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Study on Sero-Prevalence of Small Ruminant and Human Brucellosis in Yabello and Dire Districts of Borena Zone Oromia Regional State, Ethiopia

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Abstract: Cross-sectional study was conducted in Yabello and Dire districts of Borena zone Oromia regional state, Ethiopia to estimate sero-prevalence of small ruminant and human Brucellosis. Sero-survey was applied to the target population of 228 small ruminants and 86 humans. The sera samples were collected from small ruminants (sheep and goats) and humans for serological analysis of brucellosis. Samples were serially tested by RBPT and positive samples were finally confirmed by c-ELISA. The overall sero-prevalence of brucellosis were 8.8% (95% CI: 5.4-13.2) and 25.6% (95% CI: 16.8-36.1) in small ruminants and humans respectively. There was a statistically significant variation in *Brucella* infection ($p<0.05$) in humans. The higher prevalence recorded in elders. The highest recorded in small ruminant sampled from large flock size and ewes with a retained fetal membrane. These results provide evidence of the importance of brucellosis in humans and small ruminants in the study area. Overall, the existence of brucellosis, community daily practice and uncontrolled movement of animals and livelihood nature of pastoralists suggest the need for farther investigation of brucellosis in humans and animals. Furthermore, awareness creation or public health education on zoonotic importance of brucellosis and prevention techniques should be imparted continuously in pastoral communities.

Keywords: Brucella, ELISA, RBPT, Zoonotic

Introduction

Brucellosis is a worldwide zoonosis with a high degree of morbidity in humans. According to WHO about 500,000 human cases of this disease registered in the world every year (Pappas *et al*., 2006). Recent estimates from East Africa suggest that there are approximately 21 million brucellosis cases in livestock annually (McDermott and Arimi, 2002).

Livestock industry plays an important role in the economy of the country. In addition, human life is highly associated with the livestock populations in the different livestock production systems particularly in pastoral communities (Megersa *et al*., 2011). In both pastoral and mixed (agro-pastoral) livestock production systems, people haves contacts with livestock and livestock products/discharges and consume raw animal products. This could enhance the incidence of brucellosis infection (Habtamu *et al*., 2015; Terefe *et al*., 2017).

Brucellosis in human is common in rural areas because of farmers or pastoralists live in close contact with their animals and often consume fresh unpasteurized dairy products due to a lack of awareness about zoonotic importance of disease and way of transmissions (Terefe *et al*., 2017; Mantur and Amarnath, 2008; Amenu *et al*., 2010; Zeru *et al*., 2016; Tsegay *et al*., 2017). The threat of zoonotic diseases for humans is high; brucellosis is the one affecting both humans and livestock.

Infected animals are left unknown and become a source of diseases for humans and continued transmitting diseases. Among several zoonotic diseases affecting humans and animals, brucellosis is known to be an important zoonosis and economically important disease posing considerable cause of reproductive losses in animals (Terefe *et al*., 2017). Therefore, this study was designed to study seroprevalence and associated risk factors of brucellosis in small ruminants and humans in the study area.

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Materials and Methods

Description of the Study Area

The small ruminants and humans Brucellosis study was conducted in Yabello and Dire districts. Yabello and Dire districts are agro-ecologically lowlands and midland areas with an elevation ranging from 1100-1857 meters above sea level (masl). The average annual rainfall ranges from 300-700 mm mainly received in long the rainy season (ganna) from April-June and a short rainy season (Hagayya) from September-November.

In the area, pastoral livestock production system with mobility is the vital source of food and income for livelihood of people while opportunistic cultivation is practiced around Yabello and Mega Towns where the soil moisture content stays high for longer time. Relatively the altitude of Dire district is higher with moderate production of grains when sufficient rain is available. As a result farming practice is common around Mega Town of Dire district other remaining Kebeles (Pastoral Associations) are pastoralist with sedentary, transhumant and Migrant pastoralist. Dire district make international border with Kenya (Fig. 1).

Study Design and Population

A cross-sectional study was conducted on a total of 228 small ruminants and 86 human sera to estimate the prevalence of brucellosis and associated risk factors. All sera samples were collected from occupationally associated animal attendants/pastoralists. Case-control sampling techniques were applied to collect the sample from small ruminants. The animals were grouped depending on age, sex, abortion history, retained fetal membrane and other clinical signs (epididymitis and orchitis) in males. Age determination and history for the presence or absence of reproductive problems were obtained from the owners and the animal attendants. After grouping animals accordingly, samples were collected randomly from each group. This technique applied due to high abortion reported in the study area to control the high number of ewes sampling with abortion history. Based on literature (Solomon *et al*., 2006) and community knowledge flock size was the group into three; Large, Medium and small based on the number of animals in the flock.

