International Journal of Scientific Research and Engineering Development-– Volume 3 - Issue 4, July - Aug 2020

Available at www.ijsred.com

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CROSS SECTIONAL STUDY OF BRUCELLOSIS IN RUMINANTS AND HUMAN IN AND AROUND DEBREBRIHAN, ETHIOPIA

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STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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Date of Submission:_____

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my academic advisor, Dr. Gezahagne Mamo for his unreserved technical and professional advice, correction of the research work and provision of valuable reference materials that makes this thesis complete.

I am greatly indebted to Professor Gobena Ameni, for his financial support. Furthermore, special appreciation is to Addis Ababa University, Collage of Veterinary Medicine and Agriculture to overall coordination of the postgraduate program successfully and ensured enabling environment to this program.

My special thanks also goes to the Aklilu lemma Institute of Pathobiology specially board of ethical committee for commenting my proposal and providing ethical clearance. I wish to extend my heartfelt thank to the very genuine and cooperative staffs of Debrebrihan town agricultural office and Debrebrihan agricultural research center for their field assistance and encouragement.

I am deeply indebted to Dr. Getachew Tuli for his kind cooperation and offering me to use the reagents and facilities required for RBPT and CFT. Finally, the cooperation and assistances of National Animal Health Diagnostic and Investigation Center is deeply appreciated.

ABSTRACT

A cross-sectional study was conducted in and around Debrebrihan , North Shewa zone of Amhara region to determine the prevalence of brucellosis in ruminants and human in and around Debrebrihan from November 2013 to May 2014. A total of 384 ruminants (cattle, sheep and goats) and 134 human patients were randomly selected samples serologically tested for brucella antibodies. In addition, questionnaire survey involving 150 community members carried out to assess their perception about the disease. The overall seropositivity in ruminants in the study area was found to be 2.3% (95% CI: 0.01-0.04) by RBPT and 0% by CFT. The overall brucella seropositivity in a human was found to be 13.4 %(95% CI: 0.08-0.19) by RBPT and CFT. There were statistically significant difference in the prevalence of human brucellosis between different occupations ($\chi2$ = 10.26, P = 0.006), raw milk consumption ($\chi2$ = 4.56, P = 0 .033) and contact with placenta ($\chi2$ = 72.08, P = 0.000) identified as risk factors of brucella infection. The multivariable logistic regression analysis showed that being a farmer had 4.7 times the odds of being brucella reactors compared with those patients having other occupations and having raw milk consumption habit had 7.05 times the odds of being brucella reactors compared with those patients not having raw milk consumption. However, there was no statistically significant difference between male and female(x^2 =0.51, p=0.48), age groups ($\chi2$ = 1.34, P = 0.51), education levels ($\chi2$ = 5.28, P = 0.153) and raw meat consumption ($\chi2$ = 3.55, P = 0.060) in the prevalence of human brucellosis. This finding revealed that there was no ruminant and human brucellosis in Debrebrihan. However, there was high prevalence of human brucellosis in other districts of north shewa zone around Debrebrihan. Hence, the study suggests that the need for implementing control measures and raising public awareness for prevention of brucellosis in the area of high prevalence of human brucellosis.

Key words: Brucellosis, Debrebrihan, Human, Questionnaire, Ruminant, cross sectional

1. INTRODUCTION

Brucellosis is one of the most common bacterial zoonoses worldwide and it poses a major threat to human health, animal health and animal production [25]. Brucellosis causes as serious economic loses to livestock sector through reproductive failure and abortion and also in human leads to considerable morbidity across the globe and thus perpetuates poverty[26].

In domestic animals, *Brucella* infects the reticuloendothelial system and genital organs causing chronic infection and abortion (especially in the last trimester), stillbirth and infertility, which significantly affect farmers economically due to loss of production. Epididymo-orchitis is common in males and the females that have aborted show necropulurent placentitis and endometritis. Lesions in the placenta cause edema of the chorionic stroma and multifocal necrosis of allantochorion. These lesions are cardinal in the induction of abortion and eventual infertility. This is accompanied by large accumulation of neutrophils and degenerate leukocytes[35].

