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Research article

Prevalence and risk factors of brucellosis and dairy farmers' KAP in 2 townships, Myanmar

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Abstract

Brucellosis is a highly infectious zoonotic disease and has a great economic impact on dairy production. In Yangon Region of Myanmar, 0.47% seroprevalence of brucellosis in dairy cattle and 8 human IgG positive cases was reported. This study aims to determine the prevalence of brucellosis in dairy farms and its risk factors as well as to assess the current knowledge, attitudes and practices (KAP) of dairy farmers regarding brucellosis. Our study was conducted in Hlegu and Taikkyi Township during September 2019 to January 2020. Pooled milk samples were collected from 174 dairy farms. Using Milk Ring Test (MRT), *Brucella* spp. was found in 30 dairy farms and the prevalence was 17.2%. Statistically significant farm factors associated with MRT positive results were farm location in Takkyi Township (Odds ratio (OR) 3.19; 95%CI = 1.42, 7.15), pasture sharing (OR 3.43; 95%CI = 1.34, 8.76), and abortion (OR 2.68; 95% CI = 1.21, 5.98). Survey for the KAP for brucellosis in farmers indicated that good knowledge, attitudes and practices were in 35.1, 99.4, and 62.1% of the respondents, respectively. In conclusion, brucellosis was still a problem of dairy farms in study area and MRT can be used as a farm level screening test. Educational campaign for brucellosis prevention and control should be considered according to a lack of knowledge and inappropriate farm practices among dairy farmers

Keywords: Brucellosis, KAP, Milk Ring Test, Myanmar, Risk factors

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INTRODUCTION

Brucellosis is a widespread and highly infectious zoonotic disease as well as a great economic impact on dairy farming (OIE, 2009). An estimated 500,000 human infections of brucellosis per year still occurs worldwide (Cutler et al., 2005). It is caused by different species of the genus Brucella, small, Gram-negative, non-motile, non-spore forming, rod shaped (coccobacilli) bacteria (OIE, 2016). *Brucella* species are pathogenic for a wide variety of animals. Symptoms include abortion, metritis, orchitis and epididymitis (OIE, 2016). The typical symptoms in human brucellosis are mild fever, sweating, weakness, body aches, enlarged lymph nodes and weight loss (Cadmus et al., 2008). It is transmitted by ingestion of *Brucella* in aborted fetuses, vaginal discharges, contaminated feeds and water and venereal transmission by infected bulls (Omer et al., 2000). Transmission to human can occurs through breaks into the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted fetuses or placentas from infected animals (Bercovich, 1998).

The MRT is stable spot on test and readily feasible for field conditions. And then, further confirmation can be achieved by culture and Enzyme Linked Immunosorbent Assay techniques. Indirect ELISA was developed and used as the most specific serological test for brucellosis (Cadmus et al., 2008). The definitive diagnosis of brucellosis is made by the methods of culture and isolation procedures (WHO, 2006). However, it is not practicable when large numbers of animals are involved. The Milk Ring Test is being only a screening test and inexpensive preliminary test for dairy herds. Recently, MRT was occurred more 83.3% of sensitivity and 98,5% of specificity (Al-Mmashhadany, 2019). The similar study also reported that the level of sensitivity to the MRT test was positive among 85.0 % of the herd, whereas specificity was 95.0 % of the herd (Salman et al., 2012). However, Kumar et al. (2018) reported that MRT was found 53.6% sensitivity and 98.7% specificity by using mELISA as a gold standard.

