Sero-Prevalence Of And Risk Factors For Q–Fever In Dairy And Slaughterhouse Cattle Of Jimma Town, South Western Ethiopia

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Abstract

Background

Q-fever is a zoonotic disease, caused by the Gram negative bacterium *C. burnetii*, which imparts significant socio-economic burden due to production and reproductive loss (abortion, stillbirth, and infertility) in ruminants and well as debilitating clinical disease in human populations. While sheep and goats are considered the primary reservoirs of infection to humans, infection can also result from exposure to cattle. Recent studies indicate that in Ethiopia Q-ever is a disease of growing public health interest. The top cattle producing region in Ethiopia is the Oromia region and Jimma is the zone that ranks first in the population of cattle within Oromia. While in Jimma zone livestock is the main provider of people's livelihoods and an important role in nutrition (through raw milk and meat consumption) to date there is no available report on sero-prevalence of Q-fever in cattle. This is particularly important due to the low dairy farm biosecurity in Jimma town. This study aimed to evaluate the potential risk for public health from cattle production; a specific objective of this study included the estimation of the sero-prevalence of *C. burnetii* infection and its potential risk factors in dairy cattle and cattle for slaughter in Jimma Town.

Results

The sero-prevalence of *C. burnetii* in cattle present at dairy farms was significantly lower compared to cattle presented at slaughterhouse [6.17% (95% CI: 3.41–10.13) and 11.79% (95% CI: 7.63–17.17), respectively; (P = 0.04)]. As the age of dairy cattle increase by one year, they were 1.51 more likely to be positive of *C. burnetii* [OR = 1.51 (95%CI: 1.30, 1.75; (P = 0.000)]. Cattle managed in semi-intensive production systems were 8.08 more likely to be *C. burnetii* sero-positive compared to intensively managed dairy cattle [OR = 8.08 (95%CI: 1.03, 63.68); P = 0.047]. Dairy cattle with access to nuisance animals like dogs, cats and mice were 5.65 more likely to be *C. burnetii* seropositive compared to dairy cattle without access to these animals. On the other hand, dairy cattle that have no tick infestation are 93% less likely to be seropositive for *C. burnetii* [OR = 0.07 (95%CI: 0.01, 0.74); P = 0.027]. Concerning farm-level data, farms of larger herd sizes were 1.03 more likely to be *C. burnetii* sero-positive than small herd farms [OR = 1.03 (95%CI: 0.99, 1.06)]. The result from slaughterhouse indicates that as the age of cattle increase by one year their chance of being *C. burnetii* sero-positive increases by 2.27 [OR = 2.27 (95%CI: 1.93, 2.68); p = 0.000].

Conclusion

Considering its zoonotic and economic burden the sero-prevalence of Q-fever recorded in this study is of eminent public health concern. Based on modifiable risk factors identified in this study dairy farm Q-fever management plans would benefit from health education and awareness campaigns for abattoir workers and dairy farm workers. Dairy farm Q-fever management plans should also contemplate improved dairy

herd biosecurity; such as biosecurity practices to avoid cattle tick infestation, keeping different livestock species segregated and avoiding mixing of herd with others with unknown health status.

Background

Q-fever is caused by highly infectious, ubiquitous and pleomorphic intracellular Gram-negative bacterium name *C. burnetii.* The organism can persist in a spore-like form for more than 40 months (OIE, 2010; Dalton et al., 2014). The disease is classified as an emerging zoonotic infectious disease according to WHO, FAO, OIE and EFSA/ECDC (Angelakis and Raoult, 2010; EFSA, 2010). Sheep and goats are considered to be the major sources of human exposure to *Coxiella*, but cattle can also be an important reservoir of the agent to humans (Rodolakis, 2009).

Q-fever has long been considered as an occupational zoonosis of major socio-economic importance worldwide associated with exposure to livestock by farmers, veterinarians, slaughterers, and animal researchers (Van der Hoek et al., 2011). Its outbreaks have been occasionally observed in many countries throughout the world (Kosatsky, 1984; Dupuis et al., 1987; Enserink, 2010; Roest et al., 2011; Van den Brom et al., 2013). Despite the fact that the disease is widely distributed, the disease is regarded as neglected, under diagnosed and underreported because of its diverse symptoms, self-limiting course and lack of diagnostic tools (Angelakis and Raoult, 2010; CDC, 2013).

