



Prevalence of Bovine Tuberculosis among Cattle and Buffaloes in the Central Province of Sri Lanka

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ABSTRACT

The Bovine tuberculosis (bTB) is a chronic disease condition in dairy cattle and a proven global zoonosis. This study was designed to identify the prevalence of bTB in dairy cattle and buffaloes in the Central Province (CP) of Sri Lanka. Single Intradermal Comparative Cervical Tuberculin (SICCT) test was performed in 20 farms (n=616 cattle and buffaloes) in three districts (NuwaraEliya; NE, Kandy; KN, and Matale; MT) in the CP. Out of the SICCT positive samples, randomly selected serum samples (n=33) of eight farms were subjected to the rapid antibody (Ab) test for further confirmation. Results were evaluated for different risk factors; age, sex, parity, body condition score (BCS), breed, herd origin, reproductive status, herd size, type of management, and duration of farm establishment. The prevalence of bTB among individual cattle and buffaloes was 22% with a 50% herd-level prevalence. In NE and KN, 34% and 19% of individuals showed positive reactions for SICCT, respectively, while all the individuals in MT were negative. There were significant statistical associations ($P<0.05$) were observed with the prevalence of bTB and BCS, breed, herd origin, and reproductive status; however, age, sex, parity, herd size, type of management, and duration of farm establishment were not statistically significant ($P>0.05$) with the prevalence of bTB. The conclusion is that, based on the SICCT test, the estimated prevalence of bTB in cattle and buffaloes in the central province of Sri Lanka is relatively high ($>20\%$). The SICCT test could be recommended for the screening of the bTB in cattle and buffaloes in all regions of Sri Lanka to assess the island-wide prevalence of bTB, as this disease carries the risk of transmitting to humans and other susceptible animal species.

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INTRODUCTION

The dairy industry plays a key role in livestock production in Sri Lanka. The government has taken steps to increase milk production by introducing improved exotic cattle such as Holstein Friesian, Jersey, and their crossbreds. However, exposure of these cattle herds to a new tropical environment has posed a threat of attracting animal diseases.

Bovine Tuberculosis (bTB) is a disease of infectious nature (Radostits *et al.*, 2007), and has the potential of causing public health issues (Belchior *et al.*, 2016). The disease is found in the cattle population in Sri Lanka as well as in African, Asian, and American countries (OIE, 2010). Bovine tuberculosis is a chronic disease of animals caused by *Mycobacterium bovis* (*M. bovis*) (Shitaye *et al.*, 2007), a pathogen which is closely related to the bacteria that cause human and avian tuberculosis (Palomino *et al.*, 2007). This organism can affect practically all mammals, causing a general state of illness including coughing, and eventual death (Renwick *et al.*, 2007). It is contagious and spreads by contact with infected domestic and wild animals (Neill *et al.*, 2001). Usually, the path of infections is by inhaling infected droplets which are expelled from the lungs by coughing (Radostits *et al.*, 2000). Further, calves and humans could also be infected by ingesting raw milk from infected cattle (OIE, 2010).

An animal can spread the disease to many other herd mates before it begins to manifest clinical signs as the course of the disease is slow. Therefore, undetected infected domestic and wild animals are the major ways of spreading the disease. Determination of the prevalence of bTB in cattle is important to implement successful prevention and control programs (Ameniet *et al.*, 2008). Currently, there is no detailed information on the prevalence of bTB in Sri Lanka. However, preliminary epidemiological investigation (Amarasinghet *et al.*, 2014) and a confirmatory diagnosis (Kumara *et al.*, 2014a; Kumara *et al.*, 2014b) were undertaken to determine the status of bTB in the Central Province, where highly susceptible breeds of cattle are reared under intensive farm operations. Thus, the objective of this study was to investigate the prevalence of the bTB among cattle and buffalo herds in the Central Province of Sri Lanka.

METHODOLOGY

Ethics statement

The ethical clearance of this study was obtained from Ethics Review Committee, Faculty of

Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka (VER-15-012).

