

Seroprevalence of Bovine Brucellosis and its Associated Risk Factors in Jikow District, Gambella Regional State, Ethiopia

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ABSTRACT

Background: Bovine brucellosis has been a confirmed public health threat retained endemically to Ethiopia. Despite its devastating impact both on livestock economy and public health, a nationwide act of eradication/mitigation has not set out in Ethiopia yet. Attributed to its nature and the intimacy between society and their livestock in pastoral area in Ethiopia, the problem is more pronounced compared to highlands. In particular in Gambella region, one of the four pastoral states in Ethiopia, lacks a relevant brucellosis picture to date. Hence, the current study was designed cross-sectionally to study carried out from November 2016 to May 2017 in an attempt to determine seroprevalence of brucellosis in cattle managed in Jikow district, Gambella and identify associated risk factors.

Results: Of 384 sera tested, 96 showed positivity by rose bengal plate test giving an overall prevalence of 25% (96/384) and those 96 RBPT positive samples were further subjected to a recommended confirmatory test of complement Fixation Test (CFT). Accordingly, 56 were confirmed to be Brucella antibody positive by CFT providing an overall seroprevalence of bovine brucellosis in the district be 14.6% (56/384). Five (5) potential risk factors were considered for analysis amid kebeles of cattle residence, parity and history of previous abortion were found associated at a statistically significant level ($p < 0.05$), nevertheless sex and age had no statistically significant association ($p > 0.05$) with the disease.

Conclusions: The present study has given insights implying a relatively higher prevalence of the disease urging a more organized and integrated actions ahead of its disastrous consequences in both cattle productivity and public health dimensions. Public awareness, actual bacterial strain characterization circulating in Bovine species, continued surveillance and implementing control measures would bring the trajectory of the disease to an end.

Keywords: Seroprevalence, Cattle, Brucellosis, RBPT, CFT, Gambella.

1. Background

Of the highest priority diseases, both in sub-Saharan Africa and other regions of the developing world is brucellosis (McDermot *et al.*, 2002). In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange Free State of South Africa in the year 1915 (Chukuwu, 1985). In Ethiopia, there is no documented information on how and when brucellosis was introduced and established (Dinka and Chala, 2009).

Though eradicated in most developed countries, developing countries have continued to experience an increasing trend of the disease because of lack of resources and coordinated control programs, increased pastoralism and transhumance and intensification of commercial livestock farms (Ducrottoy *et al.*, 2014). Though endemic in many developing countries (Donev, *et al.*, 2010), brucellosis remains under diagnosed and under-reported.

Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide and considered as one of the most widespread zoonoses in the world (Schelling *et al.*, 2003) and being 'responsible for more sickness, misery and economic loss than any other zoonosis' (Alausa, 1979). It is the second most important zoonotic disease of the world after Rabies (FAO, 2005). Brucellosis affects man, domestic and some wild animals, and marine mammals (Bhatia and Narain, 2010 and Seleem *et al.*, 2010). Brucellosis is a disease of cattle usually caused by *Brucella abortus*, less frequently by *Brucella melitensis* and rarely by *Brucella suis* (OIE, 2008).

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 (FAO, 2003). Cross-transmission of brucellosis can occur between cattle, sheep, goats, camels and other species (Dawood, 2008).

Especially cattle herders in pastoral areas are in close contact with their animals, consumption of raw milk and handling of aborted materials is common. In animals, the disease is presented as an acute or persistent febrile illness with a diversity of clinical manifestations (Smits and Kadri, 2004). The primary sign of infection in females is abortion in the last trimester of pregnancy and in male epididymitis and orchitis (Corbel, 2006; Donev *et al.*, 2010). In highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature of the disease (Radostits *et al.*, 2000). Retention of placenta and metritis are common sequels to abortion (Walker, 1999). Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility.

The economic losses of Brucellosis is because of abortions, stillbirths, reduced milk production, infertility (McDermott and Arimi, 2002), revenue losses due to international trade impediment for animals and their products (Corbel, 2006), increased replacement costs as well as lowered sale value of infected cows (Scholz *et al.*, 2008). The epidemiology of brucellosis in livestock and humans as well as appropriate preventive measures are not well understood and such information is inadequate particularly in sub-Saharan Africa (Dinka and Chala, 2009; McDermott and Arimi (2002) and Schelling *et al.* (2003). Evidences of brucellosis in Ethiopian cattle have been serologically demonstrated by different authors. Other reports had also indicated as the disease is endemic in Ethiopia and researchers have established its prevalence rate in cattle in different regions of Ethiopia, though country wide prevalence in cattle is not yet determined (Yilma *et al.*, 2016).

