PA/Villages	Cattle Population	Production System	Number of Animals Sampled
Yabello	9575	Agro-pastoral	80
Eleweye	11,694	Agro-pastoral	104
Arero	21,660	Pastoral	184
Gomole	19,650	Pastoral	174
Teltele	237,559	Pastoral	246
Total	300,138		788

Note: Data from Central Statistics Authority Borena Zone Agricultural Bureau (unpublished data, 2020).

Serological Tests

Rose Bengal Plate Test

Approximately 7–9 mL of blood was taken in plain vacutainer tubes from each sampled animal (788) using vacutainer needles and needle holder. Each sample was then tagged and delivered to the Yabello Regional Veterinary Laboratory (YRVL), where it was kept at 37°C for 24 hours to separate blood clots and serum. Then, sera were separated in cryovials and screening with Rose Bengal Plate Test (RBPT) as recommended.[1,13] Then, suspected sera were stored in a deep freezer (−20°C) until D-ELISA tests were conducted.

In RBPT, 30 µL of serum were poured into a white enamel plate, and a micropipette was used to add an equal amount of marketable antigen to the plate. Then, serum and antigen were mixed on a white enamel plate and wait for 4 min. Finally, plate ring formation and agglutination were recorded. If a ring and agglutination were formed, the sample taken was recorded as positive for *Brucella*, and if not, the sample taken was negative for *Brucella*.[14]

Direct Enzyme Linked Immunosorbent Assay (D-ELISA)

Rose Bengal positive sera were transported to the National Animal Health Diagnostic and Investigation Center (NAHDIC) and examined to confirm and identify *Brucella*-positive samples. All sera were diluted adequately with phosphate-buffered saline before being applied to antibody-coated 96-well plates and incubated for 30 minutes at 37°C. Following that, the wells were washed with the working solution and allowed to dry before being filled with 100 µL of conjugated enzyme and incubated at 37 °C for 30 minutes. After washing and drying the wells, 100 µL of chromogenic substrate was added and incubated at room temperature for 30 minutes. Finally, 100 µL of stop solution was added to the reaction to halt it and measure the absorbance at 450 nm using a microplate reader (Belgium type).

Data Management and Analysis

The data was coded and entered into a Microsoft Excel spreadsheet before being analyzed with STATA (version 15) (StataCorp, College Station, TX, USA). Categorical variables such as sex, age, body condition score, abortion, retained fetal membrane, testicular and joint swelling, and herd size were the explanatory variables considered in the study. *Brucella* seropositivity in D-ELISA was a response variable. Since age and testicular and joint swelling hygroma did not fulfil assumptions of logistic regression, chi-square (X^2) statistics was used to estimate the association of age for the occurrence of brucellosis. Variables that fulfill the assumption of logistic regression underwent univariable logistic regression. Due to co-linearity with each other, no further multivariable logistic regression analysis was conducted for variables with a p-value <0.05 from univariate logistic regression analyses. Statistical significance was made at (P<0.05).

Results

Overall Seroprevalence of Bovine Brucellosis

The D-ELISA serological tests revealed that out of 788 Boran cattle breeds, 7.6% (60/788) were positive for brucellosis. The relationship between each risk factor and the outcome variable was determined. The number of young animals and presence of testicular hygroma and joint swelling was excluded from logistic model since all tested animals were negative and positive for brucellosis, respectively. The association of age and testicular hygroma and joint swelling with *Brucella* infection was evaluated in chi-square test, in which both age and testicular hygroma significantly influenced the disease occurrence (Table 2). The probabilities of animals with retained fetal membranes were 90.75 times higher than animals without RFM in univariate logistic regression analysis for the occurrence of brucellosis. Detail on the effect of different risk factors on the occurrence of brucellosis in logistic regression has been shown (Table 3).

Community Awareness of Bovine Brucellosis

Several risk factors for bovine brucellosis and its public health implications were reported by the majority of respondents. The majority of the participants, 91.7% (55/60) did not awared about zoonotic implications of brucellosis. Only 10% (6/60) of interviewed respondents disposed aborted fetus and RFM properly and the rest 90% (54/60) left in the environment. Ninety percent and 83.3% of the respondents revealed that they consumed raw milk and meat, respectively (Table 4).

