Seroepidemiology of Human Brucellosis among Blood Donors in Southern Ethiopia: Calling Attention to a Neglected Zoonotic Disease

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Abstract. Human brucellosis is neglected in southern Ethiopia. Although traditional food processing practices and animal husbandry which increase the risk of brucellosis are common, it has not been properly studied yet. This study was conducted to determine the seroepidemiology of brucellosis among apparently healthy individuals in southern Ethiopia. In the study, blood samples were collected to screen for serum agglutinins reactive to stained antigen of Brucella abortus. Standard tube titration was performed for reactive serum to determine the titer of the agglutinin. A structured questionnaire was used to collect data on possible risk factors for brucellosis. The seroprevalence of human brucellosis in this study was found to be 10.6% (95% confidence interval = 7.0, 14.0). Possession of domestic ruminant animals, contact with ruminant animals, and husbandry practices at home were associated with seropositivity. The higher seroprevalence of human brucellosis in the study area needs attention and additional confirmatory investigation.

Human brucellosis is one of the most common zoonotic diseases worldwide and remains endemic in areas like Europe, northern and eastern Africa, India, central Asia, Mexico, and Central and South America. 1 Although eradicated in many developed countries, brucellosis remains a major neglected zoonotic disease in low-income countries and a concern of reemergence in many countries with an increasing incidence of infection in cattle.² Both Food and Agriculture Organization and the World Health Organization considered brucellosis as one of the most widely spread zoonoses in the world.3 Annual incidence of human brucellosis may range from a few cases to more than 500 cases per 1,000,000 populations in different parts of the world. A global report estimated human cases to be 500,000 each year.¹ These figures, however, are more likely an underestimate of the true disease burden due to nonspecific clinical cases in many endemic countries where health systems are weak and misdiagnoses of cases are common.⁴ In Ethiopia, the burden of human brucellosis remains unclear and epidemiological data on the disease remain scarce.5

Humans acquire the infection mainly through contact with an infected animal and consumption of fresh unprocessed animal products. Other possible means of acquisition of brucellosis include person-to-person transmission (blood donation, tissue transplantation, etc.) and infection from a contaminated environment.⁶ Brucellosis is also an occupational hazard of livestock farmers, dairy workers, veterinarians, slaughterhouse workers, and laboratory personnel.^{7,8} Human infection may involve many organ systems and the disease usually manifests itself as an acute febrile illness characterized by a multitude of somatic complaints and nonspecific symptoms (fever, sweating, anorexia, malaise, weight loss, depression, headache, and arthralgia), and is easily confused with a wide spectrum of infectious and noninfectious diseases. The disease may persist and progress to a chronically incapacitating disease with severe complications like bone and joint involvement, neurobrucellosis, endocarditis, and epididymo-orchitis. Nevertheless, brucellosis is rarely

fatal in humans with an estimated mortality rate of less than 2%.^{5,6,9} The disease is also an important cause of travel-associated morbidity.¹

The causative agents of brucellosis are small Gramnegative intracellular coccobacillary organisms that have predilection to host species. In addition to the better-known six classical species of *Brucella* (*Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella ovis*, *Brucella canis*, and *Brucella neotomae*), newer members of the genus include *Brucella ceti*, *Brucella pinnipedialis*, *Brucella microti*, and *Brucella inopinata*. Of these species, *B. melitensis* poses the greatest risk for human infection followed by *B. suis* and *B. abortus*. However, other species have also been shown to be virulent for humans infected.^{5,10}

Ethiopia has diverse agroecological zones which have contributed to the practice of different agricultural production systems by over 85% of the population. Animal husbandry forms an integral part of agricultural production in almost all ecological zones of the country. Pastoralists also measure to a significant proportion of the country's population. Dairy product processing and animal husbandry are very traditional. Limited reports from some parts of the country have shown the existence of bovine brucellosis. Transmission from animals to humans is expected due to significant animal—human contacts and consumption of raw and undercooked animal products in Ethiopia.