In the last two decades, several serological surveys have showed that bovine Brucellosis is an endemic and wide spread disease in the country. For instance, prevalence of 18.4% around Addis Ababa, 2.4% in Jimma zone, 11.6% in Sidama region, 4.2% in south east Ethiopia, 2.9% in Central Oromia, 11.2% in east Showa zone, 4.9% in Tigray region, 7.61% in Arsi region and 1.11% in Addis Ababa and Sululta abattoir (Yohannes *et al.*, 2013), 39% in Western Ethiopia (Mayer, 1980), 8.2% in Arsi area (Bayleyegne, 1989), 22% in a dairy farm in Northeastern Ethiopia (Tariku, 1994), 8.1% in dairy farms in and around Addis Ababa (Yilkal, 1998), 11%-15% in dairy farms and ranches in south western Ethiopia (Tekelye *et al.*, 2000), and 7.7% in Tigray region (Mekonnen *et al.*, 2010), 0.77% in southwestern Ethiopia (Tadele, 2004), and 2.46% in Sidama zone (Kassahun *et al.*, 2007). Limited serological studies on livestock Brucellosis were done so far in pastoral and agro pastoral areas of East Africa as indicated by the seroprevalence of 8.2% in Eritrea and 5% in Sudan (Omer *et al.*, 2000; El-Ansary *et al.*, 2001).

In Ethiopia, though several serological surveys have showed bovine brucellosis is an endemic and widespread disease in urban, peri-urban, highland and lowland, extensive and intensive farming, small holder farms and ranches of the country (Kebede *et al.*, 2008; Tolosa *et al.*, 2008; Dinka and Chala, 2009), most of these studies on cattle brucellosis have been carried out in central and northern Ethiopia and do not provide an adequate epidemiological picture of the disease in different agro-ecological zones and livestock production systems of the country (Megersaet *et al.*, 2011). In Ethiopia, serological studies done so far are inadequate in pastoral country. For

instance, a serological study conducted in pastoral and agro-pastoral areas of East Shoa zones of Oromia Regional State reported prevalence of bovine Brucellosis to be 15.2% for pastoral and 4.1% for agropastoral areas using RBPT (Hunduma and Regassa, 2009). Similarly, a study carried out in east Showa zone of Ethiopia showed a relatively higher seroprevalence in pastoral than agro pastoral system (Dinka and Chala, 2009).

Moreover, there is a scarcity of published literature on the status of cattle brucellosis in pastoral areas of the country in general and Gambella region in particular, where large populations of cattle are reared. Reports have also revealed that the major risk factors for animal Brucellosis are age, parity, retained fetal membrane (RFM), abortion, herd size and composition across different agro-ecologies and production system (Yilma *et al.*, 2016).

Conclusively, there is limited published literature concerning seroprevalence of bovine brucellosis in Gambella region. It was hypothesized that the current study would reveal the exact picture of the disease in the study area and would lay down background data for further prospective reference and related control activities.

2. Methods

Description of the study area: A cross sectional study design was conducted in Jikow district, Gambella from October 2016 to May 2017 aimed at determining seroprevalence of Bovine brucellosis and associated risk factors. Jikow district is one of the districts under Nuer zone in Gambella region. The district possesses 25 kebeles of which six were selected for sample study and it is a district in which Gambella National Park is located. Kebele data were obtained from district agriculture office.

Study population: The study was conducted on indigenous cattle aged six months and above and maintained under extensive husbandry system in the study area, Jikow, Gambella. Individual animals of which samples drawn were privately owned (from pastoral cattle owners).

Sample size determination and sampling strategy

Both simple random and purposive sampling techniques were applied during the study. The study zone (Nuer) and district (Jikow) were selected purposely based on access and cattle population density. Study kebeles, herds and study units (individual animals) were selected using simple random fashion.

Six kebeles were selected from a total of twenty five kebeles found in Jikow district. The total number of animals to be sampled for the study was determined using Thrusfield, (2007) formula:

$$n = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2} \quad (1)$$

Where: n = required sample size, P_{exp} = expected prevalence (50%), d = desired absolute precision (5%).