Table 2 Univariate Chi-Square Analysis of Age for *Brucella* Infection

Risk Factor	Level	N^{O}. Sampled	N^{O}. Positive (%)	X^2	P-value
Age	Young (≤2)	104	0	9.8747	**0.002**
	Adult (>2)	684	60(8.8)		
Testicular Hygroma and joint swelling	Present	14	14(100)	172.9392	**0.000**
	Absent	774	46(59)		
	Total	788	60(7.6)		

Note: N^{O}, X^2; chi-square, bold indicates statistical significance.

Table 3 Univariate Logistic Regression Analysis of the Risk Factors for *Brucella* Occurrence in Bovine

Risk Factors	Level	N^{O}. Sampled	N^{O}. Positive (%)	OR	95% CI	P-value
District	Gomole	200	16(8)	Ref	-	-
	Arero	168	8(4.8)	0.575	0.24–1.38	0.215
	Yabello	144	12(8.3)	1.04546	0.48–2.28	0.911
	Eleweye	54	2(3.7)	0.442308	0.10–1.99	0.287
	Teltele	222	22(10)	1.265	0.65–2.48	0.494
Herd size	<25	116	4(3.5)	Ref	-	-
	25–40	244	22(9)	2.77478	0.93–8.25	**0.046**
	>40	428	34(8)	2.41624	0.84–6.95	0.102
Sex	Female	204	16(7.8)	Ref	-	-
	Male	584	44(7.5)	1	0.53–1.74	0.886
BCS	Poor	96	8(8.3)	Ref		
	Medium	138	14(10.1)	1.24194	0.50–3.09	0.641
	Good	554	38(6.9)	0.810078	0.37–1.79	0.604
Management	Pastoral	590	46(7.8)	Ref		
	Agro-Pastoral	198	14(7.1)	0.899811	0.48–1.67	0.739
Abortion	Present	10	8 (80)	55.8462	11.56–269.73	**0.000**
	Absent	778	52(6.7)	Ref		
RFM	Present	14	12(85.7)	90.75	19.75–417.08	**0.000**
	Absent	774	48(6.2)	Ref		

Note: Bold in the table indicates statistical significance.
Abbreviations: OR, odds ratio; CI, confidence interval; BCS, body condition score; RFM, retained fetal membrane; Ref, reference; N^{O}, number.

Discussion

The overall individual level sero-prevalence of bovine brucellosis was 7.6%. This is in line with findings by Mekonnen et al[15] and Adaneet al,[9] who found 7.7% and 5.9%, respectively, in the Tigray area and Yabello districts. This finding was higher than previous findings by Gebreyohans,[16] Degefu et al,[17] Yayeh,[18] and Berhe et al,[7] who reported seroprevalence of 1.5%, 1.38%, 0.14%, and 3.19% in Addis Ababa dairy farms in the Jigjiga zone of Somali Regional State, in the North Gondar zone, and extensive production systems in the Tigray region of Ethiopia, respectively. However, the current finding is lower than those

Table 4 Assessment of Knowledge and Attitudes Towards *Brucella* Infection in the Research Area

Variables	Level	N^{O}. Respondents	Percentage (%)
Awareness about zoonotic implications of Brucellosis	Yes	5	8.3
	No	55	91.7
Disposing aborted fetuses and RFM	Yes	6	10
	No	54	90
Consume raw milk	Yes	54	90
	No	6	10
Consume raw meat	Yes	50	83.3
	No	10	16.7

Abbreviation: RFM, retained fetal membrane; N^{O}, number.

reported by Kebede et al,[8] Eshetu et al,[19] Mussie and Hailemelekot,[20] and Junaidu et al,[21] who reported 11%, 10%, 14.96%, and 32.2% in the central highlands and Addis Ababa, Ethiopia, in extensive production systems in northwest parts of Ethiopia and Nigeria, respectively. These variations might be attributed to management variations (intensive, semi-intensive, and extensive) that favor the transmission of disease and occurrence of drought that was attacking the Borena lowlands during the study period. During drought, cattle herds are often split into groups and translocated to mitigate for pasture and water shortages. So when animals migrate in search of feed and water, there might be contact of animals during drinking of water and feed competition of herds from different places increases the risk of exposure of animals to brucellosis in the area.[22]

