Received: 14 September 2016
DOI: 10.1111/tbed.12622

ORIGINAL ARTICLE

WILEY Transboundary and Emerging Diseases

Farm-level risk factors associated with bovine tuberculosis in the dairy sector in Eritrea

M. K. Ghebremariam[1,2] | A. L. Michel[3] | M. Nielen[4] | J. C. M. Vernooij[4] | V. P. M. G. Rutten[1,3]

[1]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
[2]Department of Veterinary Sciences, Hamelmalo Agricultural College, Keren, Eritrea
[3]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[4]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Correspondence
M. K. Ghebremariam, Faculty of Veterinary Medicine, Department Infectious Diseases and Immunology, Utrecht University, Utrecht, The Netherlands.
Email: michaelvet@yahoo.com

Funding information
Netherlands Organization for International Cooperation in Higher Education.

Summary
The aim of our study was to determine the association of selected potential risk factors with the presence of bovine tuberculosis (BTB) in dairy herds in Eritrea. A case–control study was conducted in the three major milk-producing regions of the country by stratified random sampling of 61 case and 65 control herds combined with completion of a standardized pretested questionnaire pertaining 36 relevant risk factors (variables). The variables were divided into two clusters, based on potential association with either "introduction" or "establishment" of BTB on the farms to elucidate association with incident or prevalent cases separately. Subsequent to univariable analysis of the 36 risk factors at herd level, 14 of these were offered to multivariable logistic regression models. Farms with higher numbers of cows, and those with concrete floors, were 3.6, and 7.5 times more at risk for presence of BTB, respectively, compared with their references. These findings will be useful as entry points for future informed decision-making towards BTB control and eradication programme in the country.

KEYWORDS
bovine tuberculosis, case–control study, risk factors, dairy, Eritrea

1 | INTRODUCTION

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* (OIE, 2009) is a debilitating, infectious and contagious disease that affects many animal species and occasionally humans. Animal husbandry and management systems contribute to the development and dissemination of BTB. The "intensive husbandry system" has shown to be a predisposing factor to BTB in animals (Griffin et al., 1993). As the principal route of transmission of *M. bovis* is via aerosols (OIE, 2009), close contact between animals enhances the transmission of the disease (Neill, O'Brien, & Hanna, 1991). Apart from animal-to-animal transmission as a cause of infection, humans with open tuberculosis due to *Mycobacterium tuberculosis* or *M. bovis* may also be sources of infection to animals (Radostits, Gay, Hinchcliff, & Constable, 2007).

In Eritrea, BTB is one of the most important zoonotic diseases already reported by Pirani (1929), as cited by Omer, Skjerve, Woldehiwet, and Holstad (2001). Recently, Ghebremariam et al. (2016) reported 17.3% herd prevalence of bovine tuberculosis in the dairy cattle in the country. The goal of the current study was to investigate the association of potential farm-level risk factors with this herd prevalence.

2 | MATERIAL AND METHODS

2.1 | Study area and selection criteria

To define farm-level risk factors associated with positive results in the "single intradermal comparative tuberculin test (SICTT reactors)," as indicator of *M. bovis* infection, we conducted a case–control study with a 1:1 ratio. A herd was considered a "case herd" when it included at least one SICTT reactor (positive) animal during the 2011 BTB prevalence survey in the dairy sector in Eritrea (Ghebremariam et al., 2016). In the previous prevalence study (Ghebremariam et al., 2016), all herds (3,149) and all individual animals (15,354) above 6 weeks of age, from the three selected regions (Maekel, Debub and Anseba), were tested using the SICTT according to OIE. A herd was considered a "control herd" when none of the animals reacted to the test. Herds with (n = 545) and without SICTT (n = 2,604) reactors were identified in each of the three major milk-producing regions (Maekel, Debub and Anseba; Figure 1). Relative to the number of reactor herds in Maekel and Debub, the largest proportions of herds, n = 56 and n = 65, respectively, were selected from these two regions and only few (n = 5) from Anseba. Prior to the selection, all the herds in the three regions were stratified according to their herd sizes, categories being 9–20, 21–30, 31–40 and >40 animals. Only farms with a herd size of ≥9 head of cattle were selected. Numbers of herds, representative for the number of herds in each stratum, were randomly drawn using a computer-generated random table (Epi Calc 2000 version 0.1), resulting in inclusion of 61 case and 65 control herds.

To obtain information on the potential risk factors associated with the reactivity to SICTT in a standardized fashion, a pre-tested questionnaire was used. It included 36 relevant variables that captured the farmers' and farms' characteristics. The variables were divided into two clusters, based on potential association with either "introduction" (entry to the farms from an external source) or "establishment" (persistence on the farm) of BTB. The selection process was based on epidemiological importance of the factors as described by Griffin, Martin, Thorburn, Eves, and Hammond (1996). All 36 factors selected for the study and their categories are shown in Table S1.

2.2 | Data analysis

Data were first entered in Excel and then exported to SPSS IBM version 20 software and analysed.