Study area

The study sites were selected to determine the prevalence of bTB. All the three districts namely; Kandy (KN), Matale (MT) and Nuwara-Eliya (NE) in the Central Province (CP) were selected for the study (Figure1). The study period spanned from October 2013 to August 2016.

Production systems and animals tested for bTB

In Sri Lanka, the majority of dairy farmers run small-scale dairy operations (Ibrahim, 1999; Ranaweera, 2009) where herd size remains 1-10 cows (Kumara, 2017). However, medium-scale (no of cows =11-100) and large-scale (no of cows >100), (Kumara, 2017) dairy operations in the CP were included in the study. Cows reared in medium- and large-scale systems were mainly temperate purebred (Ayrshire, Holstein-Friesian, Jersey) and their crosses. Few animals belonged to tropical breeds (Sahiwal and Red Sindhi) and they were mixed with temperate breeds (Sahiwal × Jersey or Sahiwal × Holstein-Friesian). Buffalo breeds included in the study were Murah and Niliravi.

Study Design

A Cross-sectional, descriptive and analytical study was designed. The sampling frame was based on medium-scale and large-scale dairy farms in the CP.



Figure 1: Location of the study area; Three districts in the Central Province (MT: Matale, KN:Kandy, NE:Nuwara-Eliya) in Sri Lanka (source: DAPH, 2015).

Description of the study population

Cattle and buffaloes were included as the study population. Calves under 6 months of age and cows with more than 7 months of pregnancy were excluded from the study. In each sampling unit (farm), all the eligible individuals (animals) were selected for testing. At the time of Single Intradermal Comparative Cervical Tuberculin Testing (SICCT), each animal was identified by a numbered ear tag. Individual animal data on age, gender, breed, herd origin (from where the animals were purchased), reproductive status, parity, total milk production, type of management, herd category, and the farm established date were recorded, from the history records available at the farm. The breed was determined according to the phenotypic characteristics. The body condition of each individual was scored using the guidelines established by Kellogg, (2012) and Radostits *et al.* (2007).

Farms with cut and fed system were considered to be the intensive management, whereas, cut and fed system with grazing as the semi-intensive management. The grazing only system was considered as the extensive management. Selected farms were intensively and/or semi-intensively managed with continuous forages and concentrate feeding.

Calculation of sample size

All cattle and buffalo farms in the CP were included in the sampling frame. The sample size calculation was based on 50% prevalence assumption (since there was no study on bTB in the area), 95% CI and $d=0.05$ (Thrusfield, 2005). The following formula was used to estimate the sample size.

$$n = \frac{Z^2 \times P_{\text{exp}} (1 - P_{\text{exp}})}{d^2}$$

Where, n = required sample size, Z = Confidence Interval (CI at 95%; Standard value of 1.96), P_{exp} = Expected prevalence of bTB in test unit and d = Desired absolute precision (5 %) (Standard value of 0.05)

Calculated sample size was 384, nevertheless at least 500 animals need to be tested using thumb rule of 20%. Therefore, the present study included 616 animals for testing in order to increase the accuracy of the study.

Performing of SICCT test

Restraining of animals and injection site preparation

Cattle and buffaloes managed in a loose barn and/or tie-point systems were restrained only using physical methods. They were first taken into a crush-pen and head halters were applied. Two sites (dorsal and ventral) which are approximately 12 to 15 cm apart, in the left side of the neck were identified and marked (2 cm² diameter) using a permanent marker. Marked sites were clipped and cleansed with tap water in order to remove the debris prior injections. The presence of any abnormalities near to the injection sites was also recorded.

Pre-measurement of the skin thickness

A fold of skin within each clipped area was taken by measuring the thickness using digital Venire caliper while holding the skin fold between the forefinger and thumb. Then skin thickness for the Avian (A0) and Bovine (B0) injection sites for each animal was recorded accordingly.

Maintaining of cold chain

Purified Protein Derivative (PPD) kits were transported in an ice box and thawed under room temperature prior to injecting to the animals.

