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REGULAR ARTICLES

Brucellosis and associated risk factors in dairy cattle of eastern Ethiopia

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Abstract Knowing the status of Bovine brucellosis and associated risk factors is a crucial step in formulating evidence based control scheme. In this study, a total of 967 dairy cows from 307 dairy farms in eastern Ethiopia were serologically tested for *Brucella* antibodies. The screening was done first using RBPT and positive samples were subsequently subjected to CFT for confirmation. A pre-tested structured questionnaire was used to collect relevant data from 307 dairy cattle owners or attendants to assess their awareness and routine practice. The data were run using univariable logistic regression analysis using STATA version 11.0 for Windows. Accordingly, herd and individual animal seroprevalence were found to be 6.8% (95% CI = 4.28–10.28) and 1.3% (95% CI = 0.72–2.29), respectively. The prevalence of seroreactors among local breeds was observed to be higher compared to cross breed ($p < 0.05$). Herd level analysis of the risk factors indicated that in farms with large herd size (>20 animals), the odds ratio (OR) = 9.13, $p = 0.00$, CI = 3.01–27.69 of having brucellosis was 9.13 times higher than smaller size herds (<20 animals). Intensively managed herds had shown the highest seroprevalence (20.8%) than extensive (6.7%) and semi-intensive (4.2%). Experience of dairy handlers about the disease that cause abortion in late pregnancy was significantly associated ($p < 0.001$) with the occurrence of brucellosis in the herds. However, about 91% of the dairy cattle owners/attendants lack awareness about disease(s) that causes abortion in late pregnancy. Similarly risk of having brucellosis in those herds experiencing abortion was 6.3 times higher (OR = 6.3, $p < 0.001$, CI = 2.50–15.92). This study identified some of the handling practices for aborted and retained fetal materials to be risky. Therefore, the study highlights the need of comprehensive brucellosis surveillance in animal and human and institutions of public education and on farm biosafety measures in shaping proper disease control scheme.

Keywords Dairy cattle · RBPT · CFT · Brucellosis · Risk factors

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Introduction

Brucellosis is a zoonotic disease caused by bacteria belongs to genus *Brucella*. *Brucella* is a gram-negative, facultative and intracellular coccobacilli (Quinn et al. 2002). The bacteria invade placenta and fetus and multifold multiplication takes place. Hence, the aborted fetus and fetal fluids may carry large number of infectious bacteria (Radostits et al. 2007; Mariana et al. 2010). During this time, animals excrete large number of organisms through milk, fetal membranes, amniotic fluid, and uterine discharge. Cattle acquire the infection mainly due to ingestion of feed and water contaminated by aborted fetuses, fetal membranes and uterine discharges (Radostits et al. 2007). Infection in man can therefore occur by ingestion of raw milk or milk products, or by handling of infected animals and contaminated materials, especially during parturition (Yumuka and O'Callaghan 2012).

Even though Brucellosis in livestock has been eradicated in industrialized countries, it is one of the most prevalent zoonotic diseases worldwide (Pappas et al. 2006). Especially, in low-income tropical countries where agriculture is the mainstay. In this part of the world, the disease is endemic and neglected, with huge health and livelihood burdens. This is due to lack of effective control and proper disease surveillance and reporting system (McDermott et al. 2013).

A number of researchers investigated the prevalence and risk factors of bovine brucellosis in Ethiopia. In western part of the country, an individual prevalence of 3.1 and 15% herd prevalence was reported in Jima area (Ibrahim et al. (2010). A study done in Borena, southern Ethiopia indicated that 10.6, 2.2, and 1.9% seroprevalence in cattle, camel, and goats, respectively (Megersa et al. 2011a). In Jijiga district, eastern Ethiopia, 1.2% of sheep and 1.9% of goats were seropositive to *Brucella* antigen (Bekele et al. 2011). In Addis Ababa dairy farms, central Ethiopia a prevalence of 1.5% were reported by Tesfaye et al. (2011). In southern and central part of Ethiopia, an overall seroprevalence of 1.9% were reported in sheep and goats by Asmare et al. (2013a). In Tigray region, northern Ethiopia, 3.5% prevalence was reported in small ruminants (Teklue et al. 2013). Brucellosis was reported not only in ruminants but also in camel. In the north eastern part of Ethiopia, Afar region, 5.4% seropositive camels was reported by Wesinew et al. (2013).

