Journal of Animal Research: v.13 n.05, p. 653-661. October 2023

DOI: 10.30954/2277-940X.05.2023.3

Query Fever Infection: Seroprevalence and Risk Factors in Cattle in Selected Districts of Bench Sheko Zone, Southwest Ethiopia

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Received: 02 Aug., 2023 **Revised:** 12 Sept., 2023 **Accepted:** 17 Sept., 2023

ABSTRACT

Query fever (Q fever) is caused by highly infectious, ubiquitous and pleomorphic intracellular rickettsia, *Coxiella burnetii.* This imparts significant zoonotic and socio-economic burden due to production and reproductive loss (abortion, stillbirth, and infertility) in ruminant and debilitating clinical disease in human populations. The study districts, peasant associations and individual animal were selected randomly. A cross sectional study was conducted both intensive and extensive farms. Data was collected in individual animal and herd level by using questionnaire to assess possible risk factors associated with the occurrence of Query fever disease. Indirect-enzyme linked immune sorbent assay (i-ELISA) test was used to detect antibody against *C. burnetii* in the collected serum. A total of 422 animal's serum and 119 herds were observed in this study. The overall seroprevalence of *C. burnetii* was 4.97% (95% CI: 3.1%-7.5%) and 13.4% (95% CI: 7.88%-20.91%) at individual animal and herd level respectively. Multivariable logistic regression analysis revealed that cattle breed ($p = 0.04$; adjusted OR = 4.804; (95% CI: 7.07-14.54%)), tick infestation ($p = 0.018$; adjusted OR = 11.786 (95% CI: 5.51-21.48)) and multispecies mix ($p = 0.005$; adjusted OR = 9.022 (95% CI: 17.95-31.74)) were significantly associated with the occurrence of *Coxiella burnetii* infection. The present finding showed risk factors such as breed, tick infestation and multispecies mix were found to be significantly associated with *C. burnetii* seropositivity. Tick control option should be applied for cattle and other domestic animal species to decrease the dissemination of Query fever.

HIGHLIGHTS

- Seroprevalence of *C. burnetii* was significantly related with cattle breed, tick infestation and animal management system
- The rate of detection of *Coxiella burnetii* antibodies was higher in cattle intensive farms (66.7%) than semi intensive system (55.6%).

Keywords: *Coxiella burnetii*, Risk factors, Seroprevalence, i-ELISA, Bench Sheko zone, Ethiopia

Query fever is caused by highly infectious, ubiquitous and pleomorphic intracellular rickettsia, *Coxiella burnetii (C. burnetii).* It has been identified in a wide range of wild and domestic animal hosts, including arthropods (particularly ticks), birds, rodents and marsupials but the most important reservoirs as sources for human infections are cattle, sheep and goats. The disease is classified as an emerging zoonosis according to WHO and FAO (Angelakis and Raoutt, 2010).

Coxiella burnetii infection can produce both acute and chronic forms of the disease in humans. Flu like symptom and self-limiting febrile condition is the most frequent manifestation in most cases (Aitken *et al*., 2010). Spontaneous abortion, intrauterine fetal death, premature delivery or retarded intrauterine growth may occur in pregnant women. Mortality is a rare outcome of the acute form of the disease. The major clinical manifestation of

How to cite this article: Haile, T., Teklemariam, T., Soma, M., Deneke, Y. and Addis, M. (2023). Query Fever Infection: Seroprevalence and Risk Factors in Cattle in Selected Districts of Bench Sheko Zone, Southwest Ethiopia. *J. Anim. Res.,* **13**(05): 653-661.

Source of Support: None; **Conflict of Interest:** None

chronic form of Query fever is endocarditis with case fatality rate in untreated cases exceeding 10%. Beside zoonotic importance, *Coxiella burnetii* also may cause health and production problems in domestic ruminants (Almeida *et al.,* 2012).