In the African Context Q-fever was first reported in 1947, but since then the quantity and quality of epidemiological research on this pathogen has been limited (Mazeri, et al., 2013). Ethiopia was ranked highest in Africa in the health burden of zoonotic diseases (Grace et al., 2012). The first evidence of *C. burnetii* was reported in ticks collected from cattle in Ethiopia (Philip et al., 1966). As well as sero-prevalence of *C. burnetii* was found to be 6.5% by complement fixation test in workers at Addis Ababa abattoir in goat and sheep slaughterhouses and its peri-urban zone as found by (Abebe, 1990). To date the only Ethiopian study in cattle was conducted in southeast of the country reported a high sero-prevalence of *C. burnetii*, (31.6% in cattle, 90.0% in camels and 54.2% in goats) by enzyme-linked immunosorbent assay (ELISA) (Gumi et al., 2013). A 6.4% prevalence of *C. burnetii* in Ethiopia was also report from different Ixodid Ticks species by quantitative real time polymerase chain reaction targeting two different genes followed by multi-spacer sequence typing (MST) by Kumsa et al., (2015).

In recent years, reports of abortion and infertility in domestic ruminants from different corners of Ethiopia is becoming a common concern (Molalegne and Shiv, 2011; Haile et al., 2014; Alemselam et al., 2015). The Jimma zone in the Oromia Region of Ethiopia is one of such areas in from 2013–2015 it faced the worst outbreak of abortion, whereby more than 11,487 cases were recorded in domestic ruminants (cattle, goats and sheep) (Jimma zone livestock health and production agency, 2015). The Oromia Region of Ethiopia is the region with the highest population of cattle in the country and the Jimma zone of Oromia Region is the main cattle producing zone in Oromia and the second in Ethiopia with an estimated cattle population of 2,090,000 (FAO, 2018). These unusually high losses of pregnancies and the resultant infertility represent a tremendous economic loss to the nation and it is also a significant blow to the

livelihoods of livestock producers in Ethiopia. The initial suspicions of *Brucella* involvement as a cause of these abortion cases was ruled out by (Dirar et al., 2015). *Coxiella* was suspected to be one of the potential causes of such abortion episodes, as it can affect all three ruminant species. Nevertheless, to date there was no empirical evaluation of the level of sero-positivity of cattle to *C. burnetii* in this important cattle producing zone of Ethiopia.

In this study, we aimed to identify the public health risk of *C. burnetii* to dairy farmers and communities in Jimma town in the Oromia region of Ethiopia with the objective of estimating the sero-prevalence of *C. burnetii* and associated risk factors in cattle at Jimma dairy farms and slaughter-houses.

Results

A total of 227 and 195 samples were collected from dairy farms and slaughter house respectively. The overall sero-prevalence was 8.77% (95%CI: 6.07–11.47). *C.burnetii* sero-positivity was significantly lower in dairy farms (6.17%; 95% CI: 3.41–10.13) compared to slaughter house (11.79%; 95%CI: 7.27–16.32%) (p-value ≤ 0.042).

Dairy farm-level C. burnetii sero-positivity and its risk factors

Out of 227 animals included in the dairy farm analysis, the majority [n = 129 (56.83%)] originated from intensive management system and the vast majority were female [n = 223 (98.24%)]. Concerning their breed, the majority [n = 220 (96.92%)] were crossbred (Table 2) and in terms of age the minimum age sampled was 6 months and the maximum was 10 years. There was also higher sero-positivity to *C. burnetii* in male cattle compared to female and higher sero-prevalence in adult cattle compared to young. Prevalence of *C. burnetii* is found to be higher in the semi-intensive management system (8.16%; 95%CI: 3.59, 15.45) than in the intensive management system (4.65%; 95%CI: 1.73, 9.85) of dairy farms (Table 2).

Table 1
Serum and plasma samples thresholds–values and status for the interpretation of ELISA test.

Result	Status
S/P % ≤ 40%	Negative
40% < S/P % ≤ 50%	Doubtful
50% < S/P % ≤ 80%	Positive
S/P % > 80%	Strong positive

Table 2
Univariable logistic regression analysis (adjusted for herd effect) to select forward factor for final model contributing to *C. burnetii* distribution in dairy cattle and slaughter cattle of Jimma, Ethiopia (n = 227; 195 respectively)