Even though all the above findings and the metanalysis report of Asmare et al. 2014, confirmed the wide spread occurrence and importance of bovine Brucellosis in Ethiopia, there is no intervention measures under gone in the country yet. However, a prioritization study conducted recently recognized that Brucellosis is one of the five priority zoonotic diseases in Ethiopia that should be jointly addressed by animal and public health organizations (Pieracci et al. 2016). On the other hand, there are no reported studies conducted in Harari People's National Regional State and the surrounding east Hararghe areas.

Dairy sectors all over the world are challenged by expanding populations and economic growth. Growing demand for dairy products has led to a significant increase in public and private involvement in dairy cattle production in small holder farming system. It is an opportunity for small holder dairy farmers especially for rural women to improve their livelihood. Bovine brucellosis is one of the infectious diseases that partly have constrained the productivity and causes severe public health and economic burden in low-income countries (McDermott et al. 2013). Hence, dairy cattle owners, consumers, institutions promoting dairy industry, public health professionals and policy makers require baseline information about the health of dairy cattle, the public health implication and the safety of dairy products. Such information on the burden of brucellosis is very important to engage all stakeholders on the control program. However, there is no published evidence that will bridge this information gap in eastern Ethiopia. Therefore, the objectives of this study were to estimate the seroprevalence of bovine brucellosis in dairy cattle and its associated risk factors and also to highlight the occupational health implication in the study area.

Materials and methods

The study area

This study was conducted in Harari People's National Regional State and the surrounding areas such as Dire Dawa city Administrative council and East Hararghe Zone of Oromia Region that include Haramaya, Fedis (Melka Rafa), Kombolcha, Jarso (Ijersa goro), Chelenko, Qersa and Kulbi (Fig. 1).

Study animals and their management

The dairy cattle included under this study comprise apparently healthy local (zebu) and cross bred (Zebu X Holstein Friesian) dairy cattle that were kept either in extensive, semi-intensive or intensive production systems. However, most of the breeds in semi-intensive and intensive farms were cross bred dairy animals, which were highly preferred for their high milk yield. The target study populations were dairy cattle consists of pregnant, non-pregnant, lactating, dry-cow and heifers. None of these cattle have a history of vaccination against brucellosis. Body condition scoring was done by inspection and palpation and categorizing into 3-scale: poor, good, and obese (Radostits et al. 2007).

Study design and data collection

The study took place between February and September 2010 in two phases. First, a cross-sectional household survey was carried out and secondly, blood sample was taken from animals that belong to the households surveyed. Household interview was undertaken with head of the selected farm/households. The total number of herds to be included in this study was calculated using an expected herd level seroprevalence "p" of 15% (Ibrahim et al. 2010), a confidence level of 95%, desired absolute precision (d) of 0.05 and using the formula $n = (1.96)^2\ p(1 - p)/d^2$ (Thrusfield 2005). This yielded a total of 196 herds. To increase precision, the numbers of herds were inflated to be 307. From the 307 herds, all animals (male and female) greater than 6 months of age were