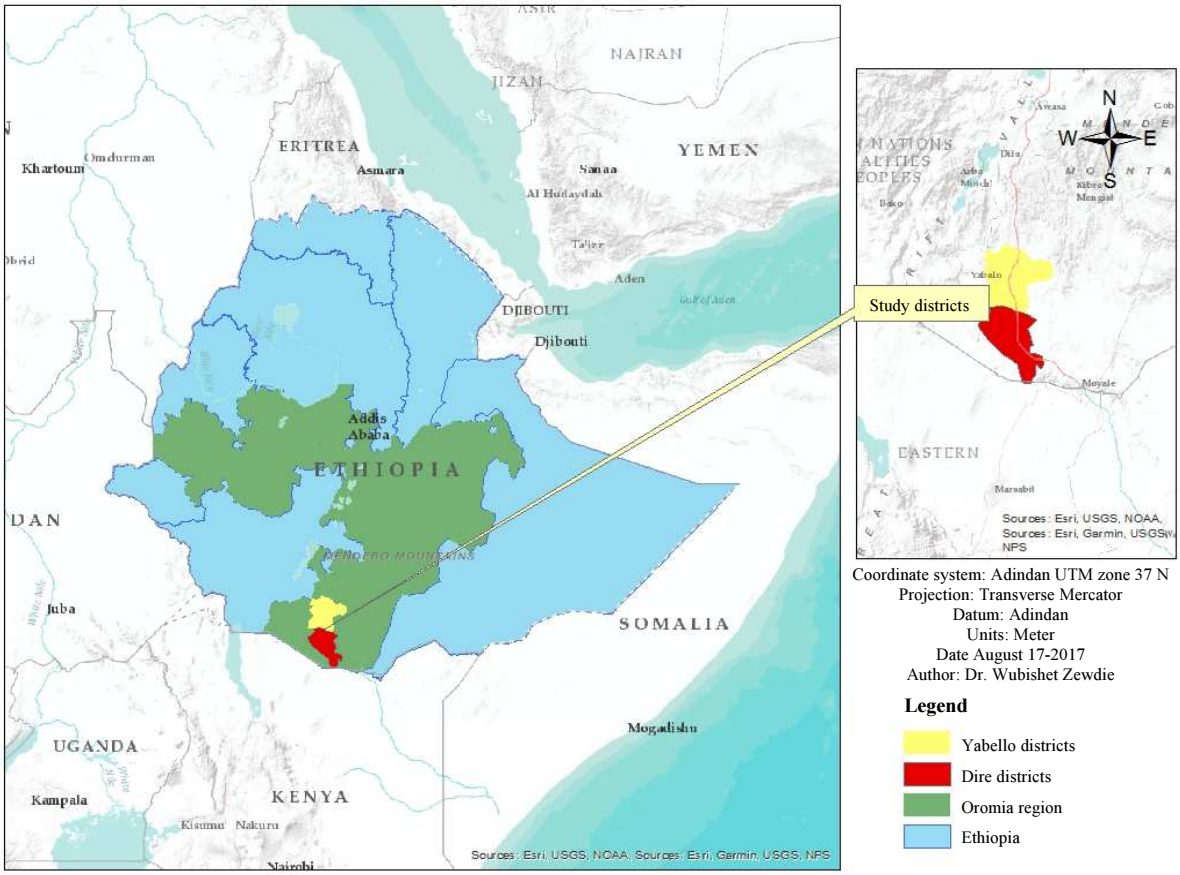

Fig. 1: Map of study area

Number of shoats greater than 30 (>30), number of shoats greater than 10 (>10) but less than thirty (< 30) and number of shoats less than or equal to ten (≤ 10); and named as large, medium and small flock size respectively. In addition, attendants/pastoralists were interviewed using a structured questionnaire to collect demographic, epidemiological and clinical data of both small ruminant and human Brucellosis. The population male in the flock is few in number because the male mostly sold only few ram remain for breeding purpose.

Type of Sample and Sampling Procedure

Before sample collection first, the purpose of the study was explained and consent to participate in the study was obtained from individuals or their parents and guardians. About 10 mL of blood samples were collected from the jugular vein of each selected animal using plain vacutainer tubes after disinfecting the area by 70% alcohol. From volunteer animal attendants/pastoralist, about 5 mL blood sample was collected by a trained medical laboratory technologist. The collected blood samples of animal and human were transported to Yabello Regional Veterinary Laboratory (YRVL) and Yabello Hospital respectively for serum separation, storage, and testing. Serum was removed from the vacutainer tube with a sterile pipette into cryovial and then code on a vacutainer tube registered properly onto cryovial tubes. Finally, the serum kept in -20°C fridges until it tested by RBPT at Yabello Regional Veterinary Laboratory and Yabello hospital. Positive sample for Rose Bengal Plate Test was submitted to National Animal Health Diagnostic and Investigation Center for c-ELISA.