Bovine brucellosis has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Gul et al., 2007). However, it remains highly endemic in areas such as Africa, the Mediterranean, the Middle East, in parts of Asia and Latin America (Refai, 2002). Brucellosis currently occurs among cattle and small ruminants in most parts of South-East Asia including Myanmar, Laos, Vietnam, Cambodia, Philippines, Thailand, Malaysia and Indonesia. In Myanmar, there were some laboratory reports of the disease but there is no routine surveillance. There were also laboratory reports of brucellosis in cattle in various parts of Myanmar by Thin (2007) the occurrence of brucellosis was 20, 2, 21, 32, 2, 5 and 26 cases in cattle in 1995, 1996, 1998, 1999, 2002, 2003 and 2006, respectively. The prevalence of bovine brucellosis in Myanmar was 4 - 5% in 2014 (Zamri-Saad et al., 2016). Previous report on seroprevalence of brucella in dairy cattle was 0.47% in Yangon Region (Tun, 2007) and 3.5% in Mandalay and Kyaukse Districts (Thin, 2007). Similarly, seroprevalence of brucella in cattle was 0.24% in Amarapura and Pyin Oo Lwin Township (Myint et al., 2010) and 2.6% in Pyawbwe Township (Win, 2015). Moreover, seroprevalence of brucella in goats were 2.0% in Mandalay and Magwe Region (Thant, 2008) and 0% in Pyawbwe Township (Win, 2015).

In the previous reports, potential risk factors of brucellosis in Myanmar were herd size (p=0.002, OR=12.40), recording of visitors (p<0.001, OR=18.06) and awareness of the disease (p<0.001, OR=18.47) were statistically significant for bovine brucellosis (Tun, 2007). The previous study reported that the prevalence of large herd size was significantly higher than the prevalence of small herd size and median herd size (p<0.05) (Thin, 2007). The prevalence in animals with a history of abortion was significantly higher than in those without such a history (p<0.01) (Thin, 2007). The previous report stated that introduction of new animal into the farm (p<0.05) was statistically significant for bovine brucellosis (Win, 2015).

Brucella immunoglobulin G antibodies was detected in 8 (10.7%) animal handlers by using Brucella IgG ELISA test in Hlegu and Taikkyi Township implied that brucellosis was transmitted to human (Myint, T. et al., 2010). These people were exposed to animals and in raw milk. These people were working in cattle farms that had history of bovine brucellosis during 2008 according to the data from Livestock Breeding and Veterinary Department. Thus, there may be possible zoonotic transmission of brucellosis in the area. Human brucellosis case was also reported in a German tourist who drunk milk and taken lassi (typical Indian dairy product) in Myanmar (Lewis et al., 2016). According to the regulations in Myanmar and the World Organisation of Animal Health (WOAH), movements of the affected animals are prohibited (Tun, 2007). However, good farming practices and management are still weak in the whole country to control the disease, thus brucellosis is still far from the most dairy farmers' attention. There are very few studies of brucellosis undertaken in dairy cattle group in Myanmar. The knowledge, attitudes and practices (KAP) of dairy farmers regarding brucellosis in Myanmar had not been evaluated. Moreover, by getting information on brucellosis, control program for brucellosis could be developed for more beneficial production and it could also be useful for poverty alleviation. According to the endemic situation of brucellosis in Hlegu and Taikkyi Townships, this study focused on herd prevalence, risk factors and the knowledge, attitudes and practices (KAP) of dairy farmers in order to understand the situation for improving brucellosis control measure in dairy farms.

MATERIALS AND METHODS

A cross-sectional study was conducted in Hlegu and Taikkyi Townships in Yangon Region between September 2019 to January 2020. A preliminary farm survey in the study area in 2019 by the Livestock Breeding and Veterinary Department (LBVD) revealed that there was a total of 211 farms: 153 farms in Hlegu and 58 farms in Taikkyi, respectively. Among them, 120 farms (78.4%) in Hlegu and 54 farms (93.1%) in Taikkyi Township were dairy farms. Farmers were contacted by phone to make appointments and to ensure participation in the study through questionnaire survey and pooled milk sample collection. All milk production farms were invited for face-to-face interview guided with a structured questionnaire. Follow by the interview, the farms were visited the next day for pooled milk sample collection. Mastitis milk, colostrum and milk from vaccinated cows were excluded to avoid false positives. The samples were sent to a laboratory on the same day.

Interviewers were trained staff from the Epidemiology Section and Township, LBVD. The questionnaire consists of four sections including general farm information, knowledge, attitudes, and practices (KAP). The questionnaire was designed in the Burmese language and it was validated by experts. The pilot test was done by 10 households in the study area and then modified accordingly. The knowledge section consisted of 18 closed-ended questions. The attitude section was measured using 5-point Likert scale. The practice section consisted of 13 open-ended questions regarding dairy farm management related to brucellosis prevention and control. Geographic coordinates of farm locations were recorded.