In human, consumption of contaminated food and occupational contact are the major risks of infection. The main routes of infection are consumption of unpasteurized dairy products from infected cattle, small ruminants and camel. It has been shown that the organism can survive in inadequate smoking ([15] and [31]. The consumption of contaminated dairy products has been widely documented as an important route of *Brucella* transmission. Contact with infected materials such as aborted foetuses, placentas, urine, manure, carcass and dead animal body has been reported in some countries to cause human brucellosis in 60–70% of cases [6]. Infection by contact has been reported to be common among veterinarians, abattoir workers, farmers, rendering-plant workers, packing-house employees, animal handlers and others who work with animals and their products ([6] and [35]).

In human, brucellosis is life threatening and presents with nonspecific symptoms, including intermittent fever, weight loss, depression, hepatomegaly and splenomegaly. The disease is characterized by a multitude of somatic complaints, such as, sweating, anorexia, malaise, headache and joint pains and is easily confused with malaria and influenza [11]. Arthritis, spondylitis, osteomyelitis, epididymitis and orchitis, as well as other more severe complications such as neurobrucellosis, liver abscesses and endocarditis, are common in some patients[1].

Brucellosis occurs worldwide, except in those countries where bovine brucellosis has been eradicated. The Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected. Although *B. melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants[12]. Furthermore, brucellosis is a re-emerging widespread and of major economic importance in most countries of the world such as Israel, Kuwait, Saudi Arabia, Brazil and Colombia, where there is an increasing incidence of *B. melitensis* or *B. suis* biovar 1 infection in cattle[12].

In Africa, brucellosis is considered to be one of the most serious health problems facing the veterinary professionals. It creates a serious economic problem in both intensive and extensive livestock production system in the tropics and a threat to public health[17]. In the sub-Saharan Africa for example, the average seroprevalence of brucellosis in cattle populations varies from 10% to 16%. Previously, a seroprevalence of 15.8% and 10.3% were reported in the southwestern and western Uganda, respectively and 41.0% has been reported in Togo ([10] and [13]).

Brucellosis is known to be endemic in Ethiopia and it first reported in 1970. Since then, a number of studies have demonstrated the presence of antibodies against Brucella in animals and humans in different parts of the country. The prevalence of animal brucellosis has been found to range from 0.2% to 38% [14]. Whereas, study on the prevalence of human brucellosis from different localities of the country indicated that between 3%-34.1%**[36]**. As compared to study of animal brucellosis, study of human brucellosis in Ethiopia is sparse with even less information on risk factors for human infection.

So far in North Shoa zoneof Amhara Region, no pervious study has been conducted on risk factors for transmission of brucellosis to human and the awareness of community with regard to brucellosis was not determined. Hence, this study was carried out to study the seroprevalence of brucellosis and to identify major potential risk factors for infection in ruminants and human.

Therefore the objectives of the study were:

- To determine the sero-prevalence of ruminants and human brucellosis
- To explore potential associated risk factors for infection and transmission of brucellosis from animal to human and
- To determine community perception about the disease in the study area

2. MATERIAL AND METHODS

A. Study Area

The study was conducted in Debre brehan town administrative of North Shewa zone of Amahara Region. Debrebrihan town administrative is located at 9 ° 41 ° N latitude and 39°31 ° E longitude 130 km from Addis Ababa on the main road from Addis Ababa to Mekele road. An average elevation is between 2800 and 2845 meters above sea level. The mean annual temperature ranges between 5 ºC and 23 ºC. The relative humidity is 73.3% [21].

The administrative is one of 24 districts of north shewa zone of amhara region (Figure.1) and it has 9 kebeles. According to the Debrebrihan town administrative agricultural office[9] human population found in urban area consisting of 72,532 and in the rural area 12,412. The numbers of animals found in the area are 21,641 cattle, 26,920 sheep, 1,100 goats, 56,576 poultry and 4,970 equines. From total population 14.6% are farmers grow crops and still maintain a few head of livestock in extensive production system and in urban area few livestock industries practices like dairy and sheep rearing. Livestock provide meat, milk, labor, income and clothing in the area[9].