Laboratory Techniques

Rose Bengal Plate Test

All serum samples were initially screened using a modified Rose Bengal Plate (25:75 µL, sera: antigen ratio) Test (RBPT) for small ruminants whereas, Rose Bengal Plate Test (RBPT) of 30:30 µL ratio (antigen: serum ratio) was used for humans. The small ruminant samples were tested at Yabello regional veterinary laboratory whereas human samples were tested at Yabello Hospital according to the procedures described (OIE, 2004), the World Organization for Animal Health (Shimeles, 2008).

Competitive ELISA (C-ELISA)

All serum samples found to be positive by RBPT was tested by C-ELISA. The test conducted according to manufacturer guidelines/SOP.

Data Management and Analysis

Factors thought to be associated with the epidemiology of brucellosis in small ruminants and humans were recorded in a Microsoft Excel® Spread Sheet. Information like reproductive problems, age of animals and parity numbers were obtained from animal owners/attendants. Data on individual person/ human sex, age, way of consumption of the animal product, methods of handling aborted fetus, means of supporting animals during parturition were also captured. STATA software version 11.0 (CRC Press LL.©2004 UK) for windows was used to analyze the data. A multivariable logistic regression model was used to identify risk factors associated with *Brucella* infection and Odd Ratio (OR) used to indicate the strength of risk factors associated with a 95% confidence interval. Descriptive and analytic statistics were also computed using software SPSS Version 15(SPSS Inc.© 2006 USA) and cross-tabulation were employed to see the association of determinant factors (habit of consumption of a raw animal product, handling aborted fetus by bare hand) with that of knowledge of the community about the disease.

Result

Serological Result

Out of 228 small ruminants and 86 human sera screened by RBT 21(9.2) and 35(40.7%), respectively, were found to be positive. With subsequent serial testing, the overall prevalence of brucellosis was 8.8% (95% CI: 5.4-13.2) and 25.6% (95% CI: 16.7-36.1), in small ruminant and human respectively by c-ELISA. In large flock size and ewes with history of retained fetal membrane, small ruminant brucellosis is higher with significant variation p-value less than 0.05 ($p<0.05$) (Table 1).

In human, Brucellosis is higher in individuals with a history of contact with retained fetal membrane and age greater than 55 years old with significant statistically variation ($p<0.05$) (Table 2).

Questioner Result

Pastoralists recognize abortion in their animals and call “sallassu” (abortion) (local language Afaan Oromo). The community consume aborted ewe milk by stimulation/forcing ewes to give milk after abortion called “Backisu” which could be the risk factor for transmission of disease from carrier animals. There were different community practices which thought to be risk factors associated with occurrence of disease in humans. Community practices involves, handling aborted fetus and aborted materials (Placenta) by bare hand; drinking of raw milk and blood including milk from aborted ewes.

Table 1: Multivariable logistic regression analysis of small ruminant brucellosis prevalence relative to different factors by c-ELISA

Factors	No	No positive (%)	Odds Ratio	P > |z|	95% Conf. Interval
Flock size					
Large	59	11(18.6)	15.10	0.011	1.9 12.1
Medium	101	8(7.9)	5.60	0.108	0.68-45.9
Small	68	1(1.5)			
Species					.
Goat	144	15(10.4)	1.50	0.458	0.54-3.9
Sheep	84	5(6.0)			
Still birth					
Ewes with still birth	11	2(18.2)	2.50	0.265	0.49-12.5
No still birth	208	17(8.2)			
Abortion History					
Ewes with abortion	20	16(8.0)	2.00	0.301	0.53-7.6
No abortion	199	3(15.0)			
Retained placenta					
Ewes with retained FM	10	5(50.0)	13.90	0.000	3.6-53.8
No retained FM	209	14(6.7)			
District					
Arero	78	4(5.1)	0.35	0.085	0.10-1.2
Dire	76	6(7.9)	0.55	0.270	0.19-1.6
Yabello	74	10(13.5)			
Sex					
Male	9	1(11.1)	1.30	0.815	0.15-10.9
Female	219	19(8.7)			

Table 2: Multivariable logistic regression analysis of human brucellosis prevalence relative to different factors by c-ELISA

		Competitive ELISA(c-ELISA)				
Factors		No +V (%)	Odds Ratio	Z	P > |z|	95% confidence interval
Consumption of raw milk						
Yes	83	22(25.6)	0.10	0.91	0.317	0.31-21.3
No	3	0				
Contact with aborted fetus						
Yes	78	19(24.4)	2.20	0.82	0.411	0.33-14.8
No	8	3(35.7)				
Touch retained placenta						
Yes	31	18(58.0)	10.80	5.19	0.000	4.38-26.3
No	55	4(7.3)				
Drink animal blood						
Yes	73	20(27.4)	0.49	1.16	0.25	0.14-1.63
No	13	2(15.4)				
Age						
Young	18	2(11.1)				
Adult	46	12(26.1)	2.10	1.52	0.126	0.29-2.9
Old	22	8(36.4)	3.40	3.21	0.030	5.4-43.3
Sex						
Male	55	14(25.5)	1.20	0.43	0.664	0.49-3.02
Female	31	8(25.8)				