Injection of Bovine PPD and Avian PPD

Aliquots of 0.1ml containing 25,000 IU/ml of bovine PPD (CZV Bovine Tuberculin PPD, CZ Veterinaria, S.A, Spain) and 0.1ml with 25,000 IU/ml of avian PPD (CZV Bovine Tuberculin PPD, CZ Veterinaria, S.A, Spain) were injected intradermal (Figure 2) to the center of the identified area using insulin syringes. Each side of the neck at identical sites was injected in young animals as there was no room to separate injection sites sufficiently on one side of the neck.

Palpation of a pea-like thickening at each site of injection was done to confirm the correct intradermal injection. The skin-fold thickness of each injection site was re-measured 72 hours after injection and recorded. The relative change in skin appearance was classified as swelling or induration followed by measurement of skin thickness at both injection sites. Skin thickness measurements on testing and reading day were performed by the same person using the same digital Venire caliper to avoid errors related to individual and technical variations.

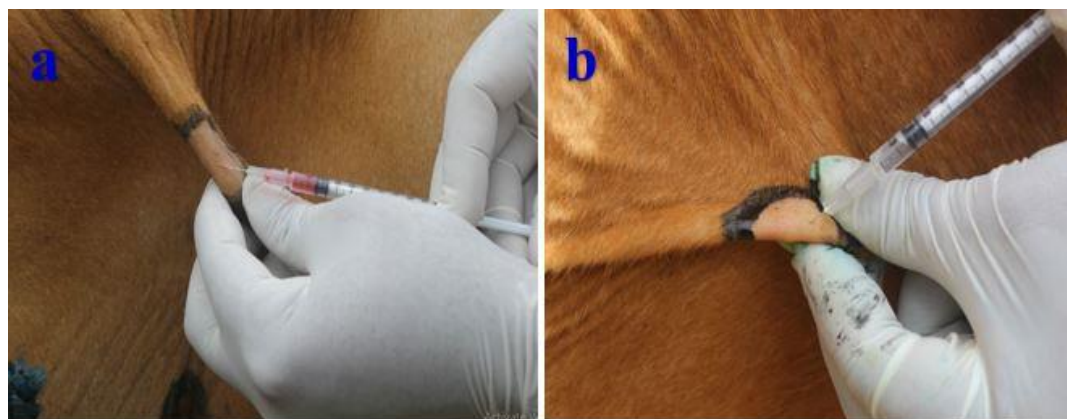


Figure 2: Injecting PPD to the marked sites; a. Avian PPD to the dorsal site, b. Bovine PPD to the ventral site.

Interpretation of the skin reaction measurements

The SICCT test results were analyzed and interpreted according to the recommendations of the OIE (OIE, 2009). When the change in skin thickness was greater at the bovine PPD injection site compared to the avian PPD injection site, the animal was considered to be 'SICCT test reactor'. However, when an increase in thickness was

observed at both sites, the difference in thickness between the two sites was considered. The reaction was considered positive, inconclusive, or negative depending on the net difference of the skin thickness at the avian PPD and bovine PPD site of injections (Table 1). Animals inconclusive to the SICCT test were subjected to another SICCT test in the right of the neck after 55-60 days (CZVaccines, 2013). Animals those were not negative to this second test were deemed to be positive to the test.

Table 1: Interpretation of skin reactions of the SICCT test.

Criteria	Clinical observation an increase in skin fold thickness; $(B_{72}-B_0) - (A_{72}-A_0)$
Positive	$\geq 4\text{mm}$ or the presence of clinical signs
Negative	$\leq 1\text{mm}$ and the absence of clinical signs
Inconclusive	$>1 - <4\text{mm}$ and the absence of clinical signs

Collection of blood samples

Cattle were bled shortly before inoculation with PPD tuberculin. Venipuncture was performed on the coccygeal vein at the medial side of the tail with an 18 G 1.5-inch vacutainer needle aseptically, and 2-3ml of whole blood sample was withdrawn into heparin vacutainer tubes (3ml). Blood samples were transported on ice to the laboratory and processed within eight hours of collection. Serum was separated by centrifugation at 3000g for 5 minutes and stored at -20°C until rapid bTB antibody (Ab) test.