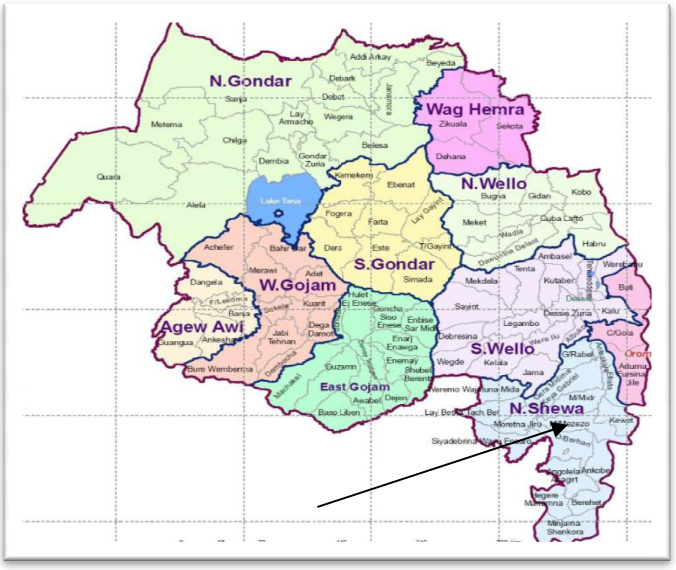

Figure 1. Districts of North Shewa zone, Amhara Regional State (Debrebrihan) [8].

B. Study design and sampling

I) Animal population. A cross sectional study was conducted from November 2013 to may 2014 using a random sampling method of three kebeles (01, 08 and 09). A herd in kebele was sampled using a stratified random sampling based on animal species (cattle, sheep and goats) regarding to extensive and semi-intensive production systems.

The total numbers of ruminants required for the study was calculated based on expected prevalence of 50% with defined precision of 5% and level of

confidence interval of 95% according to the formula given by Thrusfield [28].

$N = 1.96^2 * Pexp (1-pexp)/d^2$

Where: n = the total sample size

P = expected prevalence (50%)

d= absolute precision (0.05) at 95% CI

N= 1.962x 0.5(1.0.5)/ $(0.05)^2$ = 384 animals Therefore, the study was conducted on a total number of 384 samples consisting of 192 extensive and 192 semi-intensive production system, 165 sheep, 44 goats and 175 cattle.

II) Human population and sample collection. A total of 134 patients were selected using systematic sampling method from November 2013 to May 2014. In 6 months the hospital had been visited twice a week and 3 patients had been sampled in each day.

In this cross-sectional study, first the purpose of the investigation was explained to the Patients who come to Debrebrihan and Ayou hospitals, diagnosed with fever and whom recommended laboratory examination by physician was a sample frame.

Upon diagnosis, patients were interviewed by physicians whether they had fever and willing to participate in the study were included. A structured questionnaire were administered to study participant and information including age, sex, district, education, occupation, consumption of raw milk, consumption of raw meat, contact with animals, assisting delivery, milking and slaughtering and presence of symptoms like head ache, chills, night sweet, back ache, anorexia, weakness, joint pain were collected. After interview, the patients were requested to give blood for brucella test and 5ml peripheral blood sample was taken by experienced laboratory technician. The serum was removed from the clot blood after centrifugation at 1200rpm at room temperature, for 15 minutes by siphoning into another sterile tube (1.8ml) to which the identification was transferred. Finally, sera samples were kept at -20°C until tested by Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT).

III)Questionnaire survey. A total numbers of 150 individuals had been interviewed using purposive sampling by considering risk factors such as occupation, education, age, sex, whether other family members treated of brucellosis, assisting in delivery, livestock ownership, milking and slaughtering, history of consuming raw meat and milk, direct contact with ruminants, awareness level about zoonosis, sanitation habits, etc. Furthermore, knowledge on brucellosis, whether they use protection during handling of aborted material had been included in the questionnaire format.

C. Animal serum Sample collection

Ten milliliter of blood sample was collected from the jugular vein of each selected animal using plain vacutainer tubes and needles. Animal identity number for each animal was given and labeling was conducted on the corresponding vacutainer tube, the tubes were set tilted (45 degree) overnight at room temperature to allow for clotting. Next morning serum was removed from the clot blood after centrifugation at 1200rpm, at room temperature, for 15 minutes by siphoning into another sterile tube (1.8ml) to which the animals' identification was transferred. Finally, sera samples were kept at -20°C until tested by Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT).