Transmission in human and animal occurs mainly through inhalation of aerosolized bacteria via dust, direct contact with infected animal body-fluid and tissue (Gunaratnam *et al*., 2014). It has long been suggested that wind could play an important role in the aerosol transmission of Query fever (Clark and Magalhaes, 2018). Transdermal transmission directly through tick bites is possible, as shown by experimental infection (Marrie, 2007). Shedding and persistence infectious agent for long period of time in environment may occur during the time of parturition of infected animal. The outbreak occurred in the United Kingdom Query fever cases were reported up to 11 miles (18 km) away from the farm that was the source of the outbreak (Hawker *et al*., 1998). *Coxiella burnetii* is resistant to a variety of disinfectants, and when the bacteria are not replicating, they can form a spore-like small cell variant (SCV) that remains viable for temperature change and desiccation (Scott and Williams, 2010). The organism can persist in a spore like form for more than 40 months (Dalton *et al.,* 2014).

Coxiella burnetii was suspected to be one of the potential causes of such abortion episodes, and can affect all three ruminant species. Nevertheless, to date there was no empirical evaluation of the level of seropositivity of cattle to *C. burnetii* in most part of Ethiopia (Deressa *et al*., 2020). The objectives of study was to determine the seroprevalence and associated risk factors of *C. burnetii* infection in cattle in selected districts of Bech Sheko zone, Southwestern Ethiopia; since, there was no published previous document about seroprevalence of *C. burnetii* infection among cattle in the areas.

MATERIALS AND METHODS

Description of the study area

The study was conducted from April to December, 2021 in randomly selected three districts of Bench Sheko Zone (BSZ) namely Shei Bench district, Semen Bench district and Mizan-aman town administration. Bench Sheko zone is the part of Southwest Ethiopia Peoples Regional State of Ethiopia. Bench Sheko is bordered on the South and Southeast by West Omo zone, on the west by the Gambela region on the north by Sheka zone, and on the east by Kafa zone (Fig. 1). Bench Sheko Zone has a 4,668

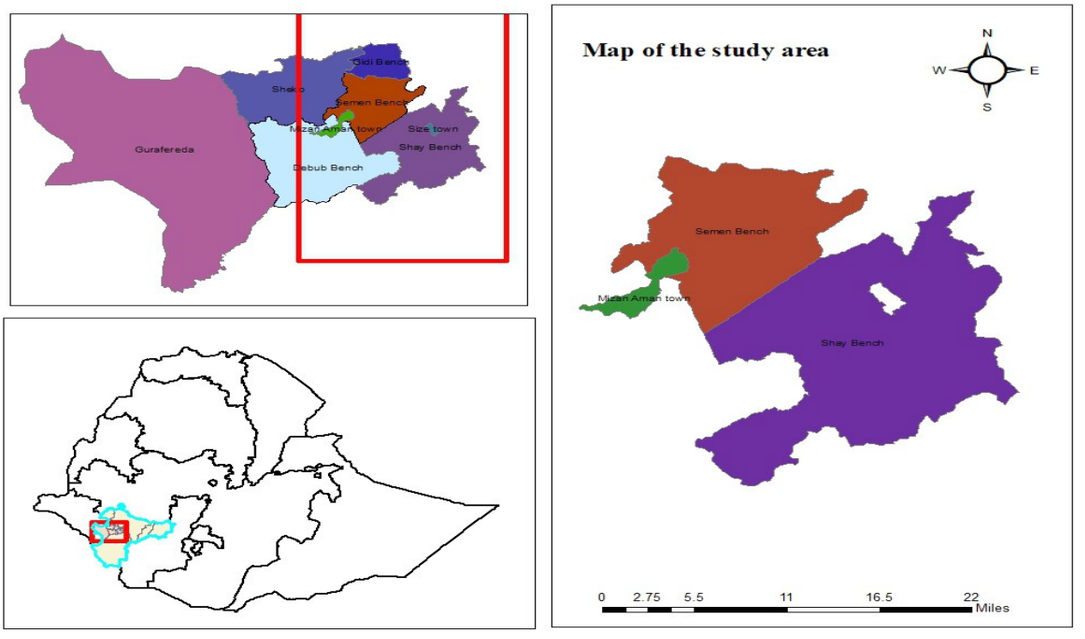

Fig. 1: Map of the study area

square kilometers area and agro-ecologically, consists of 52% lowland (<1500 masl), 43% mid altitude (1500-2300 masl) and 5% highland (>2300 masl). Geographically, it is located at 34°45'-36°10' East and 5°40'-7°40' North. The annual temperature and rain fall ranges from 15.1°C to 27.5°C, and 400 to 2,000 mm respectively. The major farming practice in the area was mixed (crop-livestock) production (BSZDA, 2021).