Rapid bTB Ab test

The testing of sera for bTB Ab was performed using Rapid bTB Ab test kit (Quicking Biotech Co., Ltd, Shanghai, China). Accordingly, 20 μl of serum

was transferred to the 200 μl of assay buffer in a centrifugal tube at room temperature and mixed gently. The test kit was taken out from the foil pouch and placed on a flat, dry surface. Three drops ($\sim 100\mu\text{l}$) of sample mixture were then added to the sample deposition area on the lateral-flow cassette. The test results were interpreted after five minutes as described in Table 2.

Data Analysis

Data were recorded and analyzed using Microsoft Excel and Minitab 16.1 version. Individual animal level prevalence was computed as the number of reactors per 100 animals tested while the herd-level prevalence was calculated as the number of herds with at least one reactor animal per 100 herds.

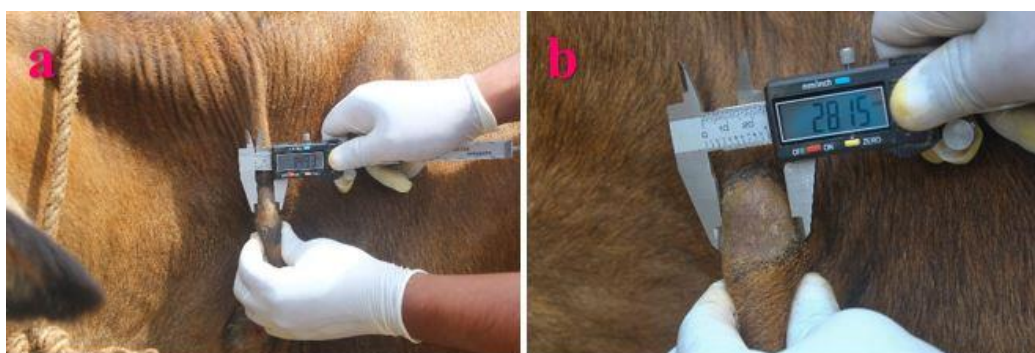
Table 2: Interpretation of the Rapid bTB Ab test results.

Interpretation	Band appearance of the test kit
Negative	The presence of only one purple band (the control band) in the result window
Positive	The presence of two bands (test and control bands) in the result window (even if the intensity of the purple colour band is faint, it should be considered as positive)
Invalid	If the purple colour band is not visible within the control area of the result window after performing the test, the test result was considered invalid and the specimen was re-tested

The variations between different factors such as age, body condition score (BCS), origin, parity, reproductive status, and sex were analyzed using Chi-square (χ^2) test to the occurrence of bTB in cattle. Categorical variables with more than two levels were coded as dummy variables. Logistic regression was employed to examine the effects of the different risk factors considered on bTB infection in cattle. In all analyses, the p-value was set at a 5% significant level.

RESULTS

Visible swelling at the injection sites was not observed in cattle with negative results (Figure 3a and 3b). However, a noticeable swelling at the injection sites was observed in cattle with positive results (Figure 4a and 4b). These observations were based on 72 hours after injecting PPD.

**Figure 3: Negative reaction to the SICCT test; a. avian site, b. bovine site.****Figure 4: Positive reaction to the SICCT test; a. avian site, b. bovine site.**

Geographical distribution of bTB based on SICCT test

The result of tuberculin test carried out in the 20 herds within the study area is depicted in Figure 5.

Among the total of 616 cattle were tested by injecting PPD, 139 (22.6%) were identified as reactors for SICCT test and the majority (77.4%) were negative for SICCT test.

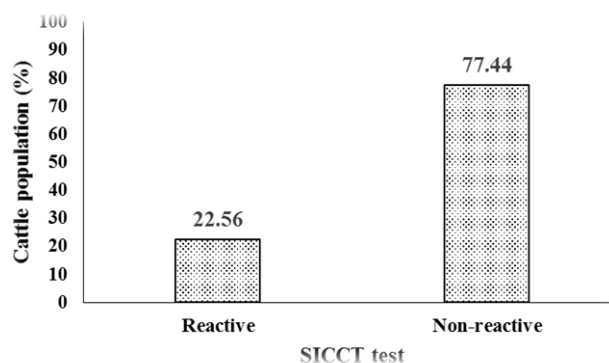


Figure 5: Prevalence of bTB in the study population.