D. Serology

Rose Bengal Plate Test (Lillidale, UK) was used as a screening test of the serum samples for the presence of *Brucella* agglutinin in the National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia (for animal serum) and National Veterinary Institute/NVI/(for human serum). For the RBPT technique, the procedure described by OIE [32] was followed. RBPT *Brucella* antigen, positive control and negative control sera was used for the RBPT. All sera samples that were considered positive by RBPT were confirmed by CFT (Adlestone, UK) following the protocol described by OIE [32]. Sera with a strong reaction more than 75% fixation of the complement (3+) at a dilution of 1:5 and with at least 50% fixation of the complement (2+) at dilutions of 1:10 and1:20 were classified as positive (+), according to the guidelines of the OIE[33].

E. Ethical consideration

Ethical clearance was obtained from the Aklilu Lemma Institute of Pathobiology ethics review committee, Addis Ababa University. Then informed written consent was obtained from each study participants. All information collected from patients cards were kept strictly confidential and names were not included in the data (Anex.1).

F. Data management and analysis

Serology result of animal and human samples as well as questionnaire survey data were recorded and coded using Microsoft Excel sheets. The data was transferred and analyzed using STATA version 11(Stata. corp., College station, TX). The prevalence of statistical analysis performed with 95% confidence interval, Pearson chi-square was used to evaluate statistical significance of the association of different categories of variables of test results. Multivariable logistic regression analysis was performed to quantify crude and adjusted effect of risk factors to positivity. P-value less than 5% was considered statistically significant. In case of estimating the effects of different risk factors in terms of odd ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its values.

3. RESULTS

A. Prevalence of ruminant brucellosis

The individual ruminant seropositivity was conducted by using RBPT and CFT serological tests. The overall individual seropositivity in the study area was established at 2.3% (95% CI: 0.01-0.04) by RBPT and all were negative by CFT. The prevalence were compared between study sites; according to RBPT result the prevalence of ruminant brucellosis for three peasant association 01, 08 and 09 were 2%, 2.6%(95% CI: 0.26-6.72) and 2.4%(95% CI: 0.24-6.65) respectively (Table 1). All the risk factors were not associated with RBPT positivity of ruminant's brucellosis. There were not statistically significant differences in proportions of ruminants RBPT positive reactor between the three Kebeles ($\chi2 = 0.123$, $P = 0.940$), species ($\chi2 = 0.008$, $P = 0.996$), sex ($\chi2 = 1.45$, $P = 0.21$), age category ($\chi2 = 0.89$, $P = 0.310$) and breed ($\chi2 = 0.81$, $P = 0.66$) (Table 2). But the only production systems showed statically significant difference ($\chi2 = 5.575$, $P = 0.015$).

The multivariable logistic regression analysis (Table 2) showed that semi-intensive production system had 8.3 times the odds of being brucella reactors compared with those ruminant living in extensive production system (adjusted OR =11.491; CI =1.33-99.23) but, the other parameters had no much odds difference. No reactor ruminant was detected from three peasant associations on confirmation test by complement fixation test.

Table 1 Association of different risk factors to RBPT positivity for ruminant's brucellosis at Debrebrihan town administrative

Variable		Number of ruminants examined	Number of Positive (%)	X^2	p-value
Kebele	01	148	3(2%)	0.123	0.940
	08	112	3(2.6%)		
	09	124	3(2.4%)		
Species	Cattle	175	4(2.3%)	0.008	0.996
	Sheep	165	4(2.4%)		
	Goat	44	1(2.3%)		
Sex	Male	112	1(0.9%)	1.454	0.208
	Female	272	8(2.9%)		
Age	Young	94	1(1.1%)	0.891	0.310
	Adult	290	8(2.7%)		
Breed	Local	231	5(2.2%)	0.808	0.668
	Exotic	132	4(3%)		
	crossed	21	0		
Production system	Extensive	192	1(0.5%)	5.575	0.015*
	Semi-inte	192	8(4.2%)		
Abortion	Yes	6	0	0.146	0.867
	No	378	9(2.4%)		
Still birth	Yes	4	0	0.097	0.909
	No	380	9(2.4%)		