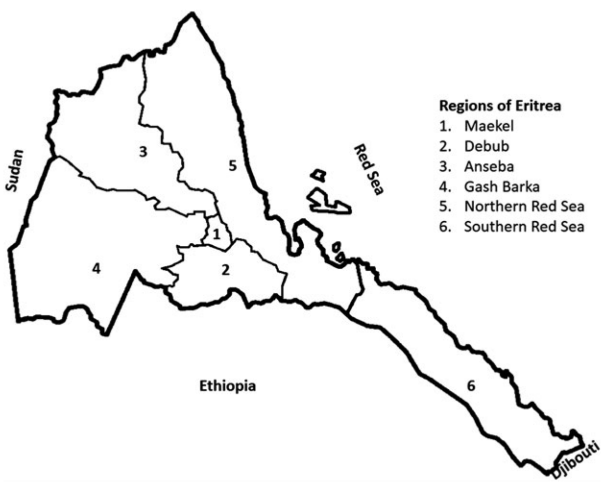

FIGURE 1 Study area map: Maekel (1), Debub (2) and Anseba (3), the three major milk-producing regions of Eritrea. Adopted from Ghebremariam et al. (2016)

Associations between the farm-level risk factors (variables) and herds with reactor(s) were tested in two stages: first, all the variables (n = 36; Table 1) were screened using Fisher's exact test in univariable analyses and those variables with p-value <.25 (n = 14) (Table 2) were tested for collinearity. The variable "reproduction method" could not provide any interpretable differences between case and control herds (Table 1). Due to the lack of artificial insemination (AI)-only reproduction methods in control herds, Firth's correction was applied to allow for estimation of the odds ratio (OR) (Table 2), but the variable ("reproduction method") was excluded from further analysis as it did not give interpretable result in the multivariable analysis. Hence, 13 variables remained to be offered to either or both of the multiple logistic regression models, for "introduction" and "establishment" of BTB (presence of SICTT reactors) on the farms (Table 3). During the multivariable logistic regression model building, using manual (Enter) and automated (Backward) procedures for the possible risk factors screened, respectively, potential confounders were identified by the changes in the coefficients (β) (i.e., if inclusion of a variable to the baseline model altered the coefficient of the model by >10%, one of the variables, depending on its importance, was retained/dropped), and p-values <.05, to decide for definite inclusion or exclusion of the variables in the models.

Two variables, "age of respondents" and "major activity," representing farmers' characteristics for "establishment" of BTB in the farms were highly related to case–control status. However, they were not used in the analysis as they overshadowed the potential influence of more biological factors. Besides, two variables ("number of bulls" and "availability of bulls") that showed high correlation with "number of cows" and "herd size" were excluded from the models, as the latter two were considered more plausible risk factors for "introduction" and "establishment" of BTB, respectively. The herd size, number of cows and number of heifers were arbitrarily categorized as shown in Table 2. The model for introduction of BTB included four variables ("number of cows," "species of animals sharing water point," "sharing of water point" and "same source of bull purchase") and that for establishment of BTB six variables ("herd size," "number of heifers," "housing type," "housing system," "building status" and "feeding system"). Finally, two variables ("number of cows" and "building status" from both "introduction" and "establishment") retained in the final model (Model 3; Table 3) as potential risk factors for the presence of BTB reactors in the farms.

3 | RESULTS

3.1 | Univariable analysis

A summary of the results of the univariable analysis is shown in Table 2.

The variables "age of respondent" and "major activity" were selected to demonstrate the role of farmers' characteristics in relation to SICTT reactivity between the case and control farms. "Age of respondents" coded in three categories (25–45 years, 46–65 years and >65 years) was significantly associated (p = .036) with the presence of SICTT reactor

TABLE 1 Names, descriptions and coding of the 36 variables and their categories included in the study as potential risk factors for "introduction" (Intro) and "establishment" (Est) of BTB in the 61 case and 65 control farms in Eritrea