Study animal and study design

The study population was cattle (local and exotic breed) with different age groups found both in intensive and extensive farms. Age tick, body condition scoring standards body condition scoring (BCS) of the animals was classified in to poor (BCS 1-3), medium (BCS 4-6) and good (BCS 7-9) based on body condition criteria to evaluate statistical association. A cross-sectional study design was used to determine seroprevalence association of risk factors of *C. burnetii* infection. Structured questionnaire was used to collect data from owners on risk factors associated with epidemiology and transmission of Query fever disease in the areas.

Sample size determination

Sample size determination was calculated based on the formula developed by (Thrusfield, 2007) of 50% expected prevalence was considered since there was no published previous study in the areas.

$$N = Z^2 * \frac{P_{\exp}\left(1 - P_{\exp}\right)}{d^2} = 1.96^2 * \frac{0.50\left(1 - 0.50\right)}{0.05^2} \approx 384$$

Where,

'*n*' is the required sample size;

'P_{exp}' is expected prevalence;

'*d*' is desired absolute precision and

1.96 is the *Z* value for the selected confidence level (95%).

According to this formula, sample size was calculated to be 384 cattle. However, to increase the precision; 10% (38) of animals were added to 384 cattle number. Therefore, a total of 422 cattle were sampled in this study.

Sampling procedure and sample collection

The study district and peasant association were selected randomly. Multistage sampling technique was used to collect the blood serum sample in extensive farms. Cattle were selected on the basis of the generated random numbers, uniformly 3 animals are randomly sampled from each herd (lottery method).In intensive dairy farm management system, a total of 31 dairy farms were included for this study. Out of which 11 medium scale (≥ 10 heads of cattle) and 20 small scale farms (≤9 heads of cattle). Cattle were randomly sampled from each farm depending on the proportion of the total herd population. About 5 ml of blood was collected aseptically from the jugular vein of each cattle using plain vacutainer tubes. The serum was separated after blood was allowed to clot for 1–2 hours at room temperature, stored horizontally overnight at 4 °C in a refrigerator. All the serum were stored at −20 °C and examined for the presence *C. burnetii* antibodies in Mizan Regional Veterinary Laboratory Center using indirect Enzyme- Linked Immuno Sorbent Assay.

Laboratory examination

All serum samples were tested using indirect Enzyme-Linked Immuno Sorbent Assay (i-ELISA) from ID Screen®Query fever Indirect Multi- Species kits (ID.vet, 310; rue Louis Pasteur–Grabels–France) for the detection of antibodies against *C. burnetii*. Interpretation of the result for each sample was calculated according to the formula stated in the manufacturer kit manual as follows.

$$\text{S/P\%} = \frac{\text{OD of sample} - \text{OD of negative control}}{\text{OD of positive control} - \text{OD of negative control}} \times 100$$

Where; S/P% = Sample per positive control percentage

OD = Optical density

Data collection, management and analysis

Data obtained from questionnaires and laboratory results were recorded, stored in Microsoft Excel 2010 and transferred to IBM SPSS statistics version 20 software for analysis. Associations between outcome and explanatory variable for all units of analysis were investigated by

using binary logistic regression model. The strength of the association between outcome and explanatory variables was assessed using the adjusted odds ratios (OR). Univariable logistic regression analysis was used to select the individual explanatory variable that may predict the outcome variable in the model. All risk factors that had no collinear effect and $p \leq 0.25$ in the univariable logistic regression analysis were subjected to multivariable logistic regression analysis to control the effect of confounding in the model. Variables that had a $p<0.05$ were considered to be significant factors for Query fever.

RESULTS

Out of 422 sampled cattle 21 were seropositive for *C. burnetii* with seroprevalence of 4.97%. While, from observed 119 herds; 16 herds were seropositive *C. burnetii* infection with seroprevalence of (13.4%).