Regarding age, the current results revealed that the occurrence of bovine brucellosis was higher in adults than in young. This is supported by Hirsh,[23] who concluded that adult and pregnant cattle are more susceptible to *Brucella* versus immature animals of both sexes. Immature animals, on the other hand, are more resistant to infection and remove diseases more frequently, notwithstanding the possibility of latent infection.[10] This could be because reproductive hormones and erythritol, which aid *Brucella* organism development and multiplication, tend to rise in concentration with increasing age and sexual maturity. The difference in sero-prevalence between the sexes was not statistically significant in the current investigation. According to Kebede et al,[8] a slightly higher prevalence rate in males was observed than in females. Males are kept for a relatively long duration in the breeding herd than females, and thus the risk of exposure during their lifetime is high. Although *Brucella* has been identified in the reproductive tract of animals with no measurable amounts of erythritol, this could be related to the level of erythritol, which stimulates the growth of the *Brucella* organism. Erythritol, a sugar alcohol generated in the ungulate placenta, is thought to be responsible for the bacterium's preferred localisation within the ruminant placenta.[24]

According to this finding, body condition had no effect on the occurrence of bovine brucellosis (P>0.05). This finding is supported by Awuh-Ndukum et al,[25] who reported that body condition had no influence on the occurrence of brucellosis in a study conducted in Adamawa and North Regions of Cameroon. This finding contradicted Kungu et al,[26] who reported that body condition affects the occurrence of brucellosis. As a result, refusing to look for food causes a loss of body condition; however, comingling with other animals reduces the risk of exposure. Muma et al[27] found a similar finding in the Kafue basin of Zambia, where animals living in communal grazing areas of the Kafue flats had generally better body condition but also had higher *Brucella* prevalence than those who grazed inferior pastures around the homesteads.

The study found that herd size affects the presence of brucellosis (P<0.05), with larger herds having higher sero-prevalence. This is in line with Asmare et al[28] and Bekele et al,[5] who agreed that herd size determines the occurrence of brucellosis conducted in southeast Ethiopia. This may be accounted by the fact that transmission of contagious diseases such as brucellosis is increased in advanced population.[29] In this study, the contribution of *Brucella* to abortion and RFM was also assessed and showed a statistically significant association. This is supported by Megersa et al[30] previously

suggested that *Brucella* infection is the likely cause of abortion in cattle, thereby contributing to reproductive losses. Abortion that occurs during the last trimester of pregnancy is the manifestation of bovine brucellosis, which may also cause RFM or yield weak calves.[31]

The questionnaire survey revealed that most respondents never used protective gloves to throw fetal membranes and aborted fetuses, and they discarded such materials thrown to open field. Besides, most of the respondents adopted to consume raw and untreated milk and meat, which is the main route for acquiring zoonotic disease. A similar finding was reported by Genene et al,[32] who concluded that the major cause of increased human brucellosis reported a high prevalence of human brucellosis in Yabello district. It has also been described that brucellosis can be a health hazard to humans, particularly to pastoral households, who in many ways are exposed to the disease.[33,34] This might suggest that people in the Borena zone have inadequate knowledge about brucellosis. Brucellosis control and elimination are hampered by a lack of knowledge about the disease.[35]

Conclusions and Recommendations

This study demonstrated that the occurrence of bovine brucellosis in the Borena zone is slightly greater than other country-level findings. In pastoral communities, this poses a high risk of infection and has major public health implications. Furthermore, the risk factor analysis demonstrated that abortion, age and retained fetal membrane had an impact on the occurrence of brucellosis in the research area ($P<0.05$). Variations in sex, herd size, district, management and body condition, on the other hand, did not have a statistical significant relationship with the occurrence of the disease ($P>0.05$). Besides, RFM and abortion were the most common brucellosis symptoms in the research location. According to the poll, the majority of respondents were uninformed about brucellosis transmission and public health concerns.

According to the fore mentioned conclusion, the following recommendations were forwarded:

- Mixing of cattle with others during feeding and drinking water should be avoided to reduce the spread of the disease.
- To cease consuming raw milk and meat, and control and prevent brucellosis, public awareness and health education, particularly among livestock owners and pastoral communities, should be stressed.
- Further research to address human brucellosis in the Borena zone is suggested.