In the present study, 248 cattle from NE, 286 from KN and 82 from MT were tested where, only 85(34%), 54 (19%) and 0 (0%) showed reactions to the SICCT test from respective districts (Figure 6).

The highest number of reactors was recorded from a herd in NE district, where 57 cattle out of 91 tested (62.6%) were positive to SICCT test. However, 10 herds which have tested for SICCT (50%) (1 herd from NE, 5 herds from KN and 4

herds from MT), were found to be negative and free from exposure to bTB within the study area.

Herd level bTB prevalence

A herd level prevalence of 50% (10/20) was resulted in tested large and medium scale dairy farms in the study area. There were no significant associations ($P>0.05$) observed with bTB prevalence and tested parameters such as herd category, duration of farm establishment and type of management practice (Table 3).

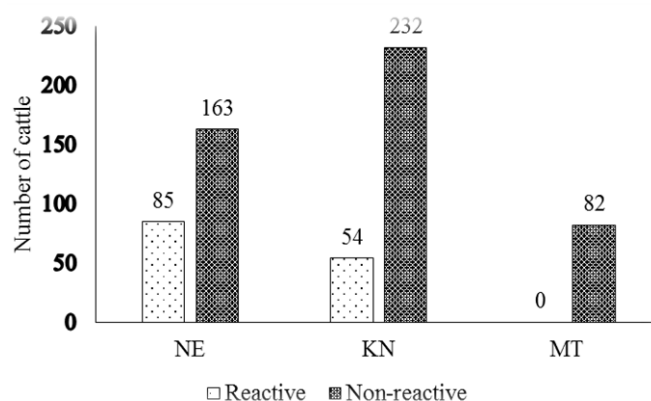


Figure 6: SICCT test reactors among the tested population in CP.

Table 3: Effects of herd classification on tuberculin reactivity.

Variable	Category	No. of herds tested	No. of test positive herds	p-value
Herd size/ category	Medium	16	6 (37.5%)	0.623
	Large	4	4 (100%)	
Duration of farm establishment	<5	5	0 (0%)	0.813
	5-10	7	3 (43%)	
	>10	9	7 (78%)	
Type of management	Intensive	14	5 (36%)	0.066
	Semi-intensive	5	5 (100%)	
	Extensive	1	0 (0%)	

Individual animal level bTB prevalence

The individual level prevalence of 22.5% was observed (139/616) in the present study. A

significant association of the prevalence of bTB with BCS ($P= 0.0001$), breed ($P= 0.0001$), origin of cattle ($P= 0.0350$) and reproductive status ($P= 0.0050$) were observed (Table 4).

Table 4: Association between individual risk factors and tuberculin reactivity in the study population.

Variables	Category	No. of animals tested	No. of animals affected	Chi-square (χ^2)	P-value
Age	≤5 (young)	301	64 (21.3%)	12.200	0.2000
	>5-10 (adult)	256	54 (21.1%)		
	>10 (geriatric)	23	12 (52.2%)		
BCS	≤2 (poor)	285	99 (34.7%)	47.698	0.0001
	>2-<3 (medium)	254	35 (13.8%)		
	3-3.5 (Good)	77	5 (6.5%)		
Breed	Ayrshire	118	75 (63.6%)	-	0.0001
	Friesian	217	28 (12.9%)		
	Jersey	135	14 (10.4%)		
	Sahiwal	6	0 (0%)		
	Murrah	46	13 (28.3%)		
	Crossbred	94	9 (9.6%)		
Origin	Born at farm	304	87 (28.6%)	12.587	0.0350
	Purchased	312	52 (16.7%)		
Parity	≤2	302	50 (16.6%)	14.737	0.9480
	3-5	215	66 (30.7%)		
	>5	25	7 (28%)		
Reproductive status	Heifer	55	5 (9%)	10.632	0.0050
	Lactating	455	115 (25.3%)		
	Dry	94	17 (18.1%)		
Sex	Male	20	6 (30%)	0.654	0.4190
	Female	596	133 (22.3%)		

Rapid bTB Ab test

A total of 33 cattle (6 males and 27 females from eight herds) which showed positive results in the

SICCT test were subjected to the rapid Ab test. Only seven cattle (2 males and 5 females from 4 different herds) out of 33 were positive for rapid bTB Ab test (Figure 7). Table 5 demonstrates the results of the rapid Ab test.