Table 1: Livestock owner response on information about their herd (frequency and percentage)

Parameter	Categories	Frequency	Precentage (%)
Multiage mix	No	47	39.49
	Yes	72	60.5
Total		119	100
Contact with other herd	No	39	32.7
	Yes	80	67.2
Total		119	100
Multispecies mix	No	60	50.42
	Yes	59	49.57
Total		119	100
Herd size	Small (≤9)	75	63.03
	Medium (≥10)	44	36.97
Total		119	100
Management system	Intensive	31	26.05
	Extensive	88	73.95
Total		119	100

Seroprevalence of *C. burnetii* was significantly related with cattle breed, tick infestation and animal management system (P<0.05) (Table 2). The variable showed P≤0.25 in the univariate logistic regression model were selected in the final multivariable logistic regression model (Table 2). Accordingly, cattle breed, sex, age, tick infestation and management system, were selected in the final model (Table 2). The probability of *C. burnetii* antibody in cattle infested with tick was 11.786 times higher than non-infested cattle (Table 2). Accordingly, seroprevalence of *C.burnetii* was significantly related with multispecies mix ($P<0.005$) and with the seroprevalence of 23.73% (Table 3). Thus, cattle herd with multispecies mix were 9.022 times more likely to have *C. burnetii* antibody than cattle herd did not mixed with the other species (Table 3).

DISCUSSION

In the present study, the seroprevalence of 4.97% result was in agreement with the finding of (Mwolol, 2016) (5%) in Kenya (Schelling *et al*., 2003) (4%). Similarly the present finding was in congruence with result of previous studies and 4.4% in Italy (Martini *et al*., 1994), 5.5% in Thailand (Nahed and Khaled, 2012), 5.8% in Turkey (Centinkaya *et al*., 2000), 6.2% in North Ireland (McCaughly *et al.,* 2010) and 6.7% in Spain (Ruiz-Fons, 2010). In contrast to this study, higher seroprevalence of *C. burnetii* has been reported in other region like 8.77% in Jimma town Deressa *et al*., 2020) 9.6% in Addadle woreda of Somali region (Ibrahim *et al.,* 2021) and 31.6% in pastoral districts of Somali and Oromia regions (Grumi *et al.,* 2013). Similarly so far higher prevalence reported in some countries 28.3% in Kenya (Knobel *et al*., 2013), 89.7% (Nakeel *et al*. 2016) in Kenya, 14.3% in Republic of Central Africa (Nakoune *et al.,* 2004), 19.3% in Egypt (Klemmer *et al*., 2018) 13.9% in Coted'ivoire (Kanoute *et al*., 2016), 39% in Zimbabwe (Kelly *et al*., 1993), 14.8% in Togo (Dean *et al.,* 2013) 11.36% in Algeria (Medani *et al*., 2020), 11.6% in Italy (Paris *et al*., 2006) 22% in Italy (Cabassi *et al*., 2006) 52% in Hungary (Dobos *et al*., 2006), 12% in Italy (Barlozzari *et al.,* 2020), 33% in China (El-Mahallawy *et al.,* 2016) and 15.6% in Bangladesh (Rahman *et al.,* 2016). The possible reasons for these variations might be the difference of sample size, sampling methods, diagnostic test used, geographical locations, management condition, the presence and absence of infectious foci, herd size, interaction of domestic animal with wild animal species at grazing pasture and watering point.

In the present study, herd prevalence of 13.4% (95% CI: 7.88% - 20.91%) was revealed and which coincides with the finding of Khalil and Sakhaee, (2009) 16.7% and Rahimi *et al*. (2011) 17.9% in Iran. In contrast high

Table 2: Univariable and multivariable logistic regression analysis of *C. burnetii* seropositivity and risk factor at individual animal level