Although studies have been conducted on the seroepidemiology of bovine brucellosis in different parts of Ethiopia, 11,13–19 only a few studies focused on humans, mainly on occupationally at-risk individuals (farmers, abattoir workers). 20–23 There is scarcity of studies on clinical cases. 12 More studies from different perspectives, including the general population, are needed to define the burden and risk factors of human brucellosis in Ethiopia. Hence, this cross-sectional study was conducted from January to March 2014 to assess the seroepidemiology and risk factors of brucellosis among blood donors at Arba Minch Blood Bank Center located in Arba Minch town, 505 km southwest of Addis Ababa. The center is the main source of transfusion blood for eight hospitals in Gamo Gofa, Wolayta, Segen, and South Omo zones.

Donors were screened based on medical history questionnaire for chronic and acute diseases, blood pressure, weight,

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and qualitative hematocrit. Blood was collected into citrate-phosphate-dextrose-adenine anticoagulated blood collection bag. The leftover sample in the blood bag tube was dispensed into silicon-coated test tube (BD Vacutainer blood collection tube, Franklin Lakes, NJ). The tube was then inverted 4–6 times gently to mix the blood with clot activator in the tube and allowed to stand for 30–40 minutes for clotting at room temperature. Subsequently, the clotted blood was centrifuged at 1,300 Relative Centrifugal Force for 10 minutes, and about 1.5 mL serum sample was carefully transferred to cryotube and immediately stored in –20°C freezer until processed within a week of sample collection.

HumaTex febrile antigen test kit (Human Diagnostic, Wiesbaden, Germany) was used for primary screening. Titer determination was done according to the manufacturer's recommendations. The kit consisted of stained bacterial suspensions of *B. abortus* that react with antibodies to all three *Brucella* species pathogenic for humans (*B. abortus*, *B. melitensis*, and *B. suis*). The serum titer was defined as the highest dilution showing agglutination. Two experienced laboratory personnel independently read and reported serological tests. Results were recorded as positive when the readings were concordant.

Data on the risk factor for human brucellosis were collected by blood donor screening professionals through face-to-face interview using a structured questionnaire. The questions mainly focused on presence and type of domestic ruminant animals at home, consumption of unpasteurized milk and raw animal products, and extent of contact with domestic ruminant animals. Variables like age, sex, blood donation history, residence, occupation, educational level, and other related data were also recorded.

Epidemiologic and laboratory data were entered into IBM SPSS Statistics for Windows version 20 (IBM Corp, Armonk, NY) for analysis. All known and common risk factors for brucellosis were assessed for their association with the serologic screening result for brucellosis using the standard binary logistic regression analysis. For contingency tables having cell frequencies of zero, exact logistic regression using the software Stata version 12 (StataCorp LP, College Station, TX) was done to identify the association between brucellosis and the respective risk factor. Odds ratios (ORs) with 95% confidence intervals (CIs) were used as measures of the presence and strength of association between variables. P value was used to indicate the probability of obtaining the observed result or a more extreme one by chance. P values less than or equal to 0.05 were considered statistically significant.

Ethical approval for this study was obtained from the Research Review Committee of the Southern Nations, Nationalities and Peoples Region Health Bureau. Informed consent was obtained from each participant before data collection. Permission to do the research was obtained from Arba Minch Blood Bank Center.

A total of 254 donors donated blood through regular programs and outreach blood collection campaigns. Majority of the study participants were males (82.3%). The mean age of the study participants was 26 years (standard deviation = 8.26). Nearly 57% were voluntary donors and the rest were replacement donors. Most of the donors were from Arba Minch town (48.8%) and the others came from wider areas around Arba Minch town, mainly from Bonke (5.9%),

TABLE 1
Sociodemographic characteristics of the study participants, Arba Minch Blood Bank Center, Ethiopia, January–March 2014

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Variables	Count	Percent	
Age, in years $(N = 243)$	16–30	182	74.9
	≥ 31–45	56	23.0
	≥ 46	5	2.1
Sex (N = 243)	Male	200	82.3
	Female	43	17.7
Donors status ($N = 244$)	Volunteer	140	57.4
	Replacement	104	42.6
Residence ($N = 239$)	AM town	124	51.9
	Outskirts of AM	24	10.0
	Near to AM	53	22.2
	Far from AM	38	15.9
Occupation (N = 239)	Student	103	43.1
	Farmer	69	28.9
	Merchant	11	4.6
	Daily laborer	7	2.8
	Pastoralist	4	1.7
	Other	45	18.8
Educational level	Illiterate	34	14.1
(N = 241)	Write, read	12	5.0
	Primary	44	18.3
	Secondary	76	31.5
	Higher education	75	31.1
Marital status ($N = 243$)	Single	134	55.1
	Married	109	44.9
History of blood donation	Yes	17	6.7
(N = 244)	No	227	93.0

AM = Arba Minch.

