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Seroprevalence and Risk Factors of Small Ruminant Brucellosis in Ambo, Adea and Fentale Districts of Oromia Regional State, Central Ethiopia

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Abstract: A retrospective study design was employed on previously collected sera samples to investigate brucellosis in small ruminant from December 2011 to March 2012 with the objectives of estimating the seroprevalence and potential risk factors for the occurrence of brucellosis in sheep and goats in selected district; Ambo, Adea and Fentale. A total of 2070 sera samples (1119 sheep and 951 goats) were tested using serological tests, screening by RBPT and confirmatory test CFT, The seroprevalence of brucellosis was calculated as the number in study population testing positive to the serological test divided by the total study units tested. The overall seroprevalence of 4.97%, 3%, and 4.05% respectively. Data including serological test result and earlier collected questionnaire survey were recorded and coded in Microsoft Excel spread sheets and then statistically analyzed using (stata^{tm} 11.0) to determine the strength of potential risk factors associated with the occurrence of brucellosis by using univariable logistic regression. Mixed flock OR=2.11(1.33-3.36 CI; p=0.002), agro-pastoral OR=4.01 (2.35-6.84CI; p=0.000) and pastoral OR=2.59(1.37-4.90 CI; p=0004) production system, larger flock size OR=1.68(1.08-2.60CI; p=0.021) were factors significantly affecting the prevalence of small ruminant brucellosis. By considering collinearity of variables, p<0.25 in univariable analysis and independent predictors of small ruminant brucellosis were further analyzed using multilogistic regression. [Seid U, Mahemmed C. Seroprevalence and Risk Factors of Small Ruminant Brucellosis in Ambo, Adea and Fentale Districts of Oromia Regional State, Central Ethiopia.
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Key words: brucellosis, CFT, RBPT, risk factors, Small ruminant, sera, unilogstic regression, multilogstic regression.

Introduction

Ethiopia is one of the countries which endowed the largest livestock population in Africa. Livestock production varies due to difference in resource endowment, climatic condition, and human and live stock population, level of economic development, research support and government economic policies. Live stock in Ethiopia provides drought power, income for farming communities, means of saving and investment and is an important source of foreign exchange earnings for nation. The sector provides an estimated 16% of total GDP (equivalent to 30% of the agricultural GDP) and generates 14% of the country's foreign exchange (QSLMSE, 2006).

The function and purpose for which livestock are reared varies considerably across the major agro-ecological and socio-economical zones and the major livestock production system; the highland crop-mixed farming and low land pastoral and agro pastoral production system. Usually the pastoral and agro-pastoral areas are found in the low lands and characterized by extensive production which is largely based on the range land (Tembely, 1998; EARO, 2000). The country hosts large number of small ruminants which constitute an estimated number of 47.83 million, of which 26.12 millions are sheep and 21.71 millions are goats. Seventy five percent of sheep were adapted to high lands and about 76% of goats adapted to low lands (CSA, 2008).

Small ruminant and their products are important export commodity significantly contributing national economy; moreover they support the livelihood of millions of pastoral peoples as a source of milk and meat. Their adaptability to broad range of environments, short generation cycle and high reproductive rates that lead to the high production efficiency made small ruminant production an attractive enterprise in pastoral production system (PFE, 2004). There was a growing export market for sheep and goats meat in the Middle Eastern Gulf States and African countries (Alemu and Markel, 2008).

The main constraints to live stock development in Ethiopia are nutritional shortage, traditional husbandry, water shortage, poor marketing and different disease that limit productivity like brucellosis.

Brucellosis is a disease caused by infection with gram negative cocco-bacillary bacteria of genus Brucella. The disease in goats and sheep is caused by *B. melitensis* although; *B.abortus* may cause clinical brucellosis, *B.ovis* cause epididymits in ram. Abortion in late term pregnancy, still birth, birth of weak offspring, acute orchitis and infertility were characteristics of the disease (Kusikula and Kambrage, 1996).