Variables	Description	Coding of the variables
Age respondent (Est)	Age of the farmer in years	25–45 years, 46–65 years and >65 years
Educational level (Both)	Farmers' educational level	Low (can/cannot read and write), medium (1–8 grade education), high (secondary/tertiary education)
Major activity (Est)	Major activity (occupation) of the farmer	Full-time dairy, part-time dairy
Breed (Est)	Breed of cattle available in the farm	Holstein Friesians (HF), others (HF crosses, local, Sudanese breeds)
Herd size (Est)	Total number of cattle in the farm (cows, heifers, bulls, etc.)	Small herds (9–26), large herds (>26)
Number of cows (Intro)	Number of matured ≥ 3 years old cattle that has calved	Small size (0–10), large size (>10)
Number of heifers (Est)	Number of matured (≥3 years old) female cattle that have not calved	Small size (0–8), large size (>8)
Availability of bulls(Both)	Availability of matured (≥2 years old) male animals at farm for natural breeding	Yes/No
Number of bulls (Intro)	Number of bulls available at farm	No bulls, one bull, ≥2 bulls
Stock density (Est)	Number of animals per given spaces	Number of cattle per square metre
Labour source (Est)	Source of work force used in the farm	Family, hired, both
Distance from other dairy farms	Distance of the dairy farms from other dairy farms in the vicinity	In metres (m)
Intro. of new animals (Intro)	Introduction of new animals in to the existing herd	Yes/No
Cattle purchase frequency (Intro)	Number of times the farm purchases cattle	No purchase, every 1–2 years, every 3 and more years
Reproduction method (Intro)	Method of reproduction used for breeding of the cattle	Artificial insemination (AI), natural method using bulls, both
Source of bulls for mating (Intro)	The sources or origins of bull(s) used for breeding in the farm	Not applicable, home-grown, not home-grown
Bull purchase frequency (Intro)	The number of times a farm purchases bull(s)	No purchase, every 1–2 years, every 3 and more years
Same source of bull purchase? (Intro)	Is the farm purchasing bull(s) from the same source(s)?	Not applicable (no purchase), yes, no
Other animals present at the farm (Intro)	Other species of animals available in the farm in addition to cattle	Not available, sheep and goats, equids, dogs and chicken, multispecies
Cattle contact with (Intro)	With which species of animals do your animals get contact	No contact, one species, multispecies
Wild animals present (Intro)	Wild animals noticed in the farm or around the farm premises	No wildlife, one species, multispecies
Source of water (Intro)	Source of drinking water for the animals in the farms	Water at farm, water outside farm, both
Sharing of water point (Intro)	Sharing of water points with animals from other farms	Yes/No
Species of animals sharing water point (Intro)	The species of animal from other farms sharing the same water point	Not sharing, one species, multispecies,
Signs of respiratory diseases?(Est)	Any clinical signs observed in relation to respiratory diseases	Yes/No
Clinical signs observed (Est)	Signs of respiratory diseases manifested by any animal(s) in the farm that were observed by the farmer	Not applicable, difficult breathing and anorexia, anorexia, listlessness and emaciation
Housing type (Est)	Dairy house types in which the animals are either kept tied (stanchioned) in the house/shade or kept at a liberty to move freely	Stanchion(tied) system, other
Housing system (Est)	System of housing that either keeps the animals all the time inside, or allows the animals to stay both inside and outside, or outside all the time	Housed indoors all the time, not all the time housed indoors

(Continues)

TABLE 1 (Continued)

Variables	Description	Coding of the variables
Building status (Est)	The floor types in the dairy houses, whether the dairy farms have concrete floors or other types of floor (e.g., sand)	With concrete floors, without concrete floors
Ventilation status (Est)	The status of the houses in terms of air supply and removal by natural means through windows and doors	Good, Bad
Frequency of cleaning (Est)	The number of times the dairy houses are cleaned	Once every day, Twice a day, Once a month
Feeding of green feeds (Est)	Provision of green feed to the animals either by grazing, by cut and carry or purchase.	Yes/No
Source of green feed(Est)	Where does the green feed come from: own farm or purchase from other farms?	Not applicable, home-grown, purchased, both
Feeding system (Intro)	The system of feeding that allows grazing of animals or that does not allow grazing but rather depends on cut and carry (bringing the green fodder to the barn).	Zero-grazing, grazing
Concentrate feeding (Est)	The provision of the animals with concentrate (formulated) feed that fulfils the requirement of dairy cattle for milk production	Yes/No, not disclosed
Mineral supplement (Est)	Provision of mineral supplements to the animals in the form of mineral licks	Yes/No

animal(s) on the farms. Farms owned by the 46- to 65-year age group (Table 2) were the most affected by BTB (OR = 3.5), followed by farms owned by those aged >65 years (OR = 1.9) when compared with farms managed by younger farmers (25–45 years).

"Major activity" of the farmers grouped into two categories (full-time dairy and part-time dairy farming) was significantly associated (p = .004) with the presence of SICTT reactor animal(s). Farms owned by full-time dairy farmers were more at risk (OR = 3.3) as compared with those owned by part-time dairy farmers.

The remaining thirteen variables described farm characteristics as risk factors for the presence of one or more SICTT reactors on the farms.

One of the potential risk factors, "number of cows" on the farms, screened in the univariable analysis, was significantly (p = .001) associated with the presence of SICTT reactors. Farms with larger (>10) numbers of cows were more at risk (OR = 4.0) compared to those with smaller numbers of cows. The mean number of cows in the dairy herds included in the study was 10.2 (range: 0–120), whereas the mean numbers of cows in the cases and controls were 13.52 (range: 0–120) and 7.09 (range: 2–20), respectively.

"Herd size" was also significantly (p = .002) associated with the presence of SICTT reactors and was coded in two categories (Table 3). The risk increased with herd sizes >26 animals (OR = 5.0). The mean herd size for cases was 27 (range: 9–180) as compared to 16 (range: 9–60) for controls.

The number of heifers was significantly associated (p = .018) with the presence of SICTT reactors in dairy farms and herds with larger (>8) numbers of heifers had a higher risk (OR = 4.1) than herds with fewer heifers. The mean number of heifers in case farms was 6.8 (range: 0–40) as compared to 4.1 (range: 0–20) in controls.