Chencha (4.3%), Gerese (3.9%), and Konso (4.7%). The predominant occupational groups were students (40.6%) and farmers (27.2%) (Table 1). One hundred and thirty-two (54.5%) of the participants reported possessing domestic ruminant animals at home. Of these, 93.9% possessed cattle, followed by sheep (56.5%) and goat (52.7%).

The overall seroprevalence of human brucellosis among the blood donors was 10.6% (27/254; 95% CI = 7.0, 14.0). For the 27 primary reactive samples, the mean titer of reactive agglutinins was 1:37 (range < 1:20-1:80) and none was reactive at the titration level equal to and above 1:160. The frequency of reactive serum at the titer of < 1:20, 1:20, 1:40, and 1:80 were 3, 10, 8 and 6, respectively. The study participants' occupation and variables related to possession of and contact with domestic ruminant animals were significantly associated with Brucella seropositivity. Being a farmer (OR = 3.68 [95% CI = 1.58, 8.58]), possession of ruminants (OR = 2.91 [95% CI = 1.12, 7.58]), history of current contact with ruminants (OR = 2.61 [95% CI = 1.04, 6.55]), and having ever assisted abortion of ruminants (OR = 2.81 [95% CI = 1.02, 7.79]) were associated with higher odds of brucellosis (Table 2).

The seroprevalence of 10.6% for human brucellosis determined by this study is quite higher compared with available epidemiological and clinical studies in Ethiopia: 2.15% in a group of healthy and nonspecific febrile patients, 20 3.78–5.30% among occupationally exposed individuals, 21–23 3–34.1% among patients with symptoms compatible with brucellosis, 12 6.3% among blood donors, 24 3.6% among febrile cases of unknown origin, 25 and 2.6% among acute febrile cases visiting health facilities.

Comparing the current serologic finding with reports on similar study groups (apparently healthy individuals), various levels of prevalence were noted. As such, low level of

TABLE 2
Factors associated with human brucellosis, Arba Minch Blood Bank Center, Ethiopia, January–March 2014

Risk factors	Category	No (%)	Nonreactive serum	Reactive serum	OR (95% CI)	P value
The state of the s	Farmer	69 (27.2)	55	14	3.68 (1.58, 8.58)	0.003
	Other	170 (66.9)	159	11	1	
Possession of ruminants at home (N = 242)	Yes	132 (54.5)	113	19	2.91 (1.12, 7.58)	0.028
	No	110 (45.5)	104	6	1	
Consumes unpasteurized milk (N = 243)	yes	159 (65.4)	143	16	0.93 (0.39, 2.21)	0.874
	No	84 (34.6)	75	9	1	
Consumes row meat (N = 243)	Yes	224 (92.2)	199	25	3.32 (0.54, + infinity)	0.233
	No	19 (7.8)	19	0	1	
Consumes "Dullet"* (N = 243)	Yes	223 (95.9)	208	25	1.64 (0.25, + infinity)	0.661
	No	10 (4.1)	10	0	1	
Current contact with ruminants (N = 242)	Yes	122 (50.4)	105	17	2.61 (1.04,6.55)	0.041
	No	120 (49.6)	113	7	1	
Ever attended parturition (N = 243)	Yes	75 (30.9)	64	11	1.89 (0.82, 4.39)	0.138
	No	168 (69.1)	154	14	1	
,	Yes	39 (16)	32	7	2.26 (0.87, 5.85)	0.093
	No	204 (84)	186	18	1	
,	Yes	28 (11.5)	22	6	2.81 (1.02, 7.79)	0.046
	No	215 (88.5)	196	19	1	
Ever attended slaughtering (N = 241) Yes No	Yes	79 (32.8)	67	12	2.05 (0.89, 4.74)	0.092
	No	162 (67.2)	149	13	1	

CI = confidence interval; OR = odds ratio.