Brucellosis is a wide spread zoonoses mainly transmitted from cattle, sheep, goats, pigs and camels through direct contacts with blood, placenta fetuses or uterine secretion or through consumption of contaminated raw animal product (especially unpasteurized milk and soft cheeses) in endemic areas (Radostits *et al.,* 2007). *Brucella melitensis* cause a fulminating disease in man which is characterized by intermittent fever (undulant and Malta fever), malaise, fatigue, osteomyelytis, is a common complication in man and is the most prevalent species owing in part to difficulties in immunizing free-ranging goats and sheep (WHO, 2005). The distribution of different species of brucella and their biovars varies with geographical areas. *B. abortus* is mostly wide spread. *B. melitensis* and *B. suis* are irregularly distributed. *B. neotomae* was isolated from desert rat (neotoma lepida) in Utah USA, and its distribution is limited to natural foci, as the infection has never been confined in man and domestic animals (George, 2001). The disease is more common in countries that do not have standardized and effective public health and domestic animal health programs. Areas currently listed as high risk are the Mediterranean basin (Portugal, Spain, Southern France, Italy, Greece, Turkey, North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean and middle east.

There are different factors associated with the occurrence of the disease in animals. Host factors; susceptibility by age (young are less susceptible), sex and reproductive status of the individual animals (sexually mature and pregnant animals are more susceptible) (Nicoletti, 1980). Placental trophoblast produces erythritol in increasing amount during the later stage of pregnancy which coincides with the period when pregnant cattle are more susceptible to infection. The preferential utilization of erythritol rather than glucose is a characteristic of pathogenic Brucella strains. Erythritol promotes the growth of some strains of Brucella, however as Brucella has been found in reproductive tracts of animals with no detectable levels of erythritol, the role of this sugar in the virulence of the organism has been in question (Sangari *et al.,* 2000).

Since it is not feasible to isolate the causative organism from infected cases; serological tests namely the RBPT, SAT, ELISA and CFT are important in routine diagnosis of the disease. Brucellosis, like tuberculosis, is a chronic granulomatus infection caused by intracellular organism and requires combined, protracted antibiotic treatment. The disease cause much clinical morbidity as well as considerable loss of productivity in animals in developing world. In this era of international tourism, it becomes common imported disease in the developed world (Georgios *et al*., 2005). The strategies for control and eradication of brucellosis in small ruminants were immunization to reduce the rate of infection in specified herd, elimination of infected animals by test and slaughter to obtain brucellosis free flocks/herds and regions, prevention of spread between animals and monitoring of brucellosis free herds and zones (European Commission, 2001)

Despite the presence of large population of small ruminants in different agro ecological regions of the country; limited research has been done on small ruminant brucellosis. Teshale and his colleagues (2006) reported a prevalence proportion of 14.6% in sheep and 16.45% in goats in Afar region and 1.6% in sheep and 1.7% in goats in Somali region. Another study in pastoral region of Afar reported a prevalence rate of 5.8% in goats and 3.25% in sheep (Ashenafi *et al*., 2007). A prevalence rate of 1.5% brucellosis in sheep was also reported from South Wollo (Yesuf *et al.,* 2010), and a 4.2% in goat from South Omo (Ashagre *et al*., 2011). Very low prevalence rate in goats (0.87%) was also reported from Bahir Dar area (Yeshawas *et al.,* 2011). Generally speaking, small ruminant brucellosis in Ethiopia, particularly in West and East Shewa zones of oromiya region, was not well studied; therefore this study was designed to add some information about the status of small ruminant brucellosis in the study area.

Objectives

- ✓ To determine the prevalence of small ruminant brucellosis in Ambo, Adaa and Fentale districts of central Ethiopia.
- ✓ To identify the associated potential risk factors for the occurrence of the disease in Small ruminants.

Materials And Methods

Description of Study Areas and Population

Study Areas

A study on seroprevalence of ovine and caprine brucellosis was conducted in three purposively selected districts of East and West Shoa Zones of Oromia Regional State, Central Ethiopia. The three districts were Fentale, Adea and Ambo, which represented low land, mid land and high lands, respectively. According to Hurni (1998) Ethiopia's tropical climate is subjected to wide topographic-induced variations. Extensive system of livestock management predominates in the area.