Risk factors	Categories	No. of tested animals	No. of positive (%)	Univariable logistic regression				Multivariable logistic regression			
				95% CI		OR	p-value	95% CI		OR	p- value
				Lower	Higher			Lower	Higher		
District	Shei Bench	153	15(9.8)	1.465	18.489	5.204	0.011				
	Semen Bench	111	4(3.6)	0.424	8.805	1.931	0.395				
	Mizan Aman town	158	3(1.9)			Ref					
Cattle breed	Indigenous	222	19(8.56)	6.13	17.30	9.26	0.003	7.07	14.54	4.804	0.04
	Cross	200	2(1)			Ref				Ref.	
Sex	Female	247	16(6.47)	0.846	6.554	2.335	0.101				
	Male	175	5(2.85)			Ref					
Age	≥3	339	20(5.9)	3.68	13.87	5.141	0.113				
	<3	83	1(1.2)			Ref					
Body condition	Good	68	7(10.29)	1.011	12.719	3.586	0.048				
	Medium	225	10(4.44)	0.446	4.732	1.453	0.535				
	Poor	129	4(3.1)			Ref					
Tick Infestation	Yes	223	19(8.5)	2.109	9.905	9.174	0.003	5.51	21.48	11.786	0.018
	No	199	2(1)			Ref				Ref.	
Management system	Extensive	281	20(7.11)	1.425	20.77	10.728	0.021				
	Intensive	141	1(0.71)			Ref					
Total		422	21(4.97%)	3.10	7.50						
Status of pregnancy	Non-pregnant	169	12(7.1)	4.441	12.533	1.414	0.560				
	Pregnant	78	4(5.12)			Ref					
Parity	>3	56	5(8.9)	0.371	10.898	2.01	0.418				
	≤3	148	9(6.08)	3.276	6.388	1.327	0.724				
	Heifer	43	2(4.6)			Ref					
History of abortion	Yes	14	1(7.14)	0.137	9.131	1.118	0.917				
	No	233	15(6.43)			Ref					
Total		247	16(6.47)	3.74	10.30						

Note: OR = Odds ratio, CI = Confidence Interval, Ref = Reference.

Table 3: Univariable and multivariable logistic regression analysis of risk factors associated with seropositivity with ***C. burnetii*** at herd level

Risk factors	Categories	Number tested animals	Prevalence (%)	Univariable logistic regression				Multivariable logistic regression			
				95% CI		OR	P value	95% CI		OR	P value
				Lower	Higher			Lower	Higher		
Multiage mix	Yes	72	12(16.67)	14.64	24.12	2.15	0.21				
	No	47	4(8.5)			Ref					
Contact with other herd	Yes	80	14(17.5)	5.84	18.21	3.92	0.081				
	No	39	2(5.13)			Ref					
Multispecies mix	Yes	59	14(23.73)	17.95	31.74	9.02	0.005	17.95	31.74	9.022	0.005
	No	60	2(3.33)			Ref				Ref	
Herd size	Medium (≥10)	44	7(15.9)	10.47	19.03	1.38	0.547				
	Small (≤9)	75	9(12)			Ref					
	Total	119	16(13.4)	7.88	20.91						

Note: OR = Odds ratio, CI = Confidence Interval, Ref = Reference.

prevalence was recorded in Denmark (59%), Switzerland 29.6% (Fretz *et al*., 2007), North Ireland 48.4% (Ruiz-Fons, 2010) was reported. In contrast, lower prevalence was reported by Rahimi *et al*. (2009) 6.2% and Edalati *et al*. (2015) 10% in Iran.

High seroprevalence was reported in Shei Bench district (9.8%) as compared to Semen Bench (3.6%) and Mizan-aman town (1.9%). This finding was similar with previous report in Turkey (Seyitoglu *et al*., 2006) Nigeria (Elelu *et al*., 2020) showed variation of seroprevalence between study areas without significantly association. This finding was in contrast with the finding of (Rahman *et al.,* 2016) in Bangladesh and (Dean *et al*., 2013) in Egypt where study area was a significantly correlated with *C. Burnetii* seropositivity. The reason for the present finding might be due to similarity of farm management system in study districts.

Cattle management system risk factor was insignificant in this study but high prevalence (7.11%) was showed in extensive cattle management system than (0.71%) in intensive system. This finding in agreement with other studies showed in dairy cattle where grazing in the field had higher seropositivity (Clark and Magalhaes, 2018; Deressa *et al*., 2020; Dhaka *et al*., 2020). Similarly this study was aligned with previous report and management system did not have a significant association with *Coxiella burnetii* seropositivity in sampled cattle (Adesiyun *et al*., 1984). Similarly the finding of (Wardrop *et al*., 2016) in Nigeria revealed that there was no statistically significant relation between the management system and *Coxiella burneti i*nfection however the rate of detection of *Coxiella burnetii* antibodies was higher in cattle intensive farms (66.7%) than semi intensive system (55.6%). This reason for high prevalence of seropositivity in cattle kept in extensive production system might be due to the probability of exposure for the disease and ticks are higher than intensive farms.