* indicate that p-value less than 0.05

Variable		Number of ruminants examined	Number positive by	Crude odds ratio(95% CI)	Adjusted odds ratio(95% CI)
Kebele	01	148	3	1	1
	08	112	3	1.33(0.26-6.72)	1.47(0.17-12.43)
	09	124	3	1.20(0.24-6.05)	1.25(0.99-8.16)
Species	cattle	175	4	1	1
	sheep	165	4	1.06(0.26-4.32)	0.59(0.08-4.19)
	goat	44	1	0.99(0.12-9.12)	0.87(0.08-9.39)
Sex	Male	112	1	1	1
	female	272	8	3.36(0.42-27.21)	4.65(0.55-38.92)
Age	young	94	1	1	1
	adult	290	8	2.64(0.33-21.37)	2.41(0.27-21.55)
Breed	local	231	5	1	1
	exotic	132	4	1.41(0.37-5.35)	1.00(0.19-5.18)
	crossed	21	0	-	-
Production System	extensive	192	1	1	1
	Semi-intensive	192	8	8.30(1.03-67.05)	11.49(1.33-99.23)

Table 2 Multivariable logistic regression analysis of ruminant's brucellosis positivity by RBPT compared with different risk factors.

PA= peasant association; CI= confidence interval

B. *Prevalence of human brucellosis*

A total of 134 human patients who had fever were selected randomly: 52 patients from Debrebrihan hospital and 82 patients from Ayou hospital. Out of 134 serum samples 18 (13.4 %(95% CI: 0.075-0.193) were identified positive reactors by RBPT and CFT. The patients come to the hospitals were from 13 districts in and around Debrebrihan town administration.

Some of the major factors contributing to the prevalence of human brucellosis in patients admitted to hospitals were assessed and among the main predisposing factors studied were departure area, age, sex, occupation, education level, habit to raw milk and meat consumption and contact with placenta were factors compared with seropositive brucellosis cases.

There were highly statically significantly difference in the prevalence of human brucellosis between districts ($\chi 2$ = 30.25, P = 0.003) (Table 3), different occupations ($\chi 2$ = 10.256, P = 0.006), habit of raw milk consumption ($\chi 2$ = 4.557, P = 0 .033) and contact with placenta ($\chi 2$ = 72.083, P = 0.000) (Table 4). But this study showed that patients who come from Mendida, Ataye, Debrebrihan town administrative and Debre sina did not react seropositivity for brucellosis test.

The multivariable logistic regression analysis (Table 5) showed that being a farmer had 4.7 times the odds of being brucella reactors compared with those patients having other occupations (adjusted OR = 2.84; CI = 0.09-87.50) and having raw milk consumption habit had 7.05 times the odds of being brucella reactors compared with those patients not having raw milk consumption (adjusted OR =1.53; CI = 0.03-75.23).

Table 3 Brucella positive reactor of human patients whocome from in and around Debrebrihan by RBPT and CFT.

District	Number of patients examined	Number of Positive (%)	Chi-square	P-value
Mendida	4	0	30.252	0.003*
Shewa Robit	7	42.8		
Ataye	1	0		
Senbete	16	25		
Eneware	2	50		
Debrebrihan	59	0		
Mehal meda	2	50		
Lemi	3	66.6		

Sheno	8	25		
Hagere mariam	5	20		
Baso	20	15		
Deneba	6	16.6		
Debre sina	1	0		
Total 134		13.4		
* indicate that p-value less than 0.05				

The overall sex-wise seropositivity was higher in females patients (15.2%) than males patients (10.9%) but no statistically significant difference(x^2 =0.511, P=0.475) prevalence of human brucellosis between male and female and in addition to the factors indicted above, there was also no statistically significant difference between age groups ($\chi 2$ = 1.341, P = 0.511), education level ($\chi 2$ = 5.276, P = 0.153) and habit of raw meat consumption ($\chi 2$ = 3.550, P = 0.060) (Table 4).

Table 4 The prevalence of human brucellosis associated with potential risk factors

Variable		Number of examined	Prevalence (%)	X^2
Sex	Male	55	10.9	0.51
	Female	79	15.2	
Age	Young	52	13.4	1.34
	Adult	53	10.5	
	Old	25	20	
Occupation	Farmer	36	23	10.25
	Private	33	6	
	Gov't employed	65	2.7	
Education	Illiterate	38	21	5.27
	<6 grade	28	17.8	
	6-12 grade	38	10.5	
	Degree/diploma	30	3.3	
Raw milk consumption	No	35	2.8	4.55
	yes	99	17.2	
Raw meat consumption	No	49	6	3.55
	yes	85	17.6	
Removal of placenta	No	104	0	72.08
	yes	30	60	