Ambo district is found in Western Shoa Zone of Oromia Regional State. The area is found at a longitude of 37^0 32' to 38^0 3' E, and latitude of 8^0 47' to 9^0 20' N and the altitude within the district ranges from 1400 to 3045 masl. The climatic condition of the area is 23% highland, 60% mid altitude, and 17% lowland with an annual rainfall and annual temperature ranging from 800 – 1000 mm and 15°C – 29°C, respectively. The mean temperature is 18.6 c°. The rainfall is bi-modal with the short rainy season from February to May and long rainy season (over 58.8% of the total annual rainfall) from June to September. Agriculture, of mixed type, is the main occupation of the human population in the area. Major livestock reared include cattle, small ruminants (sheep and goats), poultry, and pack animals (mules, horses, and donkeys) (Anonymous, 2010).

Two districts, namely Adea and Fentale, were selected from East Shoa Zone. Adea district is found at a distance of 45 kms from Addis Ababa, and situated at a longitude of 38^0, 38'E, and latitude of 08^0, 44' N. The altitude of the district ranges 1500 to over 2000 masl. The area is characterized by sub moist agro-ecology. The average rainfall is about 839 mm, while the mean minimum and maximum temperatures recorded for 27 years ranged from 7.9ºC to 28ºC with an overall average of 18.5ºC (IPMS, 2004). Farming system is mixed type in terms of crops and livestock cattle, poultry, goat, sheep, dairy and apiculture.

Fentale District is located in East Shoa Zone and 190 Km East of Addis Ababa and situated between 8^0 54’ N latitude and 36^0 23’ to 39 ^{0}54’ E longitude. It lies at an altitude range of 955 masl (at Metehara Plain) to 2007 masl (at Mount Fentale); with annual rainfall range of 560-630mm and it averages out at 553 mm. Average temperature range is 29-38^{0}C. With its arid and semi arid climate, pastoral and agro-pastoral production system predominates in the area. The rains are characterized by poor intensity and erratic distribution, the main rainfall and production season stretches from July to mid-September. The rain shower that occurs between Novembers – December locally known as “*Furamata” is* important for the regeneration of browse and shrub vegetation for goats with little importance for crop production. Fentale district has 18 peasant associations (PAs), out of which 11 are pastoral and 7 are agro-pastoral areas.