Finally, the "availability of bulls" (categories: yes/no) and "number of bulls" were associated with the presence of SICTT reactors (p-values = .199, and .077, respectively). Farms with bull(s) were more at risk (OR = 1.2) compared with farms without bulls, and those with two or more bulls were most at risk (OR = 2.8), followed by farms with one bull (OR = 1.2). The mean number of bulls in case farms was 1.2 (range: 0–4) as compared with 0.8 (range: 0–3) in control farms.

In addition to the aforementioned risk factors, related to numbers of animals within a herd, management factors associated with indoor/outdoor keeping of animals were assessed. "Species of animals sharing water point" was associated (p = .10) with the presence of reactor animals in the farms, and herds sharing with one species of animals (OR = 0.2) and those sharing with multispecies (OR = 0.8) were protective. Besides, "sharing a water point" (categories: yes/no) was associated (p = .116) with having SICTT reactor animal(s) in the farms and was a protective (OR = 0.5) factor.

The use of stanchions on the farm was identified as a risk factor (OR = 1.8) and found to be associated (p = .135) with being a case herd.

The variable "housing system" was grouped into two categories: farms that kept their animals indoor permanently, compared with those that did not do so. Farms that kept their cattle entirely indoor were at higher risk (OR = 1.8) of having SICTT reactor(s) as compared with farms that allowed animals outdoors as well.

The "building status" was grouped into two categories: dairy farms with concrete floors and without concrete floors. This variable was significantly (p = .033) associated with the presence of SICTT reactor animal(s) in the farms, and those that housed their animals on concrete floors were more (OR = 8.4) at risk.

The variable "feeding system" was categorized into two groups (zero-grazing and grazing) and was significantly associated (p = .061) with the presence of SICTT reactor(s) on the farms. Farms using zero-grazing were at higher (OR = 4.1) risk than the others.

TABLE 2 Variables (risk factors) screened by univariable analysis with *p*-values <0.25, their distribution and *p*-values of Fisher's exact test, odds ratios (OR) and 95% confidence intervals for 61 case and 65 control herds as determined by SICTT reactivity in the dairy sector of Eritrea. NI, not interpretable due to empty cells; Ref, reference

Variables	Categories	Case (*n* = 61) % (*n*)	Control (*n* = 65) % (*n*)	OR	95% CI	*p*-Value
"BTB introduction"						
Number of cows	0–10	60.7 (37)	86.2 (56)	1.0	Ref	.001
	>10	39.3 (24)	13.8 (9)	4.0	1.7–9.7	
Reproduction method	Natural and both	91.1 (56.5)	99.2 (65.5)	0.08	0.004–1.5	.024
	AI	8.9 (5.5)	0.8 (0.5)	1.0	Ref	
Number of bulls	No bulls	31.1 (19)	43.1 (28)	1.0	Ref	.077
	One bull	34.4 (21)	40 (26)	1.2	0.5–2.7	
	≥2 bulls	34.4 (21)	16.9 (11)	2.8	1.1–7.2	
Species of animals sharing water point	No sharing	86.9 (53)	75.4 (49)	1.0	Ref	.10
	One species	3.3 (2)	13.8 (9)	0.2	0.04–1.0	
	Multispecies	9.8 (6)	10.8 (7)	0.8	0.2–2.5	
Sharing water point	Yes	13.1 (8)	24.6 (16)	0.5	0.2–1.2	.116
	No	86.9 (52)	75.4 (49)	1.0		
Same source of bull purchase	Not applicable	70.5 (43)	78.5 (51)	0.3	0.1–1.1	.167
	Yes	14.8 (9)	4.6 (3)	1.0	Ref	
	No	14.8 (9)	16.5 (14)	0.3	0.1–1.3	
Availability of bulls	No	31.1 (19)	43.1 (28)	1.0	Ref	.199
	Yes	68.9 (42)	56.9 (37)	1.7	0.8–3.5	
"BTB establishment"						
Age respondent	25–45 years old	11.5 (7)	27.7 (18)	1.0	Ref.	.036
	46–65 years old	68.9 (42)	47.7 (31)	3.5	1.3–9.4	
	>65 years old	19.7 (12)	24.6 (16)	1.9	0.6–6.1	
Major activity	Full-time	80.3 (49)	55.4 (36)	3.3	1.5–7.3	
	Part-time	19.7 (12)	44.6 (29)	1.0	Ref	
Herd size	9–26	70.5 (43)	92.3 (60)	1.0	Ref	.002
	>26	29.5 (18)	7.5 (5)	5.0	1.7–14.6	
Number of heifers	0–8	78.7 (48)	93.8 (61)	1.0	Ref	.018
	>8	21.3 (13)	6.2 (4)	4.0	1.5–13.5	
Housing type	Stanchion system	72.1 (44)	58.5 (38)	1.8	0.9–3.9	.135
	Others	27.9 (17)	41.5 (27)	1.0	Ref	
Housing system	Inside the house all time	55.7 (34)	41.5 (27)	1.8	0.9–3.6	.153
	Not all the time inside the house	44.3 (27)	58.5 (38)	1.0	Ref	
Building status	With concrete floors	98.3 (58)	87.3 (55)	8.4	1.0–69.7	.033
	Without concrete floors	1.7 (1)	13.6 (8)	1.0	Ref	
Feeding system	Zero-grazing	96.7 (59)	87.7 (57)	4.1	0.8–20.3	.061
	Grazing	3.3 (2)	12.3 (8)	1.0	Ref	
Availability of bulls	No	31.1 (19)	43.1 (28)	1.0	Ref	
	Yes	68.9 (42)	56.9 (37)	1.7	0.8–3.5	.199