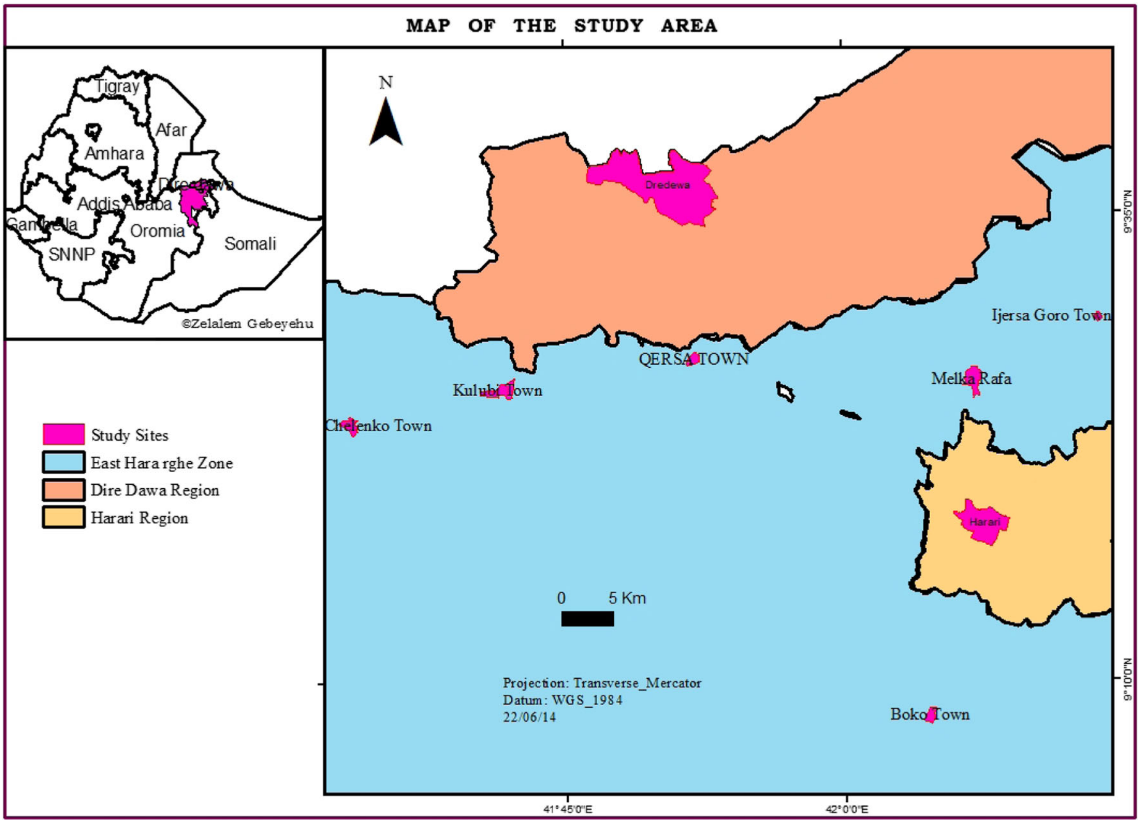

Fig. 1 Map study area

sampled. However, for individual animal level seroprevalence study, only female animal's data were analyzed.

Blood sample collection

About 7 ml of blood was collected aseptically from apparently healthy dairy cattle. The samples were labeled and kept in a slant position at room temperature for 30 min until serum starts to separate and then centrifuged at 3000 rpm for 10–15 min. Finally, the serum was stored at −20 °C at Haramaya University College of Veterinary Medicine microbiology laboratory until subsequent testing.

Serological test procedures

The Rose Bengal Plate Test (RBPT): was performed as a screening test on all serum samples collected as per the procedure described in OIE Manual (OIE 2009).

Complement Fixation Test (CFT): Serum samples screened as positive with RBPT were further tested by CFT to confirm for brucellosis infection as recommended by OIE Manual (OIE 2009). Both of the tests were conducted at Dire Dawa Regional Veterinary Laboratory, Ethiopia.

Questionnaire survey

A semi-structured questionnaire was used to obtain information on individual animal bio-data, farm level information and participants or family member perception or knowledge and practice about brucellosis. Both open- and close-ended questions were forwarded for the participant after translation to their local languages including Amharic, Orommiffaa, and Somali.