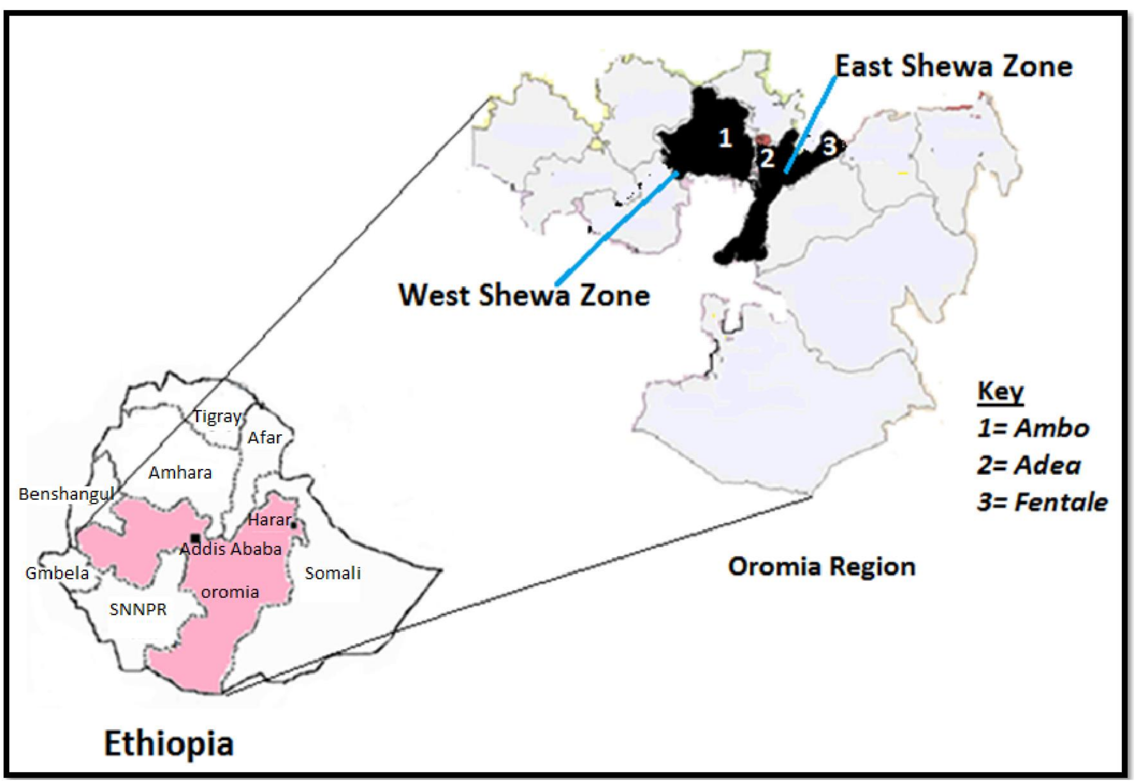

Figure 1: Study areas (districts) in East Shoa and West Shoa Zones, Central Ethiopia

The livestock and human population of the study areas are summarized in (Table 1). The data are based on the agricultural sample survey report on livestock and livestock characteristics (CSA, 2008b), and summary and statistical report of the 2007 population and housing census results (CSA, 2008a), respectively.

Table 1: Human and livestock population in the study area

Study Areas	Cattle	Sheep	Goat	Camel	Poultry	Human
West Shoa Zone						
Zone Total	2,072,485	1,035,326	1,037,159	-	1,823,471	2,072,485
Ambo District	110,796	52,714	43,339	-	NA	110,796
East Shoa Zone						
Zone Total	1,357,522	696,891	660,631	1,017,255	527,412	1,357,522
Adea District	131,273	55,305	55,491	NA	68,892	131,273
Fentale District	82,225	69,482	89,717	131,273	33,639	82,225

NA= Data Not Available

Study animals/ population

The study population consisted of small ruminants (sheep and goats). Small ruminant production in the study areas is mainly characterized by traditional and extensive type of management system, which includes sedentary, agro-pastoral and pastoral husbandry systems. Sedentary farming is a feature of the highlands while agro-pastoral and pastoral husbandry systems is practiced in Fentale district. Moreover, semi-intensive farming is practiced in the urban and peri-urban areas. Afar, Arsi-Bale and Western highland breeds of goat predominate in Central Ethiopia. Similarly sheep breeds common in the area are Afar, Arsi-Bale and Horro. All these types are known to be kept for mutton production in most parts of the country; however, pastoralists in Fentale district use goats and rarely sheep for dairy purpose. In the study, small ruminants of both sex but greater than six months were included.

Study Design

A retrospective study design was employed on previously collected sera samples. The study was conducted from December 2011 to March 2012 with the objectives of estimating the seroprevalence and potential risk factors for the occurrence of brucellosis in sheep and goats.

Sampling Method and Sample Size Determination

The peasant associations (PAs) or "Kebeles" were selected based on their accessibility for transportation and relative importance for small ruminant production. Farms and/or households in the PAs were selected using random sampling strategy. All animals in the flock were sampled, if the flock had equal or less than five sheep and goats aged above six months. However, if it had more than five animals, random samples of 5 animals were sampled.

An expected prevalence of 5.6% for sheep (Teshale *et al.,* 2006) and 4.2% for goats (Ashagrie *et al.,* 2011) and 2% absolute precision were used to calculate the required sample size followed by a two times inflation. This is because of the absence of variance data between clusters and the interest of having a more precise estimate (Thrusfield, 2007; Dahoo *et al*., 2003). The required sample size for sheep (1119) and goat (951) was allocated to each district proportionally based on their sheep and goat population. Accordingly, 382 sheep and 275 goats (total 662) from Ambo, 233 sheep and 112 goats (total 346) from Adea and 504 sheep and 540 goats (total 1049) from Fentale districts were used to test the serum samples.