KAP questionnaire survey was conducted in 174 dairy farms including 120 dairy farms in Hlegu Township and 54 dairy farms in Taikkyi Township. The general characteristics including the individual profiles of the owners such as education level, sex, age and farming experience and animal profiles such as farm location, farm size and breed were considered. Ages were categorized into 4 groups according to their abilities to improve farming system (Tun, 2007). The groups were <30 years old who are active, 30-40 years old who had some experience and trended to improve their works, 40-50 years old who had well experience and tried to update dairy farming system and over 50 years old who had well experience but did not change the farming style. The farmers' education levels were categorized into four groups according to their attended grade level in education (Tun, 2007). The groups were illiterates who didn't attend for education, low level who can read and write, middle level who passed primary school and middle school and high level who passed high school and collage/graduated. There were three types of farm size according to keeping number of dairy cattle in the farm (Tun, 2007). The groups were small size farm having up to ten cows, medium size farm keeping over ten to fifty cows and large size farm raising over fifty cows in the farm.

A pooled milk sample was collected with sterilized tube from each dairy farm in study area. For herds of up to 150 cows, 10 ml of milk was required, but for herds between 150 and 450 cows, 30 ml was collected and for herds between 450 and 700 cows, 20 ml was collected (OIE, 2018). After pooled milk samples were collected, they were kept in a cool box and then transported to the laboratory.

Sample analysis

Whole milk samples were stored for at least 24 hours at 4 °C. On the following day, the milk samples were tested with the Milk Ring Test (MRT). MRT antigen and milk samples were brought to a room temperature before performing the test. The test was carried out by mixing 30-50 µl of Brucella antigen with 1-3 ml volume of whole milk sample, followed by storage at 4 °C for at least 24 hours. The amount of milk sample used was adjusted based on the number of cattle in each herd: 1 ml bulk milk from herd size <150 cattle; 2 ml bulk milk samples for herds with 150-450 cattle and 3 ml bulk milk sample for herds with between 451 and 700 cattle (OIE, 2018). The whole milk sample and antigen mixtures were incubated at 37 °C for 1 hour. A strong positive reaction can be observed by the formation of dark blue ring above white milk column. Any blue layer at the interface of milk and cream was considered positive. The test was considered negative if the color of the underlying milk

appeared homogeneous in the milk column. If the result was regarded as inconclusive, the test should be repeated when the milk at the bottom of the tube becomes gradually whitened (OIE, 2018).

Data Analysis

Collected data was entered into Microsoft EXCEL (USA, 2010) spreadsheets. The data was analyzed by using NCSS 2020 software for descriptive statistics and analytical statistics analysis. QGIS software version 3.4.12 was used for the spatial analysis of the MRT positive results among farms. The overall scores were calculated for each knowledge, attitudes and practices section. For the knowledge, 18 closed-ended questions were used and "1" score was given for each correct answer, otherwise "0" score was awarded. For the knowledge section, only the participants who heard of brucellosis were further assessed. There are 14 questions under the attitudes section. The maximum score and the minimum score were 70 and 14, respectively. The practices section consists of 13 open-ended questions regarding dairy farm management related to brucellosis prevention and control. To assess the question regarding farmers' experiences and practices in their farms, the score was conducted with the suggestion of expert to ensure reliability. For scoring, do nothing or ignore or very poor practice= 0 score, poor practice=1 score, fair practice=2 score and good practice = 3 score. The maximum score is 39 and the minimum score is 0. Then the scores were determined using median score as a cut-off point and group as either good (>median score) or poor level (⩽cmedian score) for each KAP section of this study. To illustrate associations between MRT results and KAP levels, Chi-squared test with Yates's correlation was performed.