Data management and analysis

The epidemiological and animal bio-data collected during the study were entered into the Microsoft Excel and analyzed by using STATA version 11.0 for Windows (Stata Corp. College Station, TX). Descriptive statistics were used to summarize the data collected. Only animal positive to both Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) was classified as been *Brucella* seropositive. Since there is no history of vaccination against brucellosis in Ethiopia, seropositivity observed in this study was considered to be due to natural infection. Univariable logistic regression model was used to test the significance of the effect of different risk factors on individual and herd level sero-prevalence. A statistically significant difference between variables exists when $p < 0.05$ at 95% confidence level (CI).

Results

Seroprevalence of bovine brucellosis and its associated risk factors in dairy cattle

This serological survey indicated that from total of 967 dairy cattle, 1.3% (95% CI = 0.72–2.29) were positive for brucellosis by CFT. The highest individual animal level seroprevalence were recorded in Harari region (2%), whereas the lowest in Dire Dawa city administration (0.5%). Among the risk factors considered for individual animal level seroprevalence, breed has shown statistically significant ($p < 0.05$) difference in prevalence, while all the remaining factors were not ($p > 0.05$) (Table 1).

From the total of 307 tested herds, 21 (6.8%; 95% CI = 4.28–10.28) contain at least one animal which was positive to brucellosis by CFT. In contrast to individual level seroprevalence, the highest herd level prevalence were detected in Dire Dawa 2 (12.5%) while the lowest was detected in EHZO 7 (4.5%) (Table 2).

The association of risk factors considered with the herd level test results

With the exception of region ($p > 0.05$), all the risk factors considered at the herd level were statistically significant ($p < 0.05$). Large-sized herds have shown higher seroprevalence (33.3%) than small-sized herds (5.2%). Besides, intensively managed herds have shown the highest seroprevalence (20.8%) than extensive (6.7%) and semi-intensive (4.2%) (Table 4).

Questionnaire survey

The number of animals within the herd varies between the range of 1 and 100. From the total of 307 tested herds, 298 (97.1%) have no parturition pen, 237 (77.2%) did not have knowledge on disease that cause abortion in sheep, goat, and cattle and out of the 307 dairy cattle owner only 28 (9.1%) knew disease that causes abortion in late pregnancy. The questionnaire survey had shown that 46 (15.0%) of the respondents confirmed the presence of abortion or still birth in their farm and 255 (83.1%) of them did not take any measure for the aborted fetus whereas 28 (9.1%) of them thrown the aborted material in the surrounding area. Moreover, 69 (22.5%) of the respondents observed retained fetal membrane in their farm and the majority of them 238 (77.5%) did not take any measures, while 29 (9.4%) remove manually without any protective measures (Table 3). It is indicated that 279 (91%) of dairy owners/attendants did not have information/awareness about disease(s) that cause abortion in late pregnancy in sheep, cattle, and goat (Table 4).

Discussion

In this study, the overall individual animal level seroprevalence of dairy cattle brucellosis was low (1.3%) with moderately high herd level prevalence. Since, none of the cattle

Table 1 Univariable logistic regression analysis of risk factors for individual animal level CFT positives

Factors	Category	No. of animals tested	No. of CFT positives	Proportion in % (95% CI)	OR (95% CI)	*p* value
Region	Harari	342	7	2 (0.55–3.55)	Reference	Reference
	Dire Dawa	364	2	0.5 (−0.21–1.31)	0.26 (0.05–1.28)	0.09
	EHZO	261	4	1.5 (0.04–3.03)	0.74 (0.22–2.57)	0.64
Age	<5 years	390	5	1.3 (0.16–2.40)	1.08 (0.35–3.33)	0.89
	≥5 years	577	8	1.4 (0.43–2.34)		
Breed	Local	390	9	2.3 (0.81–3.80)	0.29 (0.09–0.97)	0.04
	Cross	577	4	0.7 (0.01–1.37)		
Physiological status	Lactating	550	7	1.3 (0.33–2.21)	1.13 (0.38–3.39)	0.82
	Nonlactating	417	6	1.4 (0.29–2.58)		
Calving	≤first calving	458	6	1.3 (0.27–2.35)	1.05 (0.35–3.15)	0.93
	≥second calving	509	7	1.4 (0.36–2.39)		
Herd size	<20	489	9	1.8 (0.65–3.03)	0.45 (0.13–1.47)	0.18
	≥20	478	4	0.8 (0.02–1.66)		
Management	Intensive	363	3	0.9 (−0.11–1.76)	Reference	Reference
	Semi-intensive	320	3	0.8 (−0.12–1.99)	1.14 (0.23–5.67)	0.88
	Extensive	284	7	2.5 (0.66–4.27)	3.03 (0.78–11.83)	0.11