Brucella agglutinins (0.33%) with only one true positive sample (0.11%) for Brucella agglutinins was reported in one of the studies,²⁷ and another study reported a relatively high prevalence (6.3%) with a titer of 1:80 thus revealing the importance of brucellosis in blood transfusion.²⁵ In Mexico, a seroprevalence of 3.6% was reported among blood donors.²⁸ It should, however, be noted that interpretation of the seroprevalence of brucellosis is made considering the serological test used, the characteristics of the study population, environmental hygiene, local prevalence of brucellosis, species of the causative agent, and other local factors. The interpretation of a positive result is also equally important. Although in the present study we considered any agglutination as positive, others have used qualitative slide agglutination titer level of 1:32 to define seropositivity.²⁶ Furthermore, the agglutination tests that were used lack specificity. Hence, all the foregoing factors separately or in combination might explain the observed difference in seroprevalence of brucellosis between the current study and the available reports both from Ethiopia and elsewhere.

Serologic cutoff values to define recent Brucella infection also vary across geographical regions. A titration level between 1:80 and 1:160 using tube agglutination method is generally considered clinically significant. The titer of 22% of individuals in this study falls within the titer range of active infection, whereas the rest may be considered as passive infection. These passive infections may be due to previous Brucella infection or exposure to infected cattle. However, the multiscreening serological test kit used in this study has been reported to yield false-negative results in the early phase of the disease, and is affected by a prozone effect of serological tests and antibiotic treatment. In addition, serum from low or nonimmune responders may also produce false-negative results, and serological cross-reactions in cases of infection or vaccination with some strains of Vibrio cholerae, Pasteurella, Proteus OX19, and Yersinia enterocolitica serotype 9 have been reported, which may overestimate the seroprevalence of brucellosis.

More than 85% of the Ethiopian population is agrarian and animal husbandry forms an integral part of agricultural activities in almost all ecological zones. Ninety-five percent of cattle are farmed under a mixed agricultural livelihood.5 These scenarios make the population at risk of zoonotic diseases. Serological evidence suggests the existence of bovine brucellosis in Ethiopia with varying prevalence in different geographical localities: 1.66% in Sidama zone, southern Ethiopia²⁹; 1.2% in Tigray region, northern Ethiopia¹⁶; 11% in Wuchale Jida District, central Ethiopia¹⁹; 5.1–18.6% in Shewa, central Ethiopia³⁰; 2.9% in central Oromiya¹⁴; 0.77% in Jimma zone, west Ethiopia³¹; and 1.9% in a large-scale study using 2,334 cattle from 273 farms.³² Although no specific data were available from the study region on bovine brucellosis, the high seroprevalence in humans indicates bovine brucellosis might be much more common than other parts of the country. This calls for large-scale studies and stronger evidence on human and animal brucellosis in the area.

Studies focusing on brucellosis are very limited in Ethiopia. These limited studies have rarely addressed risk factors for human infection.⁵ The present study showed that a very close contact with domestic ruminant animals had significant association with Brucella seropositivity. In this study, most individuals had close contact with cattle and known practices that put an individual at risk of brucellosis were common. This is in conformation with the general living standard and cultural conditions in Ethiopia that inherently predispose individuals to zoonotic diseases. In addition, animal husbandry is very traditional in Ethiopia and people most often live with their animals under the same roof and health-care support for their animals is minimal. Suboptimal handling, processing, and marketing of animal and animal products for consumption are additional factors for transmission of human brucellosis. Besides, these conditions vary under different cultural/traditional setups¹² and hence call for the need to study the epidemiology of human brucellosis in different localities in Ethiopia.

In conclusion, in southern Ethiopia, the risk of zoonotic transmission of brucellosis seems apparent. Farmers and

^{*&}quot;Dullet" is food prepared from the intestines and organs (such as liver) of cattle.

those with close contact to domestic ruminant animals were found to have higher odds of *Brucella* seropositivity. This study implies the need to consider brucellosis a locally neglected zoonotic disease. The documented high exposure of the community to *Brucella* and the considerable risk of transmission should encourage screening of donors' blood for brucellosis and to consider the disease in the differential diagnosis of acute febrile illnesses. Since brucellosis is a zoonosis, the condition in animals should be equally emphasized for a comprehensive fight against this disease. Furthermore, additional epidemiologic and confirmatory studies are needed to understand the wider picture of brucellosis in southern Ethiopia.

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