*indicate that p-value less than 0.05

Table 5 Multivariable logistic regression analysis of human brucellosis positivity by RBPT and CFT

variable		Number of patients examined	Number of positive	Crude odds ratio(95% CI)	Adjusted odds ratio(95% CI)
Occupation	Farmer	65	15	1	1
	Private	36	2	0.09(0.012-0.75)	4.91(0)
	Gov't employed	33	1	0.23(0.05-1.01)	2.84(0.09-87.50)
Education	Illiterate	38	8	1	1
	<6 grade	28	5	0.82(0.24-2.82)	0.38(0.02-10.16)
	6-12 grade	38	4	0.44(0.12-1.1.61)	0.32(0.01-10.52)
	Degree/diploma	30	1	0.13(0.02-1.20)	0.18(0)
Sex	Male	79	12	1	1
	Female	55	6	0.68(0.24-1.95)	0.18(0.02-1.67)
Age	Young	52	7	1	1

	Adult	57	6	0.77(0.24-2.42)	1.22(0.12-12.57)
	Old	25	5	1.61(0.45-5.68)	-
Raw milk consumption	No	35	1	1	1
	Yes	99	17	7.05(0.90-55.09)	1.53(0.03-75.23)
Raw meat consumption	No	48	3	1	1
	yes	85	15	3.29(0.90-11.99)	3.44(0.28-41.96)
Removal of placenta	No	104	0	1	1
	Yes	30	18	-	-

CI= confidence interval

significance difference between male and female (X^2 =2.508, P=0.113), between age groups (P=0.909,X^2 =0.013) and different Occupations (X^2 =5.203, P=0.074) were observed but, there was statically significance difference between different education level (X^2 =8.415, P=0.038) in Debrebrihan town administrative.

Table 6 Most common clinical symptoms of patients observed during examination

Sign/Symptoms		Number of examined	Prevalence (%)	X^2	P-value
Headache	No	25	0	4.769	0.029*
	Yes	109	16.5		
chills	No	15	0	2.621	0.105
	Yes	119	15		
Night sweat	No	60	8.3	2.430	0.119
	Yes	74	17.5		
Back pain	No	49	2	8.621	0.003*
	Yes	85	20		
Anorexia	No	51	2.8	6.405	0.011*
	Yes	83	19.2		
Weakness	No	71	5.6	7.899	0.005*
	Yes	63	22.2		
Joint pain	No	51	3.9	6.405	0.011*
	Yes	83	19.2		

*indicate that p-value less than 0.05

C. Questionnaire surveys

A total numbers of 150 individuals had been interviewed using purposive sampling by considering risk factors such as occupation, education, age, sex, whether other family members treated of brucellosis, assisting animals during parturition, livestock ownership, milking and slaughtering, history of consuming raw meat and milk, direct contact with ruminants, awareness level about sanitation habits and knowledge on brucellosis(Table 7).

Patients interviewed during blood sampling most of respondents mentioned at list three of the symptoms. The most common signs and symptoms were statistically significant difference between non seropositive and seropositive, headache (X^2 =4.769, p=0.029), backache (X^2 =8.621, p=0.003), anorexia (X^2 =6.405, p=0.011), weakness (X^2 =7.899, p=0.005) and joint pain (X^2 =6.405, p=0.011)(Table 6). Those symptoms of illnesses showed that significantly associated with brucellosis disease. On community awareness no statistical

Table 7 Knowledge about source of infection and transmission of brucellosis in the community

Variable		Raw milk consumption(%)	Raw meat consumption(%)	Contact with animals (%)	Awareness of brucellosis (%)
Sex	Male	67.2	61.6	77.6	28
	Female	68	40	100	44
Age	Young	63.5	68.2	61.9	30.2
	Adult	70	50.5	95.4	31
Education	Illiterate	95.3	39.5	90.7	20.9
	<6 grade	71.8	75	87.5	25
	6-12 grade	41.4	56	80.4	29.3
	Diploma/degree	58.8	67.3	64.7	50
Occupation	Farmer	86.3	43.8	94.5	21.9
	Private	59.4	72.5	78.3	37.5
	Gov't	40	70.2	60	40.5

	employee				

4. DISCUSSION

According to OIE, brucellosis is considered as the most widespread worldwide and one of the most important zoonotic diseases in the world accounting for the annual occurrence of the more than 500,000 cases[38]. In countries like Ethiopia, where there are a large number of animal populations and potential risk factors, the prevalence of zoonotic diseases such as brucellosis should be investigated both in humans and animals. History of contact with animals, animal products, materials of aborted animals without any protective measures, raw milk consumption and handling of raw meat are the main factors for disease transmission.