EHZO East Hararghie zone of Oromia Region, *CI* confidence interval

Table 2 Herd and animal level seroprevalence of bovine brucellosis in dairy cattle among the three different regions of eastern Ethiopia

Regions	Number of animal tested	Animal level seroprevalence		Herd level seroprevalence	
		RBPT+ (%)	RBPT+/CFT+ (%)	Number of herd tested	RBPT+/CFT+ (%)
Harari	342	29 (8.48)	7 (2.0)	137	12 (8.8)[a]
Dire Dawa	364	13 (3.57)	2 (0.5)	16	2 (12.5)
EHZO	261	13 (4.98)	4 (1.5)	154	7 (4.5)[a]
Total	967	55 (5.69)	13 (1.3)	307	21 (6.8)

EHZO East Hararghie zone of Oromia Region

[a] Include herds that have positive animal which are not considered in individual animal level seroprevalence (bulls)

involved for this study were vaccinated against brucellosis, *Brucella* seropositivity was considered to be as a result of natural exposure. Comparable findings were reported by Tesfaye et al. (2011) in Addis Ababa dairy farms (1.50%), central Ethiopia; Degefu et al. (2011) in Jijiga zone (1.38%), eastern Ethiopia, Tschopp et al. (2013) in Arsi zone (1.7%), central Ethiopia and Yohannes et al.(2012) in east Wollega zone (1.97%), southwestern Ethiopia, Asgedom et al. (2016) in Alage districts (2.4%), Southwestern Ethiopia and Asmare et al. (2013b) who reported 2.6% (CI, 0.4–4.9) prevalence rate in eastern Ethiopia (Harar and Dire Dawa town). In a situation where there is no viable control intervention, the low prevalence reported in this and other studies might be due to spill-over effect of other *Brucella* species in cattle as the prevalence noted to be lower in such scenario (Godfroid et al. 2013). On the other hand, higher seroprevalence reports were made by researchers elsewhere. For instance, 3.1% by Ibrahim et al. (2010) in Jimma zone, southwestern Ethiopia; 10.6% by Megersa et al. (2011a) in Borena zone, southern Ethiopia, 6.1 and 4.9% by Haileselassie et al. (2010, 2011) in western Tigray, northern Ethiopia, 3.5% by Megersa et al. (2011b) from southern (Borena) and eastern (Jijiga and Shinilie zone) Ethiopia. Similarly, the present finding has disclosed 6.8% herd level seroprevalence which was lower than the reports of Ibrahim et al. (2010) in Jimma zone (15%), southwestern Ethiopia and Megersa et al. (2011b) from southern and eastern parts of Ethiopia (26.1%). The variation in prevalence might be due to in differences in the study population, management system, study protocol, region or administrative zones, agro-ecology, keeping mixed livestock like sheep, goat and camel; differences in sample size and diagnostic tests applied among different research (Megersa et al. 2011b; Godfroid et al. 2013; Asmare et al. 2013b, 2014). In the absence of any control program and in poor hygienic practice and awareness, the low prevalence reported in this study may be explained by the actual species of *Brucella* that infecting cattle in the study area which demands further study on isolation and characterization of the agent in question. The current finding also showed that local breeds were highly affected by brucellosis than cross breed animals ($p < 0.05$). This might be due to the fact that like the majority of tropical low income countries, most local (zebu) breeds in eastern Ethiopia were managed under traditional extensive system where the sanitary measures and the awareness of the community were found to be low. It was also observed that, on the extensive production system, cattle can be mixed together for grazing, watering, and sheltering with small ruminants and other non-conventional livestock species, like camel which can increase the risk of exposure to cattle (Asmare et al. 2013b, 2014; Godfroid et al. 2013).