Finally, the univariable analysis has shown that reproduction method (AI versus AI and natural breeding) was significantly (p = .024) linked to the presence of SICTT reactor(s) in the farms. Farms using both "AI and natural breeding" had (OR = 0.08) a protective effect (Table 2).

3.2 | Multivariable analysis

From the three potential risk factors related to "introduction" of BTB on the farm that were offered to the first multivariable model (Model 1, Table 3) to analyse the occurrence of SICTT reactivity in

TABLE 3 Final logistic regression models of the risk factors associated with herds having SICTT reactor(s) in the study areas within the dairy sector of Eritrea, as they are grouped into factors for "introduction" (Model 1) and "establishment" (Model 2) of BTB with the final model (Model 3) for the presence of SICTT reactor(s) on the farms

Variables	*p*-Values	OR	95% CI Lower	95% CI Upper
Results of multivariable logistic regression for model 1				
"Number of cows"				
0–10 (reference)		1.0		
>10	.002	4.0	1.7	9.7
Results of multivariable logistic regression for model 2				
"Herd size"				
9–26 (reference)		1.0		
>26	.006	4.8	1.6	14.4
"Building status"				
Without concrete floors (reference)		1.0		
With concrete floors	.052	8.6	1.0	75.7
Results of multivariable logistic regression for model 3, based on variables from model 1 + 2				
Number of cows				
0–10 (reference)		1.0		
>10	.005	3.6	1.5	8.9
Building status				
Without concrete floors (reference)		1.0		
With concrete floors	.066	7.5	0.9	64.1

the dairy farms, the final model retained only the "number of cows" in the farms. Farms with larger numbers of cows (>10) were more at risk (OR = 4.0; 95% CI: 1.7–9.7) than farms with ten cows or less.

Likewise, from the six variables that were offered to the second model (Model 2, Table 3) as possible risk factors for "establishment" of BTB in the farms, two variables, namely "herd size" and "building status," were retained in the model. Larger herd sizes (>26) were associated with a higher risk (OR = 4.8; 95% CI: 1.6–14.4) compared with smaller herd sizes (9–26).

Herds kept on concrete floors were more at risk (OR = 8.6; 95% CI: 1.0–75.7) to have SICTT reactors when compared with those kept on other types of floors (Table 3). This variable ("building status") had a borderline significance ($p = .052$), but was retained in the model given its biological importance as a risk factor. In the final multivariable model (Model 3; Table 2), only two variables "number of cows" from the "introduction model" and "building status" from the "establishment model" were retained.

DISCUSSION

To the best of our knowledge, this is the first study in Eritrea that has systematically identified farm-level risk factors for the presence of SICTT reactor(s) in the three major milk-producing regions. Both univariable (Table 2) and multivariable logistic regression (Table 3) analyses for potential risk factors related to presence of SICTT reactor(s) on the farms have been performed. Overall, the major risks seemed to be associated either with numbers of cattle on the farms ("number of cows," "herd size," "number of heifers," "number of bulls" and "availability of bulls"), or with whether or not the animals were kept outdoors for parts of the day, for example, for grazing and/or access to and sharing of water.

"Major activity" of the farmers and "age of respondents" were the two farmers' characteristics found significant risk factors for "establishment" of BTB in the farms as identified by the univariable analysis. The majority of farmers, above 45 years old, were full-time dairy farmers. Both "age of respondents" and "major activity" were independently associated with the SICTT reactivity in the herds. The lower risk associated with farms owned by the younger age groups may be explained by younger age groups engaging more actively with and seeking advice from animal health experts and veterinary professionals if their dairy cattle showed reduced productivity or ill health. Surprisingly, farms owned by full-time dairy farmers were three times more at risk as compared to part-time dairy farmers.

"Number of cows" was identified as a risk factor for the "introduction" of BTB to the case farms (Model 1, Table 2) and was retained in the final Model 3 (Table 3) as potential risk factor for the presence of BTB reactor(s). Farms with larger (>10) numbers of cows being approx. four times more at risk than those with lower numbers of cows. This might be attributed to the inclination of farmers in Eritrea to purchase pregnant cows to cope with the high demand for milk in the market. The more pregnant cows are purchased the higher the risk of introducing a *M. bovis*-infected cow. Purchase of adult pregnant cows also implies larger numbers of older animals on the farm. The longer an animal stays in a case herd, the higher will be the cumulative increase in the chances of being infected (Cleaveland et al., 2007; Humblet, Boschiroli, & Saegerman, 2009; Proaño-Perez et al., 2009).