In this study, the prevalence of ruminant brucellosis in Debre berhan area was found to be 2.3% (95% CI: 0.01-0.038) with RBPT and all found to be negative with CFT. The current result was similar with the results of many previous observations in different places, including studies in Naztheret, Mekele and Gondor by Asmare *et al*.[5] who reported zero prevalence of bovine brucellosis. The small ruminant's brucellosis was similar with the results of studies in mille (Bekerdar) by Teshale *et al*.[17] and Afar (Assayita district) by Ashenafi *et al.[4]*. The absence of the infection might be due to the differences in management and husbandry condition in the area and the intensity of dairy production. There can also be conditions that could not facilitate the rate of transmission of the disease[23]. Brucellosis incidence varies widely not only between countries but also within countries [34], studies in Egypt [16] suggested that demographic factor may play a role.

In the present study, the prevalence of human brucellosis in the study area was found to be 13.4%(95% CI: 0.076-0.193) which is higher than the previous studies carried out in different part of the country, the reports in Adami Tulu 2.2% by Gebewo *et al*.[14], Jimma 3.6% by Tolosa *et al*.[30], Metema 3% by Regassa *et al*.[39], however, these studies lower than the report of Hammer 29.4% and Borena 34.1% by Regassa *et al*.[39].

There were highly statistical significantly difference in the prevalence of human brucellosis between 13 districts ($\chi 2 = 30.25$, $P = 0.003$), there were high prevalence of human brucellosis in patients who come from 9 districts of north shewa zone(Shewa Robit(42.8%), Senbete(25%), Eneware(50%), Mehal meda(50%), Lemi(66.6%), Sheno(25%), Hagere mariam(20%), Baso(15%) and Deneba(16.6%)). But this study showed that patients who come from Mendida, Ataye, Debrebrihan town administrative and Debre sina did not react seropositivity for brucellosis test. It is interesting to note that, the prevalence of human brucellosis was zero similar to prevalence of ruminant brucellosis in Debrebrihan town administrative showed that the source of human brucellosis were other 9 districts of north shewa zone of amhara regional state.

There was statistically significant difference in the prevalence of human brucellosis between different occupations (P=0.006, $X^2 = 10.256$) and the multivariable logistic regression analysis showed that being a farmer had 4.7 times the odds of being brucella reactors compared with patients having other occupations (adjusted OR = 2.84; CI = 0.09-87.50). This finding agreed with studies conducted in Amhara Regional State ([22], Sidama Zone of Southern People Nations and Nationalities Sate [19] and Addis Ababa[18]. Brucellosis is an occupational disease, occurring most often in veterinarians, farmers, stock inspectors, abattoir workers, laboratory personnel, butchers[7]. This could be when there is direct contact with cows at abortion, parturition or in the post parturition period from splashing of infected droplets into the eyes.

In this study, those patients who had habit of raw milk consumption ($\chi^2 = 4.557$, P = 0 .033) showed significantly at risk of brucella infection and 7.05 times the odds of being brucella reactors compared with those patients not having raw milk consumption habit (adjusted OR =1.53; CI = 0.03-75.23), as reported by Regassa *et al*.[39] the consumption of raw milk and fresh cheese were identified as risk factors for having brucellosis in the Borana and Hamer people and [2] reported raw milk consumption as the major source of infection in Kuwait. Almuneef *et al*. [3] reported that ingestion of raw milk was considered the likely source of infection among family members.

In the present study, those patients who had contact with placenta showed statistically highly significantly ($\chi^2 = 72.083$, P = 0.000) to acquire of brucella infection. In Chad, a study conducted by Schelling *et al*.[24] showed that contact with placenta of livestock was highly associated with brucellosis transmission. In Saudi Arabia, assisting animals during parturition was found to be an important risk factor for brucellosis transmission. In Nigeria the highest prevalence (20%) of brucellosis was observed among cattle handlers. Yohannes and Gill[35]in and around Ludhiana, India reported contact with parturient animal, raising animals, occupation-related mishap and eating during working hours were identified as the main risk factors. This finding is reasonably in agreement with Ali *et al*.[37] who reported contact with animals in Egypt risk factor for transmission of human brucellosis.