Variable	Category	No tested	Prevalence (%)	95%CI		OR(95% CI)	P-value
				Lower	Upper		
Age	Continuous scale	227	14 (6.17)	3.41	10.13	1.33(1.04–1.69)	0.021
Sex	Male	4	3(75.0)	19.41	99.37	57.25(10.29, 318.50)	0.000
	Female	223	11(4.93)	2.49	8.65	1	
breed	local	7	1(14.29)	0.36	57.87	2.71(0.21, 34.95)	0.444
	crossholisten	220	13(5.91)	3.18	9.89	1	
BCS	Continuous scale	57	2(3.51)	0. 43	12.11	1	0.243
Multiage mix	No	115	8(6.96)	3.05	13.25	1.33(0.52,3.43)	0.548
	Yes(ref)	112	6(5.36)	1.99	11.30	1	
MultiSpecies mix	yes	203	14(6.90)	3.82	11.30	∞	0.000
	No	24	0(0.0)	0.00	14.25	1	
Tick infest	No (Ref)	123	7(5.69)	2.32	11.37	1	0.746
	Yes	104	7(6.73)	3.30	13.25	1.21(0.08, 18.30)	
Herd size	Continuous scale	227	14 (6.17)	3.41	10.13	1.01(0.99, 1.03)	0.163
Contact other herd	No	206	12(5.83)	3.05	9.95	1	0.477
	yes	21	2(9.52)	1.17	30.38	1.71(0.38, 7.51)	
Management system	Intensive	129	6(4.65)	1.73	9.85	1	0.170
	Semi-intensive	98	8(8.16)	3.59	15.45	1.84(0.77,4.41	
Presence nuisance animals (dog, cat, mice…)	No	70	3(4.29)	0.89	12.02	1	0.314
	Yes	157	11(7.01)	3.55	12.19	1.71(0.60,4.82)	

Legend: Ref. = Reference, OR = Odds Ratio, CI = Confidence Interval

Variable	Category	No tested	Prevalence (%)	95%CI		OR(95% CI)	P-value
				Lower	Upper		
Total	cattle	227	14 (6.17)	3.41	10.13		
Female data (n = 223)							
Animal aborted	No(ref)	191	9(4.71)	2.18	8.76	1	0.722
	Yes	32	2(6.25)	0.77	20.71	1.35(0.26, 6.98)	
Parity	Heifer (ref)	65	2(3.08)	0.37	10.68	1	
	Perimiparous	42	3(7.14)	1.50	19.48	2.43(0.26, 22.34)	0.435
	Multiparous	116	6(5.17)	2.39	10.83	1.72(0.39,7.62)	0.476
Slaughterhouse data (n = 195)							
Age	Continuous scale	195	23 (11.79)	7.63	17.17	6.93(3.51, 13.66)	0.000
BCS	Continuous scale	195	23 (11.790	7.63	17.17	0.48(0.24–0.99)	0.049
Tick infest	No (Ref)	22	2 (9.09)	1.12	29.16	1	0.678
	Yes	173	21 (12.14)	7.67	17.96	1.38 (0.30–6.36)	
Total	cattle	195	23 (11.79)	7.63	17.17		

Legend: Ref. = Reference, OR = Odds Ratio, CI = Confidence Interval

The final animal-level multivariable logistic regression mixed effect model showed that *C. burnetii* sero-positivity is significantly positively associated with age (OR: 1.51(95%CI: 1.30, 1.75): p-value ≤ 0.000) (Table 3). Our results also show that cattle managed in semi-intensive system were 8.08 more likely to be *C. burnetii* sero-positive compared to intensively managed dairy cattle [OR = 8.08 (95%CI: 1.03, 63.68); P = 0.047]. Dairy cattle that have access to nuisance animals like dogs, cats, mice and other were 5.65 more likely to be *C. burnetii* seropositive compared to dairy cattle with no access to nuisance animals (Table 3). On the other hand, dairy cattle that have no tick infestation are 93% less likely to be seropositive for *C. burnetii* [OR = 0.07 (95%CI: 0.01, 0.74); P = 0.027] (Table 3).

Table 3
Results of final best fitting multivariable mixed effect generalized linear model for the probability of *C. burnetii* sero-positivity in dairy cattle (n = 227) in Jimma, Ethiopia

Variables	Category	No tested	Prevalence (%)	95%CI		OR(95% CI) **	P-value
				Lower	Upper		
Age	Continuous scale	227	14 (6.17)	3.41	10.13	1.51 (1.30,1.75)	0.000
Tick infest	No (Ref)	123	7(5.69)	2.32	11.37	1	0.027
	Yes	104	7(6.73)	3.30	13.25	0.07(0.01, 0.74)	
Management system	Intensive	129	6(4.65)	1.73	9.85	1	0.047
	Semi-intensive	98	8(8.16)	3.59	15.45	8.08(1.03,63.68)	
Presence nuisance animals (dog, cat, mice…)	No	70	3(4.29)	0.89	12.02	1	0.120
	Yes	157	11(7.01)	3.55	12.19	5.65(0.64,50.23)	
Parity	Heifer (ref)	65	2(3.08)	0.37	10.68	1	
	Perimiparous	42	3(7.14)	1.50	19.48	0.56(0.07, 4.39)	0.580
	Multiparous	116	6(5.17)	2.39	10.83	0.45(0.11, 1.88)	0.272

Legend: Ref. = Reference, OR = Odds Ratio, CI = Confidence Interval, **=Adjusted for random effect of farm

Out of twenty-five dairy farms sampled, seven of them had at least one infected animal resulting in a herd-level *C. burnetii* sero-positivity of 28% (95%CI: 12.07–49.39). Dairy farms which had at least one contact with other herds were 4.63 time more likely *C. burnetii* sero-positive than herd which had no contact [OR = 4.63 (95%CI: 0.79, 26.94)] but that difference was marginally significant (Table 4).