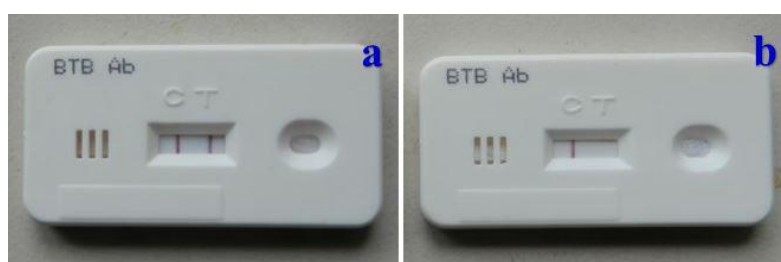


Figure 7: Rapid bTB Ab test results; Test positive (a); Test negative (b).

Table 5: Results of the rapid Ab test.

Category	Tested	Test positive %	Test negative
Total number of animals	33	7 (21%)	26 (79%)
Number of herds	8	4 (50%)	4 (50%)
Males	6	2 (33%)	4 (67%)
Females	27	5 (19%)	22 (81%)

DISCUSSION

Tuberculin screening test detects the presence of *M. tuberculosis* complex in both humans and animals. This test has traditionally been used to determine the prevalence of infection in animals and humans using the PPD tuberculin (OIE, 2009). Use of direct smear microscopy is also an inexpensive, rapid test for diagnosis of tuberculosis (Araujo *et al.*, 2014). However, it does not permit differentiation between species of *M. tuberculosis* complex. In the present study, the SICCT test was considered to examine the prevalence of bTB among the cattle and buffalo herds in the study area, since the SICCT is suitably specific to differentiate reactions between infections by *M. bovis*, *M. avium*, or *M. paratuberculosis*. In addition, the presence of avian tuberculosis or *M. paratuberculosis* (Johne's disease) is suspected, non-specific sensitization must be considered, and hence the SICCT test is the best choice (Radostits *et al.*, 2000).

ThebTB has not been reported in the CP, except for few suspected cases (DAPH, 2014). Based on this study, a high prevalence of bTB was observed at both individual and herd levels. The high prevalence (50%) of bTB at the herd level poses a high risk towards future dairy farming in Sri Lanka. However, except for the studies conducted in early 1970s (Bongso and Pinto, 1972; Pinto *et al.*, 1972), there are no considerable level of investigations done on bTB status of the country to compare the present results. Hence, the result of the current study provides a positive signal to expand the present research further to develop control measures of bTB among dairy farms.

The SICCT test was used as the principal test method for all animals enrolled in the study

while the rapid Ab test was used only for 33 SICCT test reactors. Though both the tests are ideal in testing bTB, financial constraints limited the use of rapid Ab test in the present study. However, the rapid Ab test was used as a complementary test to increase the accuracy of screening. Further, the present study included 616 cattle in the study population though 500 cattle were the minimum number required in the study population as per the sample size determination, in order to increase the accuracy of the study. All the inconclusive reactors were re-tested 55-60 days after the first inoculation of tuberculin to exclude the true test negative animals. However, Shirima *et al.* (2003) reported that some animals which were classified as inconclusive during the SICCT test, yielded typical bTB lesions when slaughtered and examined at postmortem, and the *M. bovis* was isolated from the cultured samples. De la Rua-Domenech *et al.* (2006) stated that the proportion of *M. bovis* infected cattle that are positive to the skin test were not detected by the Ab test which indicates the lower concentrations of bTB antibodies in the serum sample. Rapid Ab test was used as an improved tool for the detection of bTB infected cattle. Accordingly, 21% (7/33) of the selected SICCT test positive cattle were also positive for the Ab specific for *M. bovis* in the present study (Table 5).