Ethical Clearance

The Ethical Review Committee of Wolaita Sodo University's College of Veterinary Medicine evaluated and approved the study technique. This committee followed the protocols of the National Research Ethics Review Guideline formulated by the Ministry of Science and Technology of the Federal Democratic Republic of Ethiopia in 2014. In addition, we obtained oral consent from respondents. Animals were treated with best practice of veterinary care.

Acknowledgments

The authors acknowledge the financial support of Wolaita Sodo University and Yabello Regional Laboratory for this study. The authors appreciate the undeserved assistance provided by Yabello Regional Laboratory staff.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work. Alebachew Tilahun: comprehended the proposal, advised the activity and corrected the designed research, analyzed the data, and revised the final version. Silto Kegno: comprehended the proposal, designed the study, collected the samples and wrote the article. Dinberu Mamuye: reviewed the drafted article and suggest critical correction and was involved in analysis and interpretation of the data. Takele Adugna: reviewing the article, execution, acquisition, analysis and interpretation of data.

Disclosure

The authors report no conflicts of interest in relation to this work.

References

1. OIE. *Bovine Brucellosis: In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Paris: OIE; 2009:409–435.
2. Godfroid J, Scholz H, Barbier T, et al. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Prev Vet Med*. 2011;102(2):118–131. doi:10.1016/j.prevetmed.2011.04.007
3. OIE. *Infection with Brucella Abortus, Brucella Melitensis and Brucella Suis*. Rome: OIE (World Organization for Animal Health); 2016.
4. Ragan VE. The animal and plant health inspection service (APHIS) brucellosis eradication program in the United States. *Vet Microbiol*. 2002;90(1–4):11–18. doi:10.1016/S0378-1135(02)00240-7
5. Bekele A, Molla B, Asfaw Y, et al. Bovine brucellosis in ranches and farms in South-eastern Ethiopia. *Bull Anim Health Prod Afr*. 2000;48:13–17.
6. Kassahun A *Epidemiology of bovine brucellosis in cattle and its seroprevalence in animal health professionals in Sidama Zone, Southern Ethiopia*. Master of Science thesis Addis Ababa University, Faculty of Veterinary Medicine. Debre Zeit, Ethiopia; 2004.
7. Berhe G, Belihu K, Asfaw Y. Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. *Int J Appl Res Vet Med*. 2007;5:65.
8. Kebede T, Ejeta G, Ameni G. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). *Rev Med Vet*. 2008;159:3.
9. Adane B, Kemal K, Begna F. Survey of bovine brucellosis in pastoral and agro-pastoral production systems of Borana zone, Southern Ethiopia and its public health implications. *Ethiop Vet J*. 2012;16(1):1–3.
10. Radostits O, Gay C, Hinchcliff K, et al. *Veterinary Medicine, a Textbook of the Disease of Cattle, Sheep, Goat, Pigs and Horses*. 10th ed. London, New York: Saunders Elsevier; 2007:2047.
11. Bayemi P, Mah G, Ndamukong K, et al. Bovine brucellosis in cattle production Systems in the Western Highlands of Cameroon. *Int J Anim Biol*. 2015;1:38–44.
12. Thrusfield M. *Veterinary Epidemiology*. 3rd ed. Burgh, U.K: LTD Black well science; 2007:182–189.
13. Sayour AE, Elbauomy EM, Abdel-Hamid NH, et al. Validation of different versions of the card or rose-Bengal test for the diagnosis of Brucella melitensis infection in ruminants. *Alex J Vet Sci*. 2017;53:1
14. Nour B, Tawor B, Gebril A, et al. Evaluation of simple and rapid specific IgM/IgG flow assay and some methods used in diagnosis of brucellosis in Gezira and Blue Nile States, Sudan. *Clin Microbiol*. 2015;4:1000187. doi:10.4172/2327-5073.1000187