Table 4
Multivariable Binomial Generalized linear models of factors at farm level (n = 25 farms) for *C. Burnetii* sero-distribution in dairy cattle of Jimma, Ethiopia

Variable	Category	No tested	Prevalence (%)	95%CI		OR(95% CI)	P-value
				Lower	Upper		
Herd size	small	6	0(0.0)	0.00	45.93	ref	0.120
	Large	19	7(36.84)	16.29	61.64	1.03 (0.99, 1.06)	
Contact other herd	No	21	5(23.81)	8.22	47.17	ref	0.088
	yes	4	2(50.00)	6.76	93.24	4.63 (0.79, 26.94)	
Management system	Intensive	20	5(25.00)	8.66	49.10	ref	0.348
	Semi-intensive	5	2(40.00)	5.27	85.34	2.94 (0.31,25)	
Total	cattle	25	7 (28)	12.07	49.39		

Legend: OR = Odds Ratio, CI = Confidence Interval; ref = reference

Slaughterhouse animals' C. burnetii sero-prevalence and their risk factors

The overall sero-prevalence of *C. burnetii* antibodies from cattle sampled at slaughter houses was found to be 11.79% (95%CI: 7.63, 17.17). Out of 195 animals included in the slaughter house analysis, all were from extensive management system, males and local breeds. All *C. burnetii* seropositive cattle were adults. Prevalence of *C. burnetii* antibody was found to be higher in tick infested cattle (12.14%) than the non-tick infested cattle (9.09%). Higher prevalence was recorded in medium body conditioned (16.22%) cattle compared to good body conditioned cattle (9.09%) (Table 2).

In the multivariable model of animals sampled at slaughter house age of cattle was the only factor found to be associated with *C. burnetii* sero-positivity [OR = 2.27 (95%CI: 1.93, 2.68); p = 0.000], which means as age of cattle increase by one year, their chance of being *C. burnetii* sero-positive increases by 2.27.

Discussion

This research is the first to investigate the sero-prevalence of and risk-factors for *C. burnetii* exposure in cattle in Jimma Town the most important city in the second highest cattle production zone of Ethiopia. Overall our results demonstrate that *C. burnetii* infection is a significant public health problem in the area in that 8.77% (95%CI: 6.07–11.47) of tested animals were found with evidence of *C. burnetii* antibodies. Our results suggest that cattle in Jimma town have a high level of exposure to *C. burnetii* infection which could partly explain the observed reproductive disorders and abortions occurring in Jimma zone. Our

findings are in agreement with 7.9% report in Algeria (sample size 311, cross sectional and tested with ELISA) (Cekani *et al.*, 2008), but higher than similar studies undertaken in Bura, Tana River County, Kenya which reported 5% (Sample size 96, cross sectional study design and ELISA test) (Mwololo, 2016), and 4% in Chad (sample size 195, cross sectional with i-ELISA) (Schelling et al., 2003). However, the overall sero-prevalence reported in our study is lower compared to the previous studies in the Southeast Ethiopia (i.e. a sero-prevalence of 31.6% using cELISA) (Gumi et al., 2013), and other countries in Africa ranging between 13% and 32% (Nakouné et al., 2004; Kamga-Waladjo et al., 2010; Scolamacchia et al., 2010; Nahed and Khaled, 2012; Knobel et al., 2013; Nakeel et al., 2016). The possible reasons for these variations might be difference in sample sizes, sampling methods and diagnostic tests used, geographical locations and management systems being practiced. Our results indicate that *C. burnetii* sero-prevalence in cattle in Jimma town is significantly higher in cattle sent to slaughterhouses compared to dairy cattle in dairy farms (11.79% vs 6.17%) suggesting that management systems may play an important role at modulating exposure risk (Capuano et al., 2001). This might partly be explained by the fact that all cattle sampled at the slaughterhouse were local breeds kept under extensive management systems from a variety of different districts of Jimma zone. This finding is in line with the study conducted in Nigeria which reported a prevalence of 11% in cattle at slaughter house and a prevalence of 17.1% and 1.3% in local breed and cross breed respectively (Tukur et al., 2014). Extensive management systems allow for an increase in exposure opportunities to *C. burnetii* through aerosol transmission between animals at grazing and watering areas. The extensive management system also exposes cattle to wildlife which could play a relevant role for disease species cross-transmission (Ruiz-Fons et al., 2010).