Availability and number of bulls were significant risk factors in the univariable analyses for "introduction" of BTB in the case farms which might have been attributed to the discontinuation of artificial insemination and thus the use of bulls for reproduction by natural mating in the study area. Sharing of bulls is a common practice and those having more bulls are inclined to share them more frequently. The observed higher SICTT reactivity in those herds having bulls could be due to frequent and direct close contact between the bulls and animals in several herds (Skuce, Allen, McDowell, & Branch, 2011). Although farms not owning bulls also use bulls from other farms to breed their animals, they experience a shorter contact time and hence a lower risk of transmission from potentially infected bulls.

"Sharing of water points" and "species of animals sharing water point" were associated with a decreased risk of BTB "introduction" in the farms. The water points used in our study areas were surface waters (rivers and dams) that were indicated not to be significant risk factors for BTB transmission in cattle (Griffin et al., 1993), and in buffaloes under free ranging condition (Michel, De Klerk, van Pittius, Warren, & Van Helden, 2007). During the dry season, in our study area, farmers used their own mobile watering troughs to water their animals from water holes manually dug out from river beds. In addition, "sharing of water points" entails walking the animals to water points that may result in minimizing, within herd, animal-to-animal contacts, due to lower density of cattle on pasture, besides providing them with an opportunity of, beneficial, physical exercise "en route".

"Herd size" was identified as one of the major risk factors for the "establishment" of BTB in the case farms (Model 2). Farmers try to increase herd sizes for increased efficiency gains, but increase in herd size may lead to overcrowding causing enhanced cattle-to-cattle transmission of *M. bovis.* This finding is in agreement with those of similar studies that indicated the association of herd size with the prevalence of BTB (More & Good, 2015; Pavlik, Matlova, Dvorska, Shitaye, & Parmova, 2005; Proaño-Perez et al., 2009; Wright et al., 2015), including that of Omer et al. (2001) who identified herd size as one of the major risk factors for SICTT reactivity in the Maekel region, one of our current study areas in Eritrea. As shown by the univariable analyses, farms with larger numbers (>8) of heifers were four times more at risk to have SICTT reactors than farms with fewer heifers (Menzies & Neill, 2000; Neill, Pollock, Bryson, & Hanna, 1994) in relation to "establishment" of BTB in the farms. Increased animal-to-animal contact, normal behaviour during oestrous, might explain this finding (Hurnik, King, & Robertson, 1975).

The other major risk factor for the "establishment" of BTB in our study was "building status" (floor types) (Model 2), which was the second most important risk factor retained in the final Model 3 (Table 2). Those farms using concrete floors were about eight times more at risk when compared to those farms without concrete floors. Most of the dairy farms in the urban and peri-urban areas in Eritrea house their animals on concrete floors. Due to lack of maintenance such floors are indicated as culprits for causing digital dermatitis which was shown as one of the major constraints in the dairy farms in and around the capital by Nsahlail and Moges (2007). Eventually, due to the tendency of affected animals to lie down for prolonged times; their feed intake time may be compromised, causing emaciation and enhanced susceptibility to disease, including BTB (Cook & Nordlund, 2009; De Vries et al., 2015; Nordlund, Cook, & Oetzel, 2004).

"Feeding system" was one of the risk factors for "establishment" of BTB in the farms identified by the univariable analysis, where farms with a zero-grazing system were four times more at risk than farms that do not make use of this practice. Similarly, farms that kept their cattle permanently indoor encountered double the risk for "establishment" of BTB in the farms compared with those letting animals go outdoors. The plausible reasons for this, as already indicated by other studies (Ameni et al., 2006; Ayele, Neill, Zinsstag, Weiss, & Pavlik, 2004; Griffin et al., 1993, 1996; Skuce, Allen, & McDowell, 2012; Skuce et al., 2011), may be that indoor housing enhances closer contact between cattle, and more likely transmission of respiratory disease (Skuce et al., 2011). For infection to occur in cattle via inhalation, it requires as little as one bacillus as compared to ingestion that requires quite a large number of *M. bovis* (10^7) (Humblet et al., 2009). On the other hand, being outdoors, the animals literally reduce the contact among themselves and thus with the pathogenic agent (*M. bovis*). Besides, allowing animals to graze is directly connected with the animals' health and welfare. Relatively intense solar radiation of animals (e.g., hair, skin) (Kazda, 2010) and their excrements might play a bactericidal role and thus reduce mycobacterial burden (Fine, Bolin, Gardiner, & Kaneene, 2011).

"Housing type" was also one of the significant risk factors for "establishment" of BTB in the farms identified by the univariable analyses, where dairy farms with stanchion (tied up) were two times more at risk as compared with farms having other types of housing. Such housing might facilitate transmission of *M. bovis* from affected animals to the susceptible ones as they are tied up closely together in dairy barns. To make the situation worse, windows were very small or absent in most of the dairy barns, especially in the Maekel region.