Findings also revealed that there was a significant association ($P = 0.0001$) between the prevalence of bTB with breeds of cattle (Table 4). The highest disease percentage for bTB was observed in the Ayrshire breed (63.6%) indicating fivefold more susceptibility than the other breeds (12.85%). However, a definitive risk factor for the Ayrshire breed has not been

identified. Previous investigations have indicated that the bTB has an association with the cattle breed (Cosivi *et al.*, 1998), and temperate breeds are probably less resistant to bTB compared to indigenous cattle breeds (Kleeberg, 1984).

Herd level investigations suggested that cattle and buffaloes managed under intensive and/or semi-intensive management systems may carry a high risk of bTB transmission among animals. Possible underline causes for high herd prevalence of bTB could be intensification, stress, and overcrowding due to limited land availability. It is also suggested that these farming systems cannot avoid close contacts among animals and thereby create favourable environment in spreading of the disease from one animal to another. Water and feed sources were common for all the animals managed under semi-intensive management system in the study, where animals were herded to a common area in the yard for concentrate feeding every day. Horizontal transmission of the disease is inevitable in such situations.

The results revealed that the gender of an animal was not significantly associated with the status of bTB. This could be due to the small number of male animals included in the study since low representation of male animals in dairy farming systems. Cleaveland *et al.* (2007) and Gumi *et al.* (2011) also reported the same in their respective studies.

Among the individuals who tested positive for the SICCT, the highest percentage (34.7%) was the cattle with poor BCS (Table 6). The similar findings were reported in previous studies (Akililu *et al.*, 2014; Fikre *et al.*, 2014). This could mainly be due to the fact that animals with poor BCS are immune-compromised and thus become susceptible to diseases. Animals with good BCS (3-3.5 on the 5-point scale) were the least (5%) affected by the disease. Hence, maintaining a good BCS with optimum environmental conditions are of utmost importance to prevent the disease.

The results further indicated that the herd-level prevalence of bTB was proportionate to the herd size. Therefore, the tendency of the spreading of bTB in large-scale farms was higher than in the medium-scale farms. This finding is in line with those of Ameni *et al.*

(2002) and Asseged *et al.* (2000) who also reported a corresponding increase in the prevalence of bTB with the increase of herd size.

Though there was no significant statistical association between the ages of the cattle and positive test results, it was observed that the majority of geriatric cattle (52.2%) were positive for the SICCT test (Table 6). As described by Griffin *et al.* (1996), a young animal might be exposed to the pathogen but expresses the disease in adult age. The absence of statistical association observed in the present study could be due to the smaller number of geriatric cattle included in the study since due to non-availability of old animals with regular replacements in herds. According to the study conducted by Mohammed *et al.* (2012) the individual level prevalence of bTB changed with the age where it was increased with the age of the animal up to 7 years, and then decreased slightly.

The origin of the animal was not significantly associated with its bTB status ($p > 0.05$) in the study population. However, Tschoop *et al.* (2010) stated that the purchase of animals is highly associated with bTB positivity suggesting that the disease is likely to be spread by animal movements. Accordingly, the observation of the present study suggests that the purchase of cattle of the study population might have not involved with bTB infected herds in the past.

Reliable diagnostic tools are crucial to detect bTB from dairy farming systems. Farm owners or managers who consult veterinarians regularly are more likely to get sound advice about the health status of their animals and would help to eliminate diseased animals at an early stage, and hence reduce the level of bTB infection (Munyeme *et al.*, 2008). This process can be facilitated by implementing compensation schemes by the government.

CONCLUSIONS

The estimated prevalence of bTB among cattle in the CP of Sri Lanka was 22.6%, as determined by the SICCT test with 80% of test sensitivity. The SICCT test could be

recommended for screening of the bTB in cattle and buffaloes in all regions of Sri Lanka to assess the prevalence of bTB, as this disease carry the risk of transmitting to humans and other susceptible animal species.

COMPETING INTERESTS

The author(s) declare that they have no conflict of interests

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