As no previous study carried out on bovine brucellosis in the study district (Jikow), a 50% expected prevalence was considered. Having applied the indicated variables in the formula given, a total of 384 cattle serum samples were collected for the study. Abortion history and parity number data were provided by cattle owners whereas sex and age of animals were determined by researchers using physical observation and teeth formula, respectively.

Blood Sample collection and transportation

From January 1-20/2017, approximately, 10ml of whole blood samples per animal were collected from the jugular vein of each selected animal in a sterile plain vacutainer tube. Soon after collection, samples were labeled and packed in an ice box and transported to the nearby health center for separation and temporary storage. Samples were kept overnight at room temperature to make the blood clot. After clotting, each serum was transferred to similarly labeled cryogenic vials and stored in -20°C refrigerator. For serologic analysis, sera were transported to National Veterinary Institute (NVI) using cooling system, Bishoftu, Ethiopia.

Rose Bengal Plate tests (RBPT): RBP test was conducted at Addis Ababa University, College of Veterinary Medicine, serology laboratory. RBPT was conducted according to the procedures described by Alton *et al.* (1988). Accordingly, of the 384 sera tested by RBPT, 96 sera were found positive by RBPT. RBPT is very sensitive (>99%) but less specific (<68%). Hence, its positive results should be confirmed by a more specific test such as CFT (highly specific).

Complement Fixation Test (CFT): All sera tested positive by the RBPT were retested using CFT for further confirmation at National Veterinary Institute (NVI), Debrezeit, AAU, Ethiopia. Standard *B. abortus antigen* for CFT was used to detect the presence of anti-Brucella antibody in the sera. Those 96 RBPT positive sera were further subjected to the more specific CFT test and as a result, 56 sera were confirmed to be Brucella positive by CFT test.

Data management and analysis

Interview and laboratory result data were coded and fed into Microsoft Excel for further statistical analysis. Using IBM-SPSS version 20, frequency was operated to determine prevalence of the disease both by RBPT and CFT. Chi-square (χ^2) statistics was employed to determine the association of risk factors with seropositivity. Risk factors associated with the disease were further resolved using binary logistic regression analysis applying 95 % confidence interval (CI) to find out the degree of association between the factors and the disease.

3. Results

Prevalence determined

For prevalence determination, descriptive statistics was computed. Accordingly, of 384 samples tested by RBPT, 96 were found to be positive with a prevalence of 25% (Table 1). Since RBPT is a less specific test for Brucella antibody detection, those 96 RBPT positive samples were further subjected to a more specific and confirmatory test, CFT test. As a result, of 96 RBPT positive samples retested by CFT, 56 were found positive for *Brucella* providing an overall prevalence of 14.6% as depicted down in table 1.

Table 1: Bovine Brucellosis prevalence by RBPT and CFT

S/R	Test name	Total sample tested	Positive Result	Prevalence (%)
1	RBPT	384	96	25
2	CFT	96	56	14.6

Risk factors analysis

To determine the existence of association between risk factors and the disease (bovine Brucellosis), Chi-Square (χ^2) analysis has been done and revealed the direct association of three variables (parity, abortion history and kebeles) with disease. A similar analysis evinced the absence of impact of age and sex on disease occurrence as outlined in Table 2.

Table 2: Chi-Square analysis result for risk factors association

Risk factors	CFT result (descriptive measures)		P-value
	Negative	Positive	
Sex Female Male	257	48	0.208
	71	8	
Age 0 1 2	CFT result		0.074
	Negative	Positive	
	84	9	
	188	31	
	56	16	
Parity no parity 1-3 parity 4-8 parity	CFT result		0.012
	Negative	Positive	
	112	12	
	94	27	
	122	17	
Abortion no abortion Yes	CFT result		0.000
	Negative	Positive	
	270	14	
	58	42	
	CFT result		

	Negative	Positive	P-value
Burgile	57	7	0.017
Mading	51	13	
Bairial	49	15	
Bieyien	54	12	
Conyang	61	7	
Tongedol	56	2	

Binary logistic regression analysis

Applying 95% confidence interval (CI), binary logistic regression was employed to determine the degree of impact the risk factors have posed on the disease occurrence.

As a result, abortion history with yes and no variables was found positively associated with the disease at significance level ($P=0.000$). The analysis revealed that cows with no abortion history were found to be 98.8 % less likely at risk than cows with abortion history as shown by Table 3.