In our attempt to assess a relatively large number of risk factors, it appeared impossible to rule out all potential biases, unobserved confounders and lack of independence between some of the factors. For that reason, we retained only those factors that were most strongly associated with the risk of having SICTT reactor(s) in a case farm. Although, currently, SICTT is the most widely applied screening test for BTB in live cattle (De la Rua-Domenech et al., 2006, OIE, 2009), it is imperfect but performs better than other ante mortem tests currently available for the detection of BTB.

Although the advantages of grazing on pasture are apparent in relation to hoof health, exercise and the access for more space that might minimize animal-to-animal contact, switching from indoor housing to pasture is not a realistic option for many farms in the urban areas because of unavailability of land for pasture. In such herds, a regular test and control system related to animal movements might help to control BTB in the future.

Dairy farms that allowed their animals outdoors, that kept smaller numbers of cattle (fewer cows and smaller total herd size) or that did not have concrete floors were less at risk to be BTB positive as indicated by the SICTT. Introduction of changes in management according to these findings may lead to an improved BTB status of the dairy farms. The use of sand floors or other alternative beddings (straw or saw dust) could improve the current prevailing problems related to floor type and thus reduce the number of farms with SICTT-reactive animals. Purchasing replacement dairy cattle from farms with no SICTT reactors and testing for BTB before introduction of new cattle to farms might assist in controlling spread of BTB in the country.

ACKNOWLEDGEMENTS

We thank the Ministry of Agriculture (MOA), Animal Resources staff of Maekel, Debub and Anseba, regions, for their cooperation in the fieldwork. We thank Huruy Asghedom (Director General, MOA) for writing official letters to all the MOA offices in the six regions for cooperation, and Kahsay Negash (Head, Dairy Unit, MOA) for the timely delivery of the official information to the MOA offices in the regions and dairy associations, and for his cooperation in providing valuable information on the dairy production of the study areas. We thank Dr. Idris Ali (Head, Livestock Resources, Meakel region), Dr. Uqubazghi Kifle (Head, Livestock Resources, Debub region) and Dr. Teklemariam (Head, Animal Resources, Anseba region) for their coordination and proper guidance of their staff to cooperate during the field work and to provide the necessary information for the study. We thank Brhane Tekle (MOA, Maekel), Andebrhan and Samuel Abraha (MOA, Debub), Mulugheta (Maekel, subregion Galanefhi, MOA) Aster (Maekel, subregion Berik, MOA) for their assistance in data collection. We thank Redie, Yosief, Sebahtu and Bereket for their assistance in the data collection. We thank Hamelmalo Agricultural College for its cooperation in organizing the fieldworks through timely dispatch of official letters, and NUFFIC for funding the project.

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

Ameni, G., Aseffa, A., Engers, H., Young, D., Hewinson, G., & Vordermeier, M. (2006). Cattle husbandry in Ethiopia is a predominant factor affecting the pathology of bovine tuberculosis and gamma interferon responses to mycobacterial antigens. *Clinical and Vaccine Immunology*, *13*(9), 1030–1036.

Ayele, W. Y., Neill, S. D., Zinsstag, J., Weiss, M. G., & Pavlik, I. (2004). Bovine tuberculosis: An old disease but a new threat to Africa. *The International Journal of Tuberculosis and Lung Disease*, *8*(8), 924–937.

Cleaveland, S., Shaw, D. J., Mfinanga, S. G., Shirima, G., Kazwala, R. R., Eblate, E., & Sharp, M. (2007). *Mycobacterium bovis* in rural Tanzania: Risk factors for infection in human and cattle populations. *Tuberculosis (Edinb)*, *87*(1), 30–43.

Cook, N. B., & Nordlund, K. V. (2009). The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. *Veterinary Journal*, *179*(3), 360–369.

De la Rua-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006). Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*, *81*(2), 190–210.

De Vries, M., Bokkers, E. A. M., Van Reenen, C. G., Engel, B., Van Schaik, G., Dijkstra, T., & De Boer, I. J. M. (2015). Housing and management factors associated with indicators of dairy cattle welfare. *Preventive Veterinary Medicine*, *118*(1), 80–92.

Fine, A. E., Bolin, C. A., Gardiner, J. C., & Kaneene, J. B. (2011). A study of the persistence of *Mycobacterium bovis* in the environment under natural weather conditions in Michigan, USA. *Veterinary Medicine International*, *2011*, 765430. DOI: 10.4061/2011/76.

Ghebremariam, M. K., Rutten, V. G., Vernooij, J. C. M., Uqbazghi, K., Tesfaalem, T., Butsuamlak, T., … Michel, A. L. (2016). Prevalence and risk factors of bovine tuberculosis in dairy cattle in Eritrea. *BMC Veterinary Research*, *12*(1), 1.

Griffin, J. M., Hahesy, T., Lynch, K., Salman, M. D., McCarthy, J., & Hurley, T. (1993). The association of cattle husbandry practices, environmental factors and farmer characteristics with the occurrence of chronic bovine tuberculosis in dairy herds in the Republic of Ireland. *Preventive Veterinary Medicine*, *17*(3), 145–160.