Sample Collection and Transportation

Blood was collected from the jugular vein of sheep and goats. Sheep and goats were aseptically bled (approximately 5 ml) from the jugular vein by using vein-puncture into 10 ml vacutainer tubes which contained no anti-coagulants or preservatives, (BD Vacutainer Systems, Plymouth, UK). The blood samples were left for few hours at room temperature to allow clotting, and then centrifuged at 3000 rpm for 10 min. The serum was collected into 1.5 ml Eppendorf tubes (Eppendorf-AG, Hamburg, Germany), and transported to School of Veterinary Medicine, Debre-Zeit, using an ice box and stored at -20°C until serologically tested for the presence of anti brucella antibodies.

Serological tests

Modified Rose Bengal Plate Test (mRBPT)

All the serum samples were tested for the presence of antibodies against ovine and caprine brucellosis following the protocol of the OIE (2004). In order to improve the sensitivity of the RBPT and minimize the discrepancies between RBPT and CFT results we used three volumes of serum and one volume of antigen (e.g. 75 µl and 25 µl, respectively) in place of an equal volume of each as recommended by OIE (2004). After mixing of test and control sera with the antigen the plates/slides were rocked by hand for about 4 minutes. The results were interpreted according to (Nielson and Punkan, 1990), "0" as negative (No agglutination), "+" (Barely perceptible agglutination), "++" (Fine agglutination and some clearing), "+++" (Course clumping, definite with clearing).

Complement Fixation Test (CFT)

Rose Bengal Plate Test positive sera were stored at -20°C until tested by CFT for confirmation. The protocol described by (Mac Millan,1990) which uses standard *B. abortus* antigen (Veterinary Laboratories Agency, Addlestone, United Kingdom), Amboceptor (Biomerieux, France), 1% sheep RBC, positive and negative control antisera was used. The complement was obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany. Sera with strong reaction at dilution of 1:5 with a strong reaction of approximately 100% fixation of the complement (4+), more than 75% fixation of complement (3+) at a dilution of 1: 5 and at least 50% fixation of complement (2+) at a dilution of 1:10 and 1:20 were classified as positive (OIE, 2004). The details of CFT protocol is indicated in Annex 2).

Questionnaire survey

A systematically designed questionnaire collected earlier was accessed for the purpose of this study. Data on potential risk factors which include district (area), altitude, breed, sex, age, flock size, flock type (mixed flock), production system and management system on each animal was retrospectively accessed with questioner format (Annex1).

Data Management and Analysis

Data were recorded and coded in Microsoft Excel spread sheets before transferred to statistical software for analysis (Stata tm 11.0, Stata Corporation, College Station, Texas, USA). The database included serological test result and questionnaire responses. The seroprevalence of brucellosis was calculated as the number in study population testing positive to the serological test divided by the total study units tested. The Chi-square (χ^2) was applied to determine existence of any association between seropositivity and potential risk factors. To measure the strength of the associations, univariable logistic regression was applied to calculate Odds ratio. All non- collinear variables from univariable logistic regression with P<0.25 were further analyzed by multivariate logistic regression. For all analysis, a P-value of < 0.05 was taken as significant.

Results

Overall seroprevalence

The overall sero survey result of small ruminants brucellosis from 2070 sera samples tested 86(4.15%) with (χ^2 = 1.6526, P=0.438); i.e. out of 503 tested sera in Ambo 25(4.97%), out of 233 tested in Adea; 7(3%) and out of 1334 in Fentale districts; 54(4.05%) were positive by confirmatory test (CFT) were shown in (table 2).