Table 3 The result of questionnaire survey on dairy cattle brucellosis in eastern Ethiopia

Parameters	Number of respondents	
	Total	Yes (%)
Parturition pen	307	9 (2.9)
Knowledge of abortion in SGC	307	70 (22.8)
Knowledge of disease causing abortion in late pregnancy	307	28 (9.1)
Presence of abortion or still birth in the farm	307	46 (15.0)
Occurrence of infertility in the farm	307	87 (28.3)
Presence of RFM in the farm	307	69 (22.5)
Management of aborted fetus	307	
Buried		24 (7.8)
Throwing in the surrounding		28 (9.1)
Did nothing		255 (83.1)
Management of RFM	307	
Contact Veterinarian		31 (10.1)
Contact traditional healers		9 (2.9)
Did nothing		238 (77.5)
Manual removal		29 (9.4)

SGC sheep, goat, and cattle, *RFM* retained fetal membrane

This study indicated that intensively managed herds showed high infection rate than other two management systems. This might be associated with the larger herd size of

Table 4 Herd level seroprevalence of dairy cattle brucellosis and associated risk factors in Eastern Hararghie

Risk factors	Category	No.	CFT+ and RBPT+	Proportion (95% CI)	OR (95% CI)	(*p* value)
Region	Harari	137	12	8.8 (3.99–13.53)	Reference	Reference
	Dire Dawa	16	2	12.5 (−4.30–29.30)	1.49 (0.30–7.34)	0.63
	EHZO	154	7	4.5 (1.23–7.86)	0.49 (0.19–1.29)	0.15
Management	Intensive	24	5	20.8 (4.17–38.49)	Reference	Reference
	Semi-intensive	119	5	4.2 (0.56–7.84)	0.17 (0.04–0.63)	0.01
	Extensive	164	11	6.7 (2.85–10.56)	0.27 (0.09–0.87)	0.03
Herd size	<20	289	15	5.2 (2.62–7.76)	9.13 (3.01–27.69)	0.00
	≥20	18	6	33.3 (10.84–55.83)		
Knowledge of disease causing abortion at late pregnancy	Yes	28	10	35.7 (17.57–53.86)	13.54 (5.08–36.08)	0.00
	No	279	11	3.9 (1.65–6.24)		
Abortion in the farm	Yes	46	10	21.7 (9.64–33.84)	6.3 (12.50–16.00)	0.00
	No	261	11	4.2 (1.76–6.67)		
Measures taken for aborted fetus	Buried	23	4	17.4 (1.49–33.29)	Reference	Reference
	Burned	1	0	0 (−)	–	–
	Thrown	28	6	21.4 (5.89–36.97)	1.29 (0.32–5.29)	0.72
	Did nothing	255	11	4.3 (1.81–6.82)	0.21 (0.06–0.74)	0.02
RFM in the farm	Yes	69	9	13.04 (5.00–21.08)	2.83 (1.14–7.02)	0.03
	No	238	12	5.04 (2.25–7.84)		
Measures taken for RFM	Contact AHP	31	2	6.4 (−2.37–15.28)	Reference	Reference
	Contact TH	9	0	0 (−)	–	–
	Did nothing	238	12	5.0 (2.25–7.84)	0.77 (0.16–3.61)	0.74
	Manual removal	29	7	24.1 (8.22–40.05)	4.61 (0.87–24.42)	0.07