Similarly, for dairy cattle our results indicate a significantly increased probability of sero-positivity in crossbred dairy cattle kept under semi-intensive dairy production compared to intensive management system of dairy production. Cross breed dairy cattle are expensive and mostly kept under either intensive or semi-intensive management systems so that disease and tick are better controlled. Further, *C. burnetii* can survive in dry dusty environmental conditions for months and cattle managed in a semi-intensive system can be at greater risk of exposure to contaminated aerosols from known transmission vehicles from infected animals such as urine, feces or birthing products in the field compared to cattle managed in intensive systems (Clark and Magalhães, 2018). This is in agreement with other studies showing that dairy cows which were partially grazing in the field were more sero-positive of *C. burnetii* antibody (Capuano et al., 2001). In addition, our study demonstrated that dairy cattle with access to nuisance animals (such as dogs, cats, mice) were more likely to be seropositive to *C. burnetii* compared to dairy cattle with no access to nuisance animals. This finding is supported by evidence suggesting the ability for a range of companion animals and pest to be infected with *C. burnetii* (Riemann et al., 1979; Higgins and Marrie, 1990; Boni et al., 1998; Reusken et al., 2011; Meredith et al., 2015).

In our study we found a significant increase in the probability of *C. burnetii* exposure with increasing age in both dairy cattle and cattle sampled at slaughterhouse. This finding is in agreement with previous studies in Ethiopia and Cameroon (Gumi et al., 2013; Mazeri et al., 2013) and a more recent study by Jarelnabi et al. (2018) describing the age distribution of *C. burnetii* antibodies in camels, cattle, goats and sheep. One possible explanation is that the older the animal the greater is the potential exposure to the

pathogen infections and keep circulating antibody (Böttcher et al., 2011; Megersa et al., 2011). On the other hand, the result indicates that dairy cattle with evidence of tick infestation had significantly higher increase in the probability of *C. burnetii* sero-positivity (p-value ≤ 0.027). This result is also in line with evidence from around the world pointing for the isolation of *C. burnetii* from ticks (Ho et al., 1995; Knobel et al., 2013; Kumsa et al., 2015) indicating a potential role of tick infection in the dissemination of Q-fever in the herd.

Our results indicate that dairy farm-level sero-prevalence was marginally higher in farms with contact with other herds. Previous research reported that partial housing of the herds, contact with other herds and extensive management systems increased the likelihood of sero-positivity to *C. burnetii* (Capuano et al., 2001; Ryan et al., 2011; Taurel et al., 2011; Van Engelen et al., 2014; Jarelnabi et al., 2018). These are all modifiable farm-level bio-exclusion factors which can be acted upon by farmers to reduce the changes of *C. burnetii* transmission into the herd.

The findings of this study carry significant public health implications for the need to control Q fever in the community. Our results indicate that there is a significant risk of Q-fever particularly in slaughterhouse workers, dairy farmers and other animal workers and the consumers of dairy products in Jimma town. Our results suggest that the burden of Q-fever in these occupational groups identified is likely to be high and a collective effort is needed to investigate its impact on human health as well as to improve health promotion and education to these target community groups. Q-fever awareness campaigns and on-farm Q-fever biosecurity management plans need to be implemented in Jimma slaughterhouse workers and dairy cattle farmers with the aim of reducing their risk of exposure to *C. burnetii*. Furthermore, the level of sero-prevalence demonstrated in dairy farms necessitates more attention because these animals are the milk source for children. The veterinarian and public health sector need to work together in a One health approach to investigate the shared burden of Q-fever in the province of Oromia.

The findings of this study should be interpreted in light of its limitations. First, the cross-sectional nature of our investigation coupled with the use of serological tests for ascertainment of *C. burnetii* exposure means that we were unable to conclude on the true infection status of animals/herds. Second, from slaughterhouse survey we were unable to include female cattle which are usually managed under extensive management systems and thereby provide a more complete epidemiological picture of the level of *C. burnetii* infection in rural population.

Conclusion

The present study indicates that *C. burnetii* exposure is significantly high in cattle in an area in Ethiopia with one of the highest cattle populations in the country. Our findings demonstrate important modifiable farm-level risk factors which can be used to design farm-level Q-fever biosecurity management plans and Q-fever health promotion campaigns to reduce the public health of risk of *C. burnetii* exposure. Further studies should be designed to investigate the level of *C. burnetii* exposure in dairy farmers, slaughter house workers and consumers of dairy production in the region.