Multivariate logistic regression analysis also has been computed for parity to determine the level of association with the disease occurrence. Female cattle were set into three parity categories (no parity/heifers, 1-3 parity and 4-8 parity. The last category was considered as a reference and the first two have been compared to the reference. Accordingly, cows with parity number 1-3 were found 4.166 more likely at risk than cows with 4-8 parity numbers for the disease. Similarly, heifers/young female cattle were found 6.273 more likely at risk than cows with 4-8 parity numbers for the disease indicating cows with more parity number are more resistant to the disease (Table 3).

The degree of connectedness between the study sites and the disease was similarly done and described. Tongedol district was considered as a reference and the remaining five were compared with it. Burgile kebele is 19.4% less likely at risk than Tongedol. On the other hand, Mading is district is 3.667 times likely at risk than Tongedol for the disease occurrence. Similarly, Bairial is 3.588 times likely at risk than Tongedol. However, 33.8% and 56.1%, Bieyien and Conyang kebeles were more likely at risk for brucellosis than Tongedol, respectively, Table 3.

Table 3: Adjusted Odds ratio (AOR) by binary and multivariate logistic regression

Risk factors	Odds ratio (OR)	95% Confidence Interval (CI)	
Abortion Yes No	.012	.005	.033

Parity	4-8 parity			
	no parity	6.273	2.027	19.416
	1-3 parity	4.166	1.690	10.271
Kebele	Tongedol			
	Burgile	.806	.191	3.391
	Mading	3.667	.985	13.648
	Bairial	3.588	.956	13.466
	Bienien	1.338	.351	5.101
	Conyang	1.561	.394	6.184

4. DISCUSSIONS

Brucellosis is of the diseases that have drawn attention and concern as it causes economic losses in cattle besides its zoonotic dimension (Radostits *et al.*, 2007). The disease can be diagnosed using several serological tests including rose Bengal test (RBPT), complement fixation test (CFT), ELISA and others (Mwelwa, 2012). The present study has reported 14.6% overall seroprevalence of bovine Brucellosis by CFT which is a highly specific test recommended for confirmatory detection of brucella antibodies (Radostits, *et al.*, 2007). The present report is within the range, 10 to 15%, that was estimated for any assumed brucellosis seroprevalence for East Africa (Mangen *et al.*, 2002). As Gambella region is pastoral in nature, more supportive evidence is that in sub-Saharan Africa, the highest incidences of brucellosis were found in pastoral production systems (McDermott, J. and S. Arimi, 2002, Mangen *et al.*, 2002, Schelling *et al.*, 2003).

The present study has evinced much higher prevalence rate compared to the low prevalence reports of 1.38% in Jijjiga zone of Somalia regional state (Degafu *et al.*, 2011), 1.92% in Sidama zone (Asmare *et al.*, 2010), 2.96% RBPT and 1.97% CFT in Guto-Gida district of East Wollega Zone (Yohannes *et al.*, 2013), 2.9% in central Oromia (Jergefa, *et al.*, 2009), 3.5% in South Eastern Ethiopia (Megersa, *et al.*, 2011), 4.1% in agro-pastoral areas of East Shoa Zone (Hunduma and Regassa, 2009), 4.63% in Bahir Dar milk shed (Mussie *et al.*, 2007a), 3.19% in Tigray region (Berhe *et al.*, 2007), 0.61% in Jimma (Tadele, 2004), and no positive reactors in Selale and Addis Ababa (Belihu, 2002).

The high prevalence of the disease observed in the present study could be attributed to the pattern of pastoral community characterized by clustering of households with close proximity of herds in the pastoral camps, the diversity of livestock species reared as part of a coping mechanism for uncertainties and risks. Such conditions certainly would increase aggregation and interaction of different animals at villages, pasture fields and water points facilitating the transmission of the disease. The dynamics and frequent migration of pastoral herds might increase the chance of coming into contact with other potentially infected herds in different areas. Mobility also increases the opportunity of interactions with wild animals (Bekele *et al.*, 2000; Samui *et al.*, 2007). The higher prevalence observed in the current report could also be due to the large herd size as similar observations made by several researchers (Maiga *et al.*, 1996; Bekele *et al.*, 2000; Tolosa, 2004; Asmare, 2004). According to one finding, large herd size enhances the exposure potential, especially following abortions through increased contact and common feeding and watering points promoting transmission of *Brucella* organisms (Hellmann *et al.*, 1984).