To identify risk factors, univariable analysis and multivariable logistic regression analysis were used. Variables with p-value <0.10 in the univariable analysis were further evaluated in a multivariable logistics regression analysis. Forward stepwise variable selection was performed. The variable selection was continued until the most parsimonious model with p-values for all remaining variables less than 0.05. The removed variables were individually added back to prove that the variables were not removed due to the order of the removal. Finally, Pearson's Chi-squared test was performed between the retained variables to prove a lack of multicollinearity. Final model was evaluated. For statistical analyses, a binomial logistic regression (BLR) model was performed with MRT result, positive or negative, as the outcome. The best model was chosen based on the Likelihood Ratio Test and Goodness of Fit Test was used to check the validity of the BLR model. Saturated models were fitted by evaluation of likelihood ratio test statistic and Receiver Operating Characteristics (ROC) curve was used to evaluate the accuracy of the model.

RESULTS

Descriptive information

Results showed that most of the owners were male, accounting for 157 out of 174 (90.2%). The group of owners aged over 50 years was the largest group, occupying 41.4%, age group 40-50 was 28.2%, age group 30 – 40 was 22.4% and age group of up to 30 possessed 8.0%.

As for the farming experience, 38.5% of the owners had up to five years' experience, followed by groups of 5-10 years (27%), 10-20 years (25.3%), and more than 20 years (9.2%) respectively. The farmers' levels of education revealed that 14.4% were illiterate, 62% had low level, 20.7% had middle level and only 2.9% had high level of education. The dairy farms profiles indicated three different farm sizes including, 109 (62.6%) of small size farms (up to 10 cows), 55 (31.6%) of medium size farms (>10 – 50 cows) and 10 (5.7%) of large size farms (>50 cows). All studied farms raise Holstein-Friesian and Jersey cross-bred cattle. The geographic distribution of different farm sizes located in these 2 townships was shown in Figure 1.

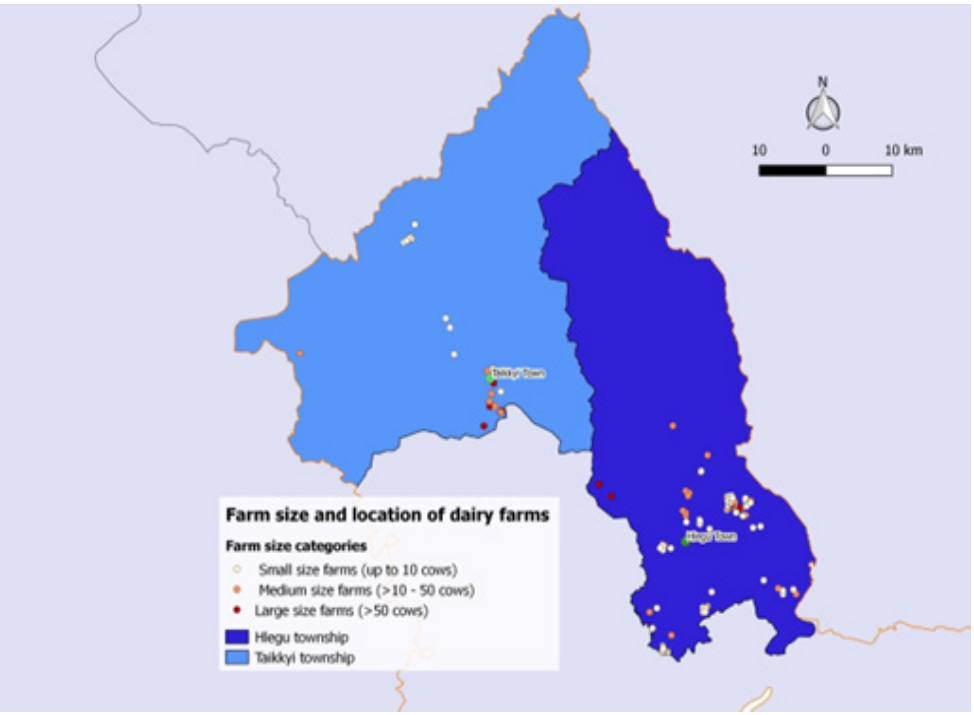

Figure 1 Locations of dairy farms in Hlegu and Taikkyi townships classified by farm sizes.