Methods

Study area and period

This study was conducted in Jimma Town from October 2016 to October 2017. The town is located in the Jimma zone of Oromia Regional State, South Western Ethiopia (Fig. 1). Jimma town is situated at a distance of 356 Km, South West of Addis Ababa, the capital city of Ethiopia, between 7º41"N latitude and 36º50"E longitudes and has an altitude of 1704 meters above sea level. The climate of the area is a tropical humid climate characterized by heavy rainfall which ranges from 1200–2000 mm per annum. With the mean annual minimum and maximum temperature ranging from 6ºC and 31ºC respectively, the overall average temperature is approximately 18.5ºC. Jimma zone is one of the largest in livestock populations in Ethiopia with cattle population estimated 2,212,962 heads (CSA, 2016). Dairy cattle are more under production in Jimma town and the surroundings small towns but more than 95% of the cattle populations are under extensive management which are used for mixed dairy and meat production as well as cash income generation for the rural communities.

Target and study population

The target population was apparently healthy crossbred dairy cattle kept under intensive and semi-intensive management systems and local breed cattle which are kept under extensive management system. These involved smallholder dairy farms and Jimma Dairy Development Enterprise (JDDE) and the local breed of male cattle presented to slaughter house aged between 3 and less than 10 years.

Sample size determination

The sample size to arrive at the study population was determined using the formula described by (Dohoo et al., 2009). The conservative estimate of 50% prevalence, 95% level of confidence and 5% absolute precision was used. Accordingly, the estimated sample size of 384 animals was obtained. The calculated sample size was oversampled by 10% to account for possible problems with non-response or missing data (Naing et al., 2006). This allowance was added summing up to the total of 422 samples. These samples were approximately halved to be distributed to dairy farms and slaughter house for blood sample collection. The proportion of required number of samples from each dairy farm was obtained by multiplying 28.3% expected prevalence of *C. burnetii* in cattle reported from Kenya (Knobel et al., 2013) to the total number of cattle in each dairy farm. Then, 9 animals were sampled from each dairy farm on average.

Study design and sampling strategy

Two cross sectional studies were designed to achieve the objectives of this study. First, a slaughterhouse survey was designed in the following way: on each day of visit to the slaughter house a representative percentage of 25% of animals were picked by simple random sampling technique from the lairage during ante mortem inspection. The sampling frame was constructed by listing the total number of animals in the lairage of each visiting day. The total numbers of slaughtered animals in Jimma slaughter house

ranged from 55–85 per day. On average, 14 samples were sampled per day to attain the total samples required and after sampling, animal level data like age, sex, tick infestation, breed, body condition score, production system were recorded.

Second, a farm-level survey was designed to measure Q fever exposure in the following way: a list of all 61 dairy farms and their contact details and location (ie. Kebele) was obtained from Jimma town livestock and fisheries resources development office. Thus a total of 25 dairy farms were selected by simple random sampling technique out of the 61 farms on the list to satisfy the total sample required from dairy farms. All targeted farms are business oriented dairy farms with crossbred and/or pure exotic breeds of dairy cattle (Holstein- Friesian). Based on Mulisa (2011) herd size was categorized as small (if the animals number in the herd were 3–10 animals), and large (if the animal number in the herd were 11 and above). A questionnaire to the farm owners was used to collect risk factors data for Q-fever infection, these included individual-level data and farm-level data. For individual-level data animals' age in year by the means of dentition as described by (Lawrence et al., 2001) and also asking the owners, sex, body condition score (BCS) categorizes as (poor, good and very good) (Roche et al., 2004) and breed. For farm-level data these included multi species mix, multi age mix, tick infestation status of the animals and farms, history of contact with other herd, herd size (continuous scale), production system (intensive and semi-intensive, extensive), presence of nuisance animals in the farm (dogs, cats, rodents and others), parity, and abortion status were included in the questionnaire/check list (Appendix 1).

Specimen collection procedure

About 10 ml of blood sample was collected from the jugular vein of each selected cattle using plain vacutainer tubes and multipurpose disposable blood collection needle 21Gx1 1/2" plus needle holder (Zhejiang Kanshi) Medical Devices Co. Ltd. (HENSO). Before and after sample collection, 70% ethanol alcohol was applied as disinfectant. Each specimen was labeled with unique identification number. The tubes were transported to Jimma University College of Agriculture and Veterinary Medicine laboratory in an icebox and the tubes were put in an oblique position of 45˚, for overnight at room temperature, to allow clotting of blood, the next morning sera was gently pipetted into cryovials and stored in deep freezer at -20^{0}C, until diagnosis was made in the laboratory of National Veterinary Institute (NVI) at Debre-Zeit, Ethiopia.