EHZO East Hararghie zone of Oromia, *CI* confidence interval, *RFM* retained fetal membrane, *AHP* animal health professionals, *TH* traditional healers

intensive farms. In farms with large herd size (>20), the risks (odds ratio = 9.13, $p = 0.00$, CI = 3.01–27.69) of having brucellosis was 9.13 times higher than smaller size herds (<20), (Table 4). The higher seropositivity of large herds is in accordance with previous findings (Ibrahim et al. 2010; Megersa et al. 2011b; Teklue et al. 2013; Lindahl et al. 2014) and can be justified by the fact that an increase in herd size is accompanied by high stocking density that can facilitate *Brucella* transmission especially following abortion or parturition (Radostits et al. 2007). Surprisingly the knowledge/experience of the dairy handlers about a disease that cause abortion in late pregnancy was significantly associated ($p = 0.00$) with the occurrence of brucellosis in the herds. The risk of having brucellosis in those herds experiencing abortion was 6.31 times higher than those did not experience abortion (odds ratio = 6.31, $p = 0.00$, CI = 12.50–16.00) (Table 4). This finding was in agreement with previous reports (Ibrahim et al. 2010; Haileselassie et al. 2011; Tesfaye et al. 2011; Lindahl et al. 2014). Hence, the presence of positive animals within large herd together with the existence of abortion may increase the dissemination potential of the bacteria in the environment common to the healthy animal and human. A study from Macedonia and Pakistan clearly indicated that there is a statistically significant association on occupational risk of brucellosis between *Brucella* seropositivity in human and where individuals are working with animals during parturition or abortion (Karadzinska-Bislimovska et al. 2010; Mukhtar 2010).

This survey documented different predisposing factors that can significantly contribute for further spread of bovine brucellosis in the herd. Unsafe handling practices and inappropriate removal measures for aborted and retained fetal materials; the lack of awareness among animal owner/attendant about brucellosis in sheep, goat, and cattle; absence of parturition pen and the presence of abortion or still birth or retained fetal material in the farms are some of the factors need due attention in disease control. Among the measures taken for the aborted material, not taking any measures were significantly contributed for brucellosis infection in the herds. Similarly, the presence of retained fetal membrane in the farm was also significantly associated with the occurrence of brucellosis. This was in agreement with the findings of Tesfaye et al. (2011) and Haileselassie et al. (2010). Poor hygiene, prevalence of the disease in animal, risky handling practices influence the occurrence of the disease in animals and humans (Radostits et al. 2007; Swai and Schooman 2009; Adesokan et al. 2013). A study from Tanzania indicated that family members in seropositive livestock household were 3.3 times more likely to be seropositive than those with seronegative (Shirima et al. 2010). About 91% of animal owner/attendant lack awareness about brucellosis in sheep, goat, and cattle and this seeks attention on the institution of public education in the disease control campaigns. Creating clear understanding about brucellosis has been shown to have controlling effects in the transmission (Kozukeev et al. 2006).

Despite of the limitations on not studying occupationally exposed human and on milk consumption habit, this study revealed low (1.3%) individual level prevalence of bovine brucellosis in dairy cattle. However, in light with the infectious and contagious nature of the disease, moderately high herd level prevalence (6.8%), poor dairy handler's awareness and risky handling of parturition and aborted material can contribute greatly for further spread of bovine brucellosis in the herd and to occupationally exposed groups. Therefore, further study on the causative agent identification and occupational health significance of brucellosis will be warranted.

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Compliance with ethical standards

Statement of animal care This study involved a questionnaire-based survey of farmers as well as blood sampling from their animals. The study protocol was assessed and approved by Haramaya University, research and extension office. Participants provided their verbal informed consent for animal blood sampling as well as for the related survey questions. Collection of blood samples was carried out by veterinarians adhering to the regulations and guidelines on animal husbandry and welfare.

Conflict of interest The authors declare that they have no competing interests.

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