Out of the 174 participants, 169 (97.1%) indicated that they heard about brucellosis, and some of them knew about symptoms related to brucellosis in cattle including decrease in milk production (41.4%), orchitis (58%), joint inflammation (72.2%), and abortion (88.1%). For brucellosis transmission in animals, 57.4% of the participants knew that cattle could get infected by licking aborted fetus. In addition, 66.9% of them knew infection can occur through licking genital discharge and placenta. Interestingly, 71.6% were aware that brucellosis could be prevented by using vaccine. Sixty-two participants (36.7%) considered brucellosis as a zoonotic disease, the symptoms also identified including night sweating (82.3%), mild fever (85.5%), weight loss (90.3%) and muscle pain (95.2%). About transmission in humans, 96.8% of respondents have knowledge that humans can contract infection by drinking raw milk and almost of participants indicated that wearing mask and gloves can prevent brucellosis.

The attitudes of participants found that more than 55% of them disagreed to dispose aborted placenta and fetuses without protective sheet and most of

them refused to eat aborted placenta. More than 80% strongly disagreed to drink raw milk from aborted cows. More than 90% of participants agreed to cull the infected cattle and 59.8% agreed that brucellosis is more important in human health. All farms used various vaccines to prevent significant infectious diseases such as foot and mouth disease, hemorrhagic septicemia, anthrax, and black quarter but none of them used anti-brucellosis vaccine.

There were three farm types based on their management including backyard farms (79.3%), pasture sharing (14.4%) and intensive farms (6.3%). All farms practiced on similar manual feeding system. Our results showed that 35.1% of farms used artificial insemination (AI) methods to breed cows and the rest relied on natural mating using either their own or shared bulls. Most of the farms collected milk by hands and only 3.5% reported the use of milking machine. More than 95% of farms had no practice for brucellosis management by using Rose Bengal test (RBT) or MRT, and 67.8% of the farms did not have preventive measures to ensure brucellosis free cattle when new cows were introduced to their herds. Even though more than 60% of the farmers did not experience having aborted cows on their farms, only 13.8% reported local veterinarians or LBVD. The management practice on aborted placenta showed that 33.9% of the farmers buried the placenta but 66.1% of them managed by eating, selling, giving it to others, feeding it to dogs or throwing away. To dispose aborted placenta, 63.8% of farmers did not wear neither gloves nor masks.

Prevalence of brucellosis in dairy farms or households

Pooled milk samples from 174 farms were examined by MRT and 30 dairy farms were found positive for brucellosis, giving a prevalence of 17.2% (95% CI: 11.6-22.9) at a farm level. The prevalence was different between the two townships, where Taikkyi Township was significantly higher (29.6%) than Hlegu Township (11.7%) (Table 1).

Table 1 Prevalence of brucellosis at farm level by MRT

Township	No. dairy farms	No. of MRT positive farms	Prevalence (95% CI)
Hlegu	120	14	11.7 (5.9-17.4)
Taikkyi	54	16	29.6 (17.5-41.8)
Total	174	30	17.2 (11.6-22.9)

Knowledge, attitudes and practices about brucellosis of dairy farmers

The overall median scores of knowledge, attitudes and practices among dairy farmers was 33% (Interquartile range (IQR) 22-78%), 86% (IQR 81-90%) and 49% (IQR 44-54%), respectively (Figure 2). For overall KAP regarding brucellosis, after grouping with its median score, the results showed 35.1% of the respondents were considered to have good level of knowledge, 99.4% were considered to have good attitudes while 31.0% were categorized as good practices.

Among 30 MRT positive farms, 11 farms (36.7%) have good knowledge, while 19 farms (63.3%) possess poor knowledge related to brucellosis. All positive farms revealed good attitude and 11 positive farms (36.7%) raised cows with good practice.