Laboratory Analysis and Interpretation

All serum samples were tested using Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA) from ID Screen®Q-Fever Indirect Multi- Species kits (ID.vet, 310; rue Louis Pasteur–Grabels–France) for the detection of antibodies against *C. burnetii*. All reagents were prepared and results were interpreted according to the manufacturer's instructions. Briefly, the optical densities (OD) were read at 450 nm in a micro-plate photometer (Multi Skan Ex, Thermo Electron Corporation, Finland). Negative control (NC), and positive control (PC) were run as duplicates in the micro – plate wells A, B and C, D respectively whereas sera were run as a single spot in the remaining micro plate wells. Interpretation of the result for each

sample was obtained as the percentage of the ratio between the sample Optical Density (OD) and positive control OD, according to the formula. The negative and positive samples were determined based on the laboratory test thresholds–values for its status (Table 1). The sensitivity (Se) and specificity (SP) of the test was claimed 100% as described by the manufacturer using serum from confirmed infected animals but other authors cited the test sensitivity and specificity for serum as 100% and 95%, respectively, compared to PCR (García-Pérez et al., 2009).

$$\frac{S}{P}\% = \frac{ODsample - ODnegativecontrol}{ODpositivecontrol - ODnegativecontrol} \times 100$$

The coloration quantity depends on the presence of antibodies in the specimen; positive sample will remain colored after addition of stop solution, while the light yellow negative sample will be colorless or white (Fig. 2).

Data management and statistical analysis

All data collected during the sero-surveys were entered into MS Office Excel 2010. Data were analyzed separately for cattle sampled in dairy farms and cattle sampled at the slaughterhouse. The overall prevalence was calculated as a total number of positive samples for *C. burnetii* divided by the total number of samples tested multiplied by 100. For each prevalence, binomial 'exact' 95% confidence interval (CI) was calculated using Epitools (Sergeant, 2019). To statistically test the difference between the overall prevalence in dairy farms and slaughter house, a test for two sample proportions was calculated using the proportion test calculator in the statistical software STATA versio 13 (StataCorp., 2013)

Univariable mixed effect logistic regression analysis was used to select individual explanatory variable that may predict individual *C. burnetii* sero-positivity. Variables with a p-value ≤ 0.25 at the univariable screening were taken forward to a multivariable mixed effect generalized linear model (farm as random effect) with Bernoulli family with a logit link. A separate multivariable binomial generalized linear model was used to model herd level prevalence data. Slaughterhouse data was analyzed using logit generalized linear model. Furthermore, multicollinearity was also assessed for any correlation between the explanatory variables with Spearman's rank correlation and between management system and contact with other herds shows there is a correlation (Spearman's rho =-0.6001; P-value ≤ 0.0015). Interaction terms between explanatory variables were entered into the model to investigate the presence of effect modification. Statistical significance in the multivariable model was set at a P–value ≤ 0.05. All statistical analyses were performed in Stata statistical software version 13 (StataCorp., 2013).

Abbreviations

21Gx1

21 Needle Gauge
BCS
body condition score
C. burnetii
coxiella burnetii
CDC
Centers for Disease Control and Prevention
cELISA
competitive Enzyme-linked Immunosorbent Assay
CI
confidence interval
Co. Ltd
Company Limited
CSA
Central Statistical Agency
E
East
ECDC
European Centre for Disease Prevention and Control
EFSA
European Food Safety Authority
FAO
Food and Agricultural Organization
ID.vet
diagnostic kits producing company for farm animals; Louis Pasteur, France
I-ELISA
Indirect Enzyme-linked Immunosorbent Assay
JDDE
Jimma Dairy Development Enterprise
Km
kilometer
MS
Microsoft
N
North
NC
Negative control
NVI
National Veterinary Institute
ºC

degree Celsius
OD
Optical densities
OIE
World organization for animal health
OR
odds ratio
P
predictive value (p-value)
PC
Positive control
PCR
Polymerase chain reaction
Q-fever
Query fever
QLD
Queensland
Se
sensitivity
SP
specificity
UQ
The University of Queensland
WHO
World Health Organization

Declarations

Ethics approval and consent to participate

The research work plan received ethical review and approved by the Jimma University College Agriculture and Veterinary Medicine's Ethical Review Board. Oral consents were taken from cattle owners after explaining the objectives of the study and its benefit and all safety procedure was followed during sample collection from the study units. The sero-status of the study unit was kept anonymous.

Consent for publication

Consent to publish the finding of the data was obtained from all the owners of the farms during the collection of sample and data orally.

Availability of data and material

The data generated and analyzed to support the findings of this study are not available publicly due to ethical reason and are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that there is no conflicting interest.