Discussion

Confirmed sero-prevalence of brucellosis in small ruminant was 8.8%. This result is closely in agreement with previous serological study result of 9.6% in Yabello district of Borena zone, southern Ethiopia (Ragassa *et al*., 2009). On the contrary, this result is higher than previous research result of 4.8% in Afar region, Eastern Ethiopia (Mohammed *et al*., 2007), 1.37% Somali region, Eastern Ethiopia (Animut *et al*., 2009), 4.89% Amhara region, northern Ethiopia (Teshale *et al*., 2006) and 3.3% Borena, southern Ethiopia (Sintayehu *et al*., 2015). This difference between the previous result and current result may be due to study area difference, sampled animal composition and the difference in sensitivity or specificity of the serological test used.

In the present study, the sero-prevalence of human brucellosis were 25.6% by C-ELISA. The current

study result recorded is lower than the result recorded of 34.1% and 29.4% in Borena and Hamer pastoral area of southern Ethiopia respectively (Animut *et al*., 2009). The lower prevalence of the current study compared to the previous studies can be explained as variation in the study population; the previous study was conducted on pastoralists with febrile illness. The current study result is higher than most studies conducted in high land areas. This can be explained as higher prevalence is due to difference in the ecosystem and community practice (consumption of raw milk and blood, handling aborted fetus and fetal membrane) which are considered to be the main way of transmission of disease from animals to humans (Mantur and Amarnath, 2008; Animut *et al*., 2009; Tibeso *et al*., 2014). Consequently, drinking raw milk and blood, handling aborted fetuses/retained fetal membranes by bare hand, leave/place aborted materials in the environment and touch vaginal discharges while support parturition. This questioner result is in agreement with the study documented (Kassahun *et al*., 2006; Mussie *et al*., 2007; Kassahun *et al*., 2007).

Same of pastoralist in Pastoral Association (PA) complain about the presence of undulant fever, back pain, joint and muscular problem which could be the compatible clinical sign of brucellosis (Teshale *et al*., 2006; Yohannes, 2012). This implies that there are a large number of undiagnosed cases of febrile diseases, osteoarticular complication (joint problems) and certain generalized complications in pastoral communities that might be associated with brucellosis (Ragassa *et al*., 2009; Tolosa *et al*., 2007; Kassahun *et al*., 2007).

In this study, the challenge encounter was a financial problem to collect sufficient samples or samples with the best representation of Brucellosis situation in community. It was also advantageous to characterize the bacterial species circulating in human and small ruminate as well but I haven't succeeded to do this test due to financial limitations and absence of well-equipped laboratory nearer to study area for zoonotic disease diagnosis and investigation.

Conclusion and Recommendations

Animals are the main source of *Brucella* infection for humans. The sero-prevalence recorded in the present study revealed that Brucellosis is a widespread and established disease in small ruminants and humans in the study area. The possibility of Brucellosis sero-positivity for humans was contacted with retained fetal membrane and age. In small ruminants, herd size and female animals with a history of the retained fetal membrane are prone to be seropositive relative to other groups of animals. In humans, drinking raw animal blood, handling of aborted fetus and contact with the vaginal discharge of infected animals had not shown statistical significance. Traditional husbandry and poor management practices, the same grazing area of different species of animals, letting aborted material in environment and unrestricted movement of animals were thought to support the spread of the disease between animals as well as a human in the study area. The prevalence of human brucellosis is higher than the prevalence in animals. This could be due to community close contact and ingestion of raw animal products from the single infected animals within whole family members. In conclusion, community close contact with livestock, handling cases with bare hands, consumption of the raw animal product and letting aborted materials in the environment could enhance the spread of disease between animals and humans. Therefore, the disease situation and species of bacteria circulating in the area should be assessed more widely in all animal species and humans to know the transmission dynamics of disease in the animal; and humans.

Awareness creation of public health education on zoonotic importance of brucellosis and prevention techniques should be imparted continuously in the pastoral community.

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Ethics

Ethical Clearance for animal sampling was obtained from the College of Veterinary Medicine and Agriculture, Addis Ababa University. Whereas human sampling ethical clearance was obtained from the Aklilu Lema Institute of Pathobiology, Addis Ababa University. The same human sample collected from the area was also used for Ph.D. research on Brucellosis from the Borena area.

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