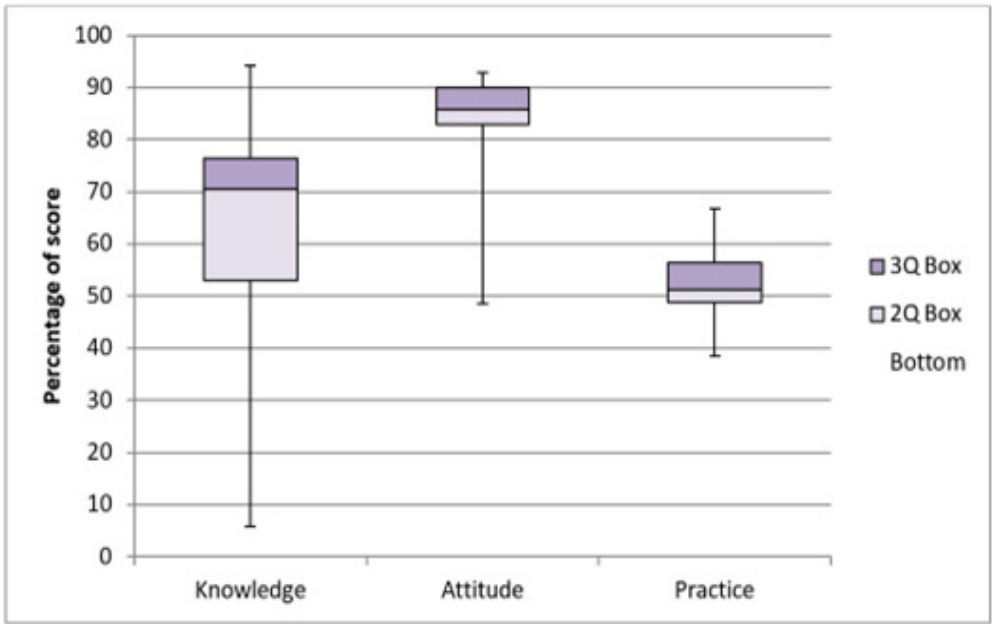

Figure 2 Knowledge, attitude and practice score distribution regarding brucellosis of dairy farmers in study area.

Association between KAP results and experimented factors

The statistically significant association were found between farmers' practices and owner age ($\chi2 = 4.69$, p=0.030), township ($\chi2 = 7.52$, p=0.006) and rural area ($\chi2 = 6.85$, p=0.009). The significant association were also found between education level ($\chi2 = 4.73$, p=0.030) and farmers' attitudes but no association was found between knowledge and study factors (Table 2). Our results showed that participants' age over 50 years were likely to have good practice (40.5%) as well as farms located in Taikkyi (46.3%). and the farmers from urban area (48.8%). There was no correlation found between knowledge, attitudes and practices regarding brucellosis among dairy farmers in this study.

Univariable analysis for risk factor of brucellosis

The potential risk factors related to MRT positive farms were determined using Chi-square test. There were three factors of statistically significant association with brucellosis, which were farm locations (OR=3.39, *p*=0.007), pasture sharing (OR=3.43, *p*=0.017) and farms with history of abortion (OR=2.68, p=0.024) (Table 3).

In the study, sixteen farms in Taikkyi Township were found to be infected with brucellosis, and 38 farms tested negative. Thus, the ratio of brucellosis positive farms to negative farms in Taikkyi Township was 1:2.4. Nine of the farms that practiced pasture sharing were brucellosis positive and 16 were negative. Therefore, the ratio of brucellosis positive farms to negative farms that practiced pasture sharing was 1: 1.8. Fifteen of the unburied placenta farms were brucellosis positive farms and 98 farms were negative. Therefore, the ratio of brucellosis positive farms to negative farms that practiced unburied placenta was 1: 6.5.

Multivariable analysis for risk factors of brucellosis

Using the five potential risk factors which had p-value less than 0.1 from univariable analysis including farm locations, pasture sharing, farm with history of abortion, bury placenta and exposure to placenta without protective sheet were analyzed using multivariable logistic regression. In the first binary logistic regression (BLR) model, farm locations, pasture sharing, history of abortion, bury placenta and exposure to placenta without personal protective equipment were entered as independent variables. The final multivariable model retained two risk factors associated with having MRT positive comprised of farm locations and pasture sharing (Table 4). The model had a good fit according to the AUC of the ROC curve that is 0.67 in the study.