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Authors' contributions

FB and BD conceive the research idea, wrote the proposal, analyzed and interpreted the data analysis results and prepared the article.

DO was a major contributor in data collection and write up.

RJS contributed in article edition, interpretation of the analysis results and study map preparation. All authors have read and approved the manuscript for publication.

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Figures

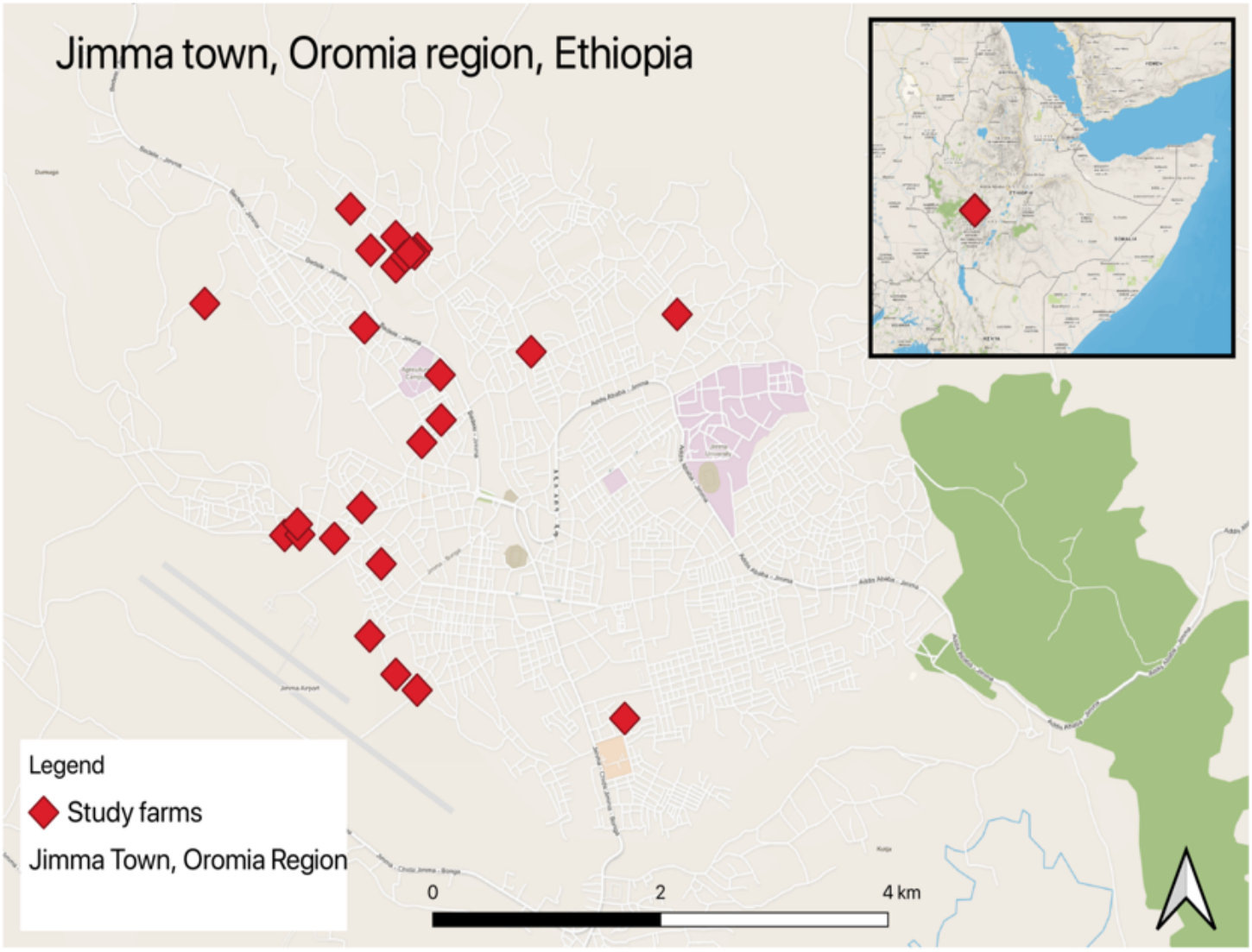

Figure 1

Study area and location of study farms (Map created by ArcGIS® software Esri version 10.8 (https://desktop.arcgis.com/en/arcmap/))

Figure 2

Multi- Species Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA) test kits from ID Screen®Q-Fever (ID.vet, 310; rue Louis Pasteur–Grabels–France). A. ELISA Kits Micro – Plate loaded with sera, NC and PC, and B are reagents plus NC and PC. Legends: NC = Negative control, PC = Positive control

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