Conversely, the present report was by far much lower than the previous reports of Rashid (1993) with 38.7% in cattle owned by institute of agriculture research farm, Gebremariam (1985) with 18.4% in the dairy farms of around Addis Ababa, and in Abernosa cattle breeding ranch and East Shoa where a seroprevalence of 19.5 % was reported by Yirgu (1991). Higher than the present study prevalence was also reported in other African countries as 46.8% in Uganda by Kungu *et al.* (2010), 41% in Togo by Domingo (2000), while a relatively similar prevalence to the current one that was 14.2% in South Africa have been reported (Manhica, 2010).

The observed variation of the reported prevalence among different regions of Ethiopia could be attributed to the difference of animals' age, sex, breed system, pregnancy status and the study area, animal management practices, herd size, reproductive diseases and serological tests applied (Gul and Khan, 2007; Kebede *et al.*, 2008).

Among potential risk factors considered for the present study, location/kebeles of cattle, parity, and story of abortion evinced statistically significant association ($p < 0.05$) with seropositivity of bovine Brucellosis whereas sex and age had no statistically significant association with the disease.

The observed significant association of the disease with different locations would be related to the pastoralists' husbandry practices involving activities of sharing grazing areas and drinking from the same water points, more importantly in densely cattle populated areas like Bieyien, Mading and Bairial. These areas have high cattle population because of the abundant pastures and grazing lands, thus allowing different animals to intermingle enhancing possible transmission of the organism among cattle. A survey conducted in Kura local government area of Kano which is also a cattle concentration point (Bakari, 2010) discovered similar findings.

Parity of animals had also shown statistically significant variations in seroprevalence with the odd of the disease being 6.2 and 4.16 times higher in animals with no parity and in animals with 1-3 parity respectively, when compared with animals with 4-8 parity. This finding is in line with the finding of Shewit *et al.* (2008), who reported the association of seropositivity with parity.

The history of previous abortions in animals was also significantly associated with the seropositivity to *Brucella* antibodies (Radostits *et al.*, 1994 and Swell and Brocklesby, 1990). Statistically, aborted cows were found to be 98.8% more likely to be seropositive when compared to none aborted cows as of the present study. Thus, the present finding is consistent with the findings of Tolosa (2004) and Hailemeleket (2005) who have reported significant association between abortion and seropositivity. Similar results were also obtained by other investigators (McDermott and Arimi, 2002; Kubuafor *et al.*, 2000 and Schelling *et al.*, 2003).

5. Conclusions

Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide particularly developing countries as one of the most widespread zoonoses in the world following rabies. The present study came up with an intermediate prevalence record of 14.6% by CFT in bovine kept by extensive management system in the study area. As part of the present study, a statistical computation has figured out the connected association of parity, previous history of abortion and areas/locations with the disease whilst

insignificant value for age and sex. The relatively higher prevalence recorded in the district warns a demand of integrated act and full community awareness.

As of the above conclusive remarks, the following recommendations could be forwarded:

- ✘ Region wise regular brucellosis surveillance and control measures need to be launched in a view to minimize the economic and public health impacts posed by the disease
- ✘ A strategic control system of livestock movements within the region as well a cross of inter-regional boundaries need to be devised and put in place
- ✘ Programs targeted at public awareness on brucellosis economic and public health impacts have to enhanced
- ✘ Confirmatory bacterial isolation and characterization studies need to be done at larger scale

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Our research samples (blood samples were drawn of animals, cattle) with full awareness and knowledge of each animal owner about the study. However, ethical clearance would not be applicable as Ethics approval committee has not established in our University yet (Samara University).

Source of animals

It so not applicable to get written informed consent from animal owners.

Consent for Publication: Not applicable

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: I am not sure of other authors' stance. I need support to go for it.

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Authors' contributions

S/N	Author's name	Contributions
1	Gatluak Reath	Proposal writing, data collection and paper write up
2	Wossene Negash	Proposal evaluation, advising, data analysis and interpretation, paper evaluation and editing and manuscript writing
3	Getachew Terefe	Title selection and funding
4	Gezahegn Mamo	Title selection and funding

ABBREVIATIONS

FAO FOOD AND AGRICULTURAL ORGANIZATION

RBPT Rose Bengal Plate Test

OIE Office International des Epizootics

ELISA Enzyme Linked Immunosorbent Assay

CFT Complement Fixation Test

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