The overall sex-wise seropositivity in human was higher in females patients (15.2%) than males patients (10.9%) but no statistically significant difference(x^2 =0.511, p=0.475) in the prevalence of human brucellosis between male and female. In a study in Kampala, (Uganda), significantly more females than males were found to show seropositively for brucellosis [20], report from Adami Tulu, Central Ethiopia by Gebewo *et al.*[14] also showed the prevalence of human brucellosis was a bit higher in females than male, even if statistically insignificant.

Although the age group of old appears to be the most commonly affected, the different age groups included in our study were not significantly associated with brucellosis. These findings agreed with report of Yohannes and Gill[25] in and around Ludhiana, India age group did not significantly associated brucellosis infection.

In this study, association of brucella seropositivity to the symptoms of illnesses where patients experienced such as headache, fever, joint pain, back pain, chills, night sweat, anorexia and weakness were assessed. The most common signs and symptoms showed statistically significant difference were head ache (X^2 =4.769, p=0.029), back pain (X^2 =8.621, p=0.003), anorexia (X^2 =6.405, p=0.011), weakness (X^2 =7.899, p=0.005) and joint pain (X^2 =6.405, p=0.011). Those symptoms of illnesses indicated that having significantly associated with brucellosis disease. The disease is often treated as fever of unknown origin and frequently misdiagnosed as other common febrile diseases such as malaria and typhoid fever[29].

On awareness survey no statistical significance between male and female (X^2 =2.508, P=0.113), between age groups (X^2 =0.013, P=0.909) and different occupation (X^2 =5.203, P=0.074) but there is statistically significant difference between different education level (X^2 =8.415, p=0.038) in Debrebrihan town administrative. On education categories illiterate (20.9%), 0-6 grade (25%), 6-12 grade (29.3%) and degree/diploma holder (50%), indicate that the illiterates are at risk of getting zoonotic diseases like brucellosis. None of the patients had any knowledge about the cause, prevention and treatment of brucellosis, regardless to living conditions and dietary habits of these communities, control of brucellosis in the livestock population may be the main option to prevent diseases.

5. CONCULUSION AND RECOMMENDATIONS

The present study revealed that there was no ruminant and human brucellosis in the Debrebrihan town administrative, North Shewa zone of amhara regional state. However, there was high prevalence of human brucellosis outside Debrebrihan. This finding indicates that the source of human brucellosis was from other districts of north shewa zone of Amhara regional state. Besides, the study also showed that occupation, level of education, habit of raw milk consumption, contact with animals and their product were important risk factors associated with the prevalence of the infection in other districts of north shewa zone where the human brucellosis was found highly prevalent. Moreover, the study indicated that the disease was prevalent in human beings, who were among the high risk groups of contracting the infection. This emphasizes that the impact of brucellosis in public health and the need to control and prevention of the disease in the areas. Therefore the following recommendations will be forwarded:-

- Proper disposal of aborted fetuses and fetal membranes, personal hygiene of the workers through the use of detergents, safe handling of potentially infected materials and wearing of protective cloths are an important measure for the prevention of human brucellosis

- Milking should be in separate tanks and by separate personnel with the proper sanitary care and an establishment of parturition pen to avoid further cross contamination among non-infected animals is also mandatory

- Public education on the transmission and source of infection of the disease need to be undertaken on the area of prevalence of human brucellosis is high. The necessary precautions should be taken to reduce occupational risks. Pasteurization of milk should be widely practiced to prevent human infections

- There is also a need for further study to investigate the link between animal and human brucellosis and cross infection between different species.

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7. ANNEXES

Annex-1 Consent form

CODE _____

Name of the study participant_____Age_____Sex _____

Physician Name_____

I have been informed about a study that plans to investigate the **Cross sectional study of brucellosis in ruminants and human in and around Debrebrihan, Ethiopia,** which will help in understanding the zoonotic importance of brucellosis

The investigator has briefed me that there is no major risk associated with the sampling procedure except very minimum pain when inserting the needle. Sampling will be done under aseptic conditions by well experienced nurses. I have been informed that there is no direct benefit provided to me.

I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my informed consent and cooperate at my will in the course of the conduct of the study.

Name (participant) _____Signiture_____ Date_____

Name (investigator) _____Signiture_____ Date_____

Name (witness) _____Signiture_____ Date_____