Indigenous cattle were 4.804 times more likely to be seropositive compared to cross breed cattle ($P<0.05$). The present findings concomitant with the previous study (Deressa *et al.,* 2020) in which cattle breed was significant risk factor with the prevalence of 14.29% in local breed as compared to cross breed cattle (5.9%). Similarly in Nigeria the prevalence of 17.1% and 1.3% in local breed and cross breed respectively and significantly associated (Wardrop *et al*., 2016). In contrast to this finding seropositivity to *C. burnetii* among cattle breed was not statistically significant (Ruiz-Fons, 2010; Paris *et al.,* 2006). This finding might be due to the probability of indigenous cattle kept in extensive management system exposure to tick and wildlife is higher than exotic breed.

The present finding revealed that cattle with tick infestation had significantly associated with the probability of *C. burnetii* seropositivity ($P<0.018$; OR = 11.786). This present study result was similar with previous study (Deressa *et al*., 2020) and revealed that *C. burnetii* antibody was found to be significantly associated and higher in tick infested cattle. This result was also in line with the finding of (Tukur *et al.,* 2014) and prevalence rate was higher in herds with no ectoparasite control than in herds with ectoparasite control. Also the finding was parallel with evidence around the world and the isolation of *C. burnetii* from ticks and animal infested with tick were more seropositive than non-infested cattle (Larson *et al*., 2019).In contrast to this finding, tick infested cattle were not significantly associated with *C. burnetii* seropositivity (Elelu *et al.,* 2020). Based on the present finding tick might have a role in the dissemination of query fever in the herd.

In this finding the seropositivity with *C. burnetii* in the female animals were higher than male with ($P<0.05$). This result was in line with previous finding of (Kanoute et al., 2016; Deressa *et al.*, 2020). In contrast to this finding sex considered as a significant association and female animal were more prone than male (Lee *et al.,* 2019).

In this study findings age factor was not significant association for *C. burnetii* seropositivity. This finding similar with the previous study (Kanoute *et al*., 2016; Grumi *et al*., 2013). In contrast to this finding, the probability of *C. burnetii* exposure increases with increasing age (Deressa *et al*., 2020); in Cameroon. In contrast to this finding the older animal the greater the risk of exposure to the pathogen and keep circulating antibody (Elelu *et al.,* 2020).

The multivariable logistic regression analysis revealed that practicing multispecies mix was a significant risk factor for *C. burnetii* seropositivity. This finding was in contrast with previous report (Tukur *et al*., 2014; Wardrop *et al*., 2016) stated that multispecies mix were not-significant risk factor with *C. burnetii* seropositivity. The present finding might be due to sheep and goats were a reservoir

of Query fever and important role for the transmission of the disease to cattle.

Teaching the livestock owners aboutgood animal husbandry practices and how to prevent the occurrence Query fever disease. Tick control option should be applied for cattle and other domestic animal species to decrease further dissemination of Query fever. Further national wide Query fever disease research should be conducted in regards to different animal production systems, geographical variation and species to determine the magnitude and distribution of the disease.

CONCLUSION

The present study revealed that there was sero-positivity of *C. burnetii* in the areas. The finding of positive serological reactors indicates the presence of foci of infection that could serve as source of infection for the spread of the disease into unaffected animals and herds. The risk factors such as cattle breed, tick infestation and multispecies mix were found to be significantly associated with *C. burnetii* seropositivity in this study. Further, study should be needed to investigate the antigen of circulating species of the pathogen.

ACKNOWLEDGEMENTS

The authors would like to thank Mizan Regional Veterinary Laboratory Center and Jimma University College of Agriculture and Veterinary Medicine for allocating the resources to conduct this research work. Further, we acknowledge district farmers for their participation during data and sample collection.

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