Griffin, J. M., Martin, S. W., Thorburn, M. A., Eves, J. A., & Hammond, R. F. (1996). A case-control study on the association of selected risk factors with the occurrence of bovine tuberculosis in the Republic of Ireland. *Preventive Veterinary Medicine*, *27*(3), 217–229.

Humblet, M. F., Boschiroli, M. L., & Saegerman, C. (2009). Classification of worldwide bovine tuberculosis risk factors in cattle: A stratified approach. *Veterinary Research*, *40*(5), 1–24.

Hurnik, J. F., King, G. J., & Robertson, H. A. (1975). Estrous and related behaviour in postpartum Holstein cows. *Applied Animal Ethology*, *2*(1), 55–68.

Kazda, J. (2010). *The ecology of mycobacteria: Impact on animal's and human's health*. Dordrecht: Springer Science & Business Media.

Menzies, F. D., & Neill, S. D. (2000). Cattle-to-cattle transmission of bovine tuberculosis. *Veterinary Journal*, *160*(2), 92–106.

Michel, A. L., De Klerk, L. M., van Pittius, N. C. G., Warren, R. M., & Van Helden, P. D. (2007). Bovine tuberculosis in African buffaloes: Observations regarding Mycobacterium bovis shedding into water and exposure to environmental mycobacteria. *BMC Veterinary Research*, *3*(1), 1.

More, S. J., & Good, M. (2015). Understanding and managing bTB risk: Perspectives from Ireland. *Veterinary Microbiology*, *176*(3), 209–218.

Neill, S. D., O'Brien, J. J., & Hanna, J. (1991). A mathematical model for *Mycobacterium bovis* excretion from tuberculous cattle. *Veterinary Microbiology*, *28*(1), 103–109.

Neill, S. D., Pollock, J. M., Bryson, D. B., & Hanna, J. (1994). Pathogenesis of *Mycobacterium bovis* infection in cattle. *Veterinary Microbiology*, *40*(1–2), 41–52.

Nordlund, K. V., Cook, N. B., & Oetzel, G. R. (2004). Investigation strategies for laminitis problem herds. *Journal of Dairy Research*, *87*, E27–E35.

Nsahlail, I., & Moges, A. (2007). Dairy constraint analysis in Eritrea, with special emphasis on Asmara and surrounding dairy farms. In B. Ghebru & T. Mehari (Eds.), *Innovative agricultural approaches of promoting food security in Eritrea: Trends, challenges and opportunities for growth: Proceedings of the Workshop of the Association of Eritreans in Agricultural Sciences (AEAS)*. Bern, Switzerland: Geographica Bernensia. BORIS DOI: 10.7892/boris.71045 (Website visited: 31/08/2016).

OIE (2009). http://www.oie.int/fileadmin/Home/eng/Healthstandards/tahm/2.04.07BOVINETB.pdf.

Omer, M. K., Skjerve, E., Woldehiwet, Z., & Holstad, G. (2001). A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea. *Tropical Animal Health and Production*, *33*(4), 295–303.

Pavlik, I., Matlova, L., Dvorska, L., Shitaye, J. E., & Parmova, I. (2005). Mycobacterial infections in cattle and pigs caused by *Mycobacterium avium* complex members and atypical mycobacteria in the Czech Republic during 2000-2004. *Veterinarni Medicina-Praha*, *50*(7), 281.

Proaño-Perez, F., Benitez-Ortiz, W., Celi-Erazo, M., Ron-Garrido, L., Benitez-Capistros, R., Portaels, F., … Linden, A. (2009). Comparative intradermal tuberculin test in dairy cattle in the north of Ecuador and risk factors associated with bovine tuberculosis. *American Journal of Tropical Medicine and Hygiene*, *81*(6), 1103–1109.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W., & Constable, P. D. (2007). A textbook of the diseases of cattle, horses, sheep, pigs and goats. *Veterinarni Medicina*, *10*, 2045–2050.

Skuce, R. A., Allen, A. R., & McDowell, S. W. (2012). Herd-level risk factors for bovine tuberculosis: A literature review. *Veterinary Medicine International*, *2012*. DOI: 10.1155/2012/621210.

Skuce, R. A., Allen, A. R., McDowell, S. W., & Branch, B. (2011). *Bovine tuberculosis (TB): A review of cattle-to-cattle transmission, risk factors and susceptibility*. London, UK: Thai Agricultural Commodity and Food Standard to Thai Agricultural Standard in accordance with the enforcement of The Agricultural Standards, 2551.

Wright, D. M., Reid, N., Montgomery, W. I., Allen, A. R., Skuce, R. A., & Kao, R. R. (2015). Herd-level bovine tuberculosis risk factors: Assessing the role of low-level badger population disturbance. *Scientific Reports*, *5*, 13062. DOI: 10.1038/srep13062.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Ghebremariam MK, Michel AL, Nielen M, Vernooij JCM, Rutten VPMG. Farm-level risk factors associated with bovine tuberculosis in the dairy sector in Eritrea. *Transbound Emerg Dis*. 2018;65:105–113. https://doi.org/10.1111/tbed.12622