15. Mekonnen H, Kalayou S, Kyule M. Serological survey of bovine brucellosis in barka and arado breeds (Bos indicus) of Western Tigray, Ethiopia. *Prev Vet Med*. 2010;94(1–2):28–35. doi:10.1016/j.prevetmed.2009.12.001
16. Gebreyohans T. *A survey of bovine brucellosis in Addis Ababa dairy farms*. DVM Thesis FVM, AAU. Debre-Zeit, Ethiopia; 2004.
17. Degefu H, Mohamud M, Hailemelekot M, et al. Seroprevalence of bovine brucellosis in agro pastoral areas of Jijjiga zone of Somali National Regional State, Eastern Ethiopia. *Ethiop Vet J*. 2011;15. doi:10.2147/VMRR.S338930
18. Yayeh T. A survey of bovine brucellosis in selected areas of North Gondar Zone, Ethiopia. Debre Zeit, Ethiopia: DVM Thesis Faculty of Veterinary Medicine, Addis Ababa University; 2003.
19. Eshetu Y, Kassahun J, Abebe P, et al. Seroprevalence study of brucellosis on dairy cattle in Addis Ababa, Ethiopia. Bulletin of Animal health and Production in Africa; 2005.
20. Mussie H, Hailemelekot M. *Sero-prevalence study of brucellosis in cattle and human in Bahirdar milk shed*. Master's Thesis, FVM, AAU. Debre Zeit, Ethiopia; 2005.
21. Junaidu A, Oboegbulem S, Salihu M. Seroprevalence of brucellosis in prison farm in Sokoto. Nigeria; 2008.
22. Bedane AK, Feyissa K, Fanos B, Demelash T, Asseged B. Survey of bovine brucellosis in pastoral and agro-pastoral production systems of borana zone, southern Ethiopia and its public health implications. *LARCJI*. 2012;3:53–59.
23. Hirsh D, Zee Y. *Veterinary Microbiology*. USA: Black well science. Inc Pp; 1999:115–249.
24. Megersa B, Biffa D, Abunna F, et al. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Trop Anim Health Prod*. 2011;43:651–656. doi:10.1007/s11250-010-9748-2
25. Awah-Ndukum J, Mouiche M, Bayang H, et al. Seroprevalence and associated risk factors of brucellosis among indigenous cattle in the Adamawa and north regions of Cameroon. *Vet Med Int*. 2018;2018. doi:10.1155/2018/3468596
26. Kungu JM. Seroprevalence and risk factors of brucellosis in cattle in Gulu and Amuru Districts, Northern Uganda. Makerere University; 2011.
27. Muma J, Munyeme M, Matope G, et al. *Brucella* seroprevalence of the Kafue lechwe (Kobus leche kafuensis) and Black lechwe (Kobus leche smithemani): exposure associated to contact with cattle. *Prev Vet Med*. 2011;100(3–4):256–260. doi:10.1016/j.prevetmed.2011.03.013
28. Asmare K, Asfaw Y, Gelaye E, et al. Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. *Afr J Agric Res*. 2010;5:257–263.
29. Ibrahim N, Belihu K, Lobago F, et al. Sero-prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. *Trop Anim Health Prod*. 2010;42:35–40. doi:10.1007/s11250-009-9382-z
30. Megersa B. An epidemiological study of major camel diseases in the Borana lowland, Southern Ethiopia. Oslo: Drylands Coordination Grou; 2010.
31. Seifert H. *Brucellosis. Trop Anim Health*. 2nd ed. Dordrecht Kluwer Academic Publishers group; 1996:358–362.
32. Genene R, Desalew M, Yamuah L, et al. Human brucellosis in traditional pastoral communities in Ethiopia. *Int J Trop Med*. 2009;4:59–64.
33. Abbas B, Agab H. A review of camel brucellosis. *Prev Vet Med*. 2002;55(1):47–56. doi:10.1016/S0167-5877(02)00055-7
34. Habtamu T Pastoralism and agro-pastoralism systems. *In the Proceedings of the 18 Annual Conference of the Ethiopian Veterinary Association (EVA) held in Addis Ababa*. Ethiopia; 2004.
35. Khan HAA, Akram W, Shad SA, et al. A cross sectional survey of knowledge, attitude and practices related to house flies among dairy farmers in Punjab, Pakistan. *J Ethnobiol Ethnomed*. 2013;9:1–10. doi:10.1186/1746-4269-9-18