Table 2. Overall seroprevalence of small ruminant brucellosis in study districts

Districts	Number tested	RBPT		CFT	
		Number Positive	Percent seroprevalence	Number Positive	Percent seroprevalence
Ambo	503	32	6.36	25	4.97
Adea	233	7	3.0	7	3.0
Fentale	1334	62	4.65	54	4.05
Total	2070	101	4.88	86	4.15
		χ^2 =4.3012, P= 0.116		χ^2 = 1.6526, P=0.438	

Risk factors

Univariable Logistic Regression

Logistic regression showing seroprevalence of small ruminant brucellosis with associated risk factor including; district, species, altitude, breed, age, flock size, production system, mixed flock. Ambo districts show higher prevalence, 4.97 % than Fentale (4.05%) and Adea (3%) districtst. Similarly goats have higher prevalence 4.84 % than sheep, but this result was not statistically significant.

Prevalence of brucellosis by altitude was higher 6.55% in low land areas, than mid and highlands. To similar way afar breed small ruminants show high prevalence 6.38 % than horro, arsi-bale, and western highlands, which were statistically significant. Female show relatively higher prevalence 4.19 % than males, similarly adults has higher 4.50% than young, which was not statistically significant.

Small ruminant brucellosis was higher in large flock sized than small sized similarly agro-pastoral

7.32 %and pastoral4.85 % have relatively higher prevalence than sedentary and were statistically significant. Semi- intensive management system show higher 4.21 % than extensive managemental system but not statistically significant. In other words mixed flock has higher prevalence 5.52% than sheep and goats kept alone and this was statistically significance. The details of statistical output for each risk factor were summarized in (Table 3).

Multivariable Logistic Regression

The following explanatory variables were found collinear: altitude vs. district, breed vs. altitude, production system vs. district and altitude and mixed flock vs. altitude and breed. Thus considering collinearity, P<0.25 in univariable analysis and comparable frequency of each category of every variable (>10) only production system, mixed flock, flock size, age and species were offered to the final model. Accordingly production system and flock size were found to be independent predictors of small ruminant brucellosis (Table 4).

Table 3. Univariable logistic regression analyses of explanatory variables of small ruminant brucellosis

Variable	Level	No. tested	No. Positive (%)	OR	95% CI of OR	P-value
District	Adea	233	7 (3.0)	1.0	-	-
	Fentale	1334	54 (4.05)	1.36	0.61 – 3.03	0.449
	Ambo	503	25 (4.97)	1.69	0.72 – 3.96	0.229
Species	Sheep	1119	40 (3.57)	1.0	-	-
	Goat	951	46 (4.84)	1.37	0.89 – 2.11	0.153
Altitude	Highland	344	6 (1.74)	1.0	-	-
	Midland	688	12 (1.74)	1.17	0.43 – 3.14	0.759
	Lowland	1038	68 (6.55)	4.15	2.23 – 7.72	0.000
Breed	Western highland	308	1 (0.32)	1.0	-	-
	Horro	379	8 (2.11)	6.62	0.82 – 53.22	0.076
	Arsi-Bale	348	11(3.16)	1.51	0.60 – 3.81	0.378
	Afar	1035	66 (6.38)	3.16	2.23 – 7.72	0.002
Sex	Male	422	17 (4.03)	1.0	-	-
	Female	1648	69 (4.190	1.04	0.61 – 1.79	0.884
Age	Young (<1yr)	469	14 (2.99)	1.0	-	-
	Adult (.1>yr)	1601	72 (4.50)	1.53	0.86 – 2.74	0.152
Flock size	Small	1097	35 (3.19)	1.0	-	-
	Large	973	51 (5.24)	1.68	1.08 – 2.60	0.021
Production system	Sedentary	1036	20 (1.93)	1.0	-	-
	Pastoral	392	19 (4.85)	2.59	1.37 – 4.90	0.004
	Agro-pastoral	642	47 (7.32)	4.01	2.35 – 6.84	0.000
Management	Extensive	1785	74 (4.15)	1.0	-	-
	Semi-intensive	285	12 (4.21)	1.02	0.54 – 1.90	0.959
Mixed flock	No	1002	27 (2.69)	1.0	-	-
	Yes	1068	59 (5.52)	2.11	1.33 – 3.36	0.002

Table 4. Multivariable logistic regression analyses of potential risk factors of small ruminant brucellosis

Variable	Level	OR (95% OR)	P
Species	Sheep	1.0	-
	Goats	1.17 (0.75, 1.82)	0.487
Age	Young	1.0	-
	Adult	1.43 (0.79, 2.57)	0.232
Flock size	Small	1.0	-
	Large	1.58 (1.01, 2.47)	0.048
Production system	Sedentary	1.0	-
	Pastoral	3.19 (1.42, 7.20)	0.005
	Agro-pastoral	4.45 (2.19, 9.002)	0.000
Mixed flock	No	1.0	-
	Yes	1.26 (0.84, 2.48)	0.474

Discussion

In this study the overall seroprevalence of small ruminant brucellosis in the study areas were 4.97%, 3%, 4.05% in Ambo, Adea and Fentale districts respectively This findings was higher when compared with the report 1.7% in goats and 1.6% in sheep in Somali region and lower than14.6%in sheep and 16.45% finding in goats in afar region by Teshale et al (2006).

Sera survey of small ruminant brucellosis show relatively higher prevalence in adults than young's; this is because susceptibility increases after sexual maturity especially with pregnancy, the presence of erythritol hormones and other substances in the uterus, placenta and fetal fluids favors the proliferation of *B.melitensis* which is the principal agent causing infection in sheep and goats. (kusiluka and kambrage, 1996)

Small ruminants categorized in larger flock size were have higher prevalence (OR=1.68, 95% CI 1.08-2.6, P=0.021) than categorized in small flock size, this is due to close contact between animals which contribute to the contagious nature of the infecting agent getting access to affect large num.

Mixed flock (sheep and goats kept together) show a higher seroprevalence (OR=2.11, 95% CI: 1.33-3.36, P=0.002) than kept alone, this finding can agree with the following statements; As with bovine brucellosis, higher prevalence brucellosis associated with larger, more freely mixing goat and sheep flocks in arid and semi-arid pastoral areas while Smaller, more restricted-grazing flocks have a lower prevalence. McDermott and Arimi (2002).

In species category, goats show higher seroprevalence 4.84% (OR=1.37, 95% CI; 0.89-2.11, P=0.153) than sheep 3.57% although statistically not significant, this is in agreement with findings 5.8% in goats and 3.2%in sheep in afar region by Ashenafi *et al*., 2007)

Seroprevalence of small ruminant brucellosis was significantly higher (OR= 4.15, CI: 2.23-7.72, P =0.000) in low land than mid and highland.

The seroprevalence of small ruminant brucellosis is significantly higher in agro-pastoral (OR=4.45, 95% CI: 2.19 – 9.02; P=0.000) and pastoral (OR=3.19, 95% CI: 1.42 – 7.20; P=0.005) systems as compared to sedentary production system. This is in agreement with the findings of McDermott and Arimi (2002).

Conclusion And Recommendations

Sheep and goats brucellosis is a zoonotic infection which was transmitted mainly by contact with discharges from placenta and aborted material with important effects on public health, animal health and production, and is wide spread disease in the country causing a serious economic loss. The sero-survey result in study district reveals as brucellosis in small ruminant were present in a spreading infection, although there was relative difference in prevalence among districts. Sexually mature sheep and goats were affected more this condition can greatly affect individual and national economy; due to reduction in reproductive efficiency and infertility, which contribute to the great loss. Due to cross infection between species of brucella organism, keeping sheep and goats together in one flock can increase the occurrence of infection. The contagious nature of the infectious agent can increase the prevalence of infection in flocks with large number of small ruminants. Sero-survey indicates that goats were affected more, this condition contribute to a risk of zoonosis in areas were goat milk were consumed, especially in pastoral areas being a serious public health problem.

Therefore, based on the above specified conclusion the following recommendations have been forwarded:

✓ Even though the sero- prevalence in the study areas was not as such higher, there should a strict control measures to be taken in order to limit the infection level.

✓ Avoid improper handling and disposal of infective contaminated material in order to limit spread of infection and risk of zonoosis.

✓ Avoid the habit of drinking row (unpasteurized) milk which was obtained from small ruminants, especially goat's milk.

✓ Avoid mixing of sheep and goat together to minimize risk of infection.

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