Table 2 Associations between farmers' characteristics and knowledge, attitudes and practices levels regarding brucellosis

General characteristics	Knowledge level		p-value	Attitude level		p-value	Practices level		p-value
	Good *n* (%)	Poor *n* (%)		Good *n* (%)	Poor *n* (%)		Good *n* (%)	Poor *n* (%)	
Gender									
Male	55 (35.5)	100 (64.5)	0.935	154 (99.4)	1 (0.6)	1.000	48 (31.0)	107 (69.0)	0.957
Female	6 (31.6)	13(68.4)		19 (100.0)	0 (0.0)		6 (31.6)	13 (68.4)	
Age									
Up to 50	36 (36.0)	64 (64.0)	0.887	99 (98.0)	1 (2.0)	1.000	24 (24.0)	76 (76.0)	0.030
>50	25 (33.8)	49 (66.2)		74 (100.0)	0 (0.0)		30 (40.5)	44 (59.5)	
Education level									
Under Graduate	58 (34.9)	108 (65.1)	1.000	166 (100.0)	0 (0.0)	0.030	50 (30.1)	116 (69.9)	0.426
Graduate	3 (37.5)	5 (62.5)		7 (87.5)	1 (12.5)		4 (50.0)	4 (50.0)	
Farming experience									
Up to 5	24 (35.8)	43 (64.2)	0.997	67 (100.0)	0 (0.0)	1.000	20 (29.9)	47 (70.1)	0.921
>5	37 (34.6)	70 (65.4)		106 (99.1)	1 (0.9)		34 (31.8)	73 (68.2)	
Farm Locations									
Taikkyi	19 (35.2)	35 (64.8)	1.000	54 (100.0)	1 (0.0)	1.000	25 (46.3)	29 (53.7)	0.006
Hlegu	42 (35.0)	78 (65.0)		119 (99.2)	1 (0.8)		29 (24.2)	91 (75.8)	
Rural area									
Yes	49 (36.8)	84 (63.2)	0.483	132 (99.3)	1 (0.7)	1.000	34 (25.6)	99 (74.4)	0.009
No	12 (29.3)	29 (70.7)		41 (100.0)	0 (0.0)		20 (48.8)	21 (51.2)	
Farm/Herd size									
Up to 50	60 (36.6)	104 (63.4)	0.171	163 (99.4)	1 (0.6)	1.000	50 (30.5)	114 (69.5)	0.780
>50	1 (10.0)	9 (90.0)		10 (100.0)	0 (0.0)		4 (40.0)	6 (60.0)	
Knowledge level									
Good	-	-		61 (100)	0 (0.0)	1.000	20 (32.8)	41 (67.2)	0.845
Poor				112 (99.1)	1 (0.9)		34 (30.1)	79 (69.9)	
Attitude level									
Good	61 (35.3)	112 (64.7)	1.000				54 (31.2)	119 (68.8)	1.000
Poor	0 (0.0)	1 (100)					0 (0.0)	1 (100.0)	

Table 3 Potential risk factors of MRT positive farms

Risk factors	No. of farms	No. of positive	% of positive	Chi-square	p-value	Odds ratio (95% CI)
Farm locations						
Taikkyi	54	16	29.6	7.21	0.007	3.19
Hlegu	120	14	11.7			(1.42 – 7.15)
Pasture sharing						
Yes	25	9	36.0	5.746	0.017	3.43
No	149	21	14.1			(1.34 – 8.76)
Bury placenta						
Yes	61	15	24.6	2.806	0.094	0.47
No	113	15	13.3			(0.21 – 1.04)
Expose to placenta without protective sheet						
Yes	111	14	12.6	3.751	0.053	0.42
No	61	16	26.2			(0.19 – 0.94)
History of abortion						
Yes	59	16	27.1	5.101	0.024	2.68
No	101	14	13.9			(1.21-5.98)

Table 4 Multivariable logistic regression model

Variable	Levels	Odds ratio (95% CI)	P-value
Farm Locations			
	Hlegu	1(Ref.)	
	Taikkyi	2.86 (1.25-6.53)	0.013
Pasture sharing			
	No	1(Ref.)	
	Yes	2.91 (1.11-7.68)	0.031

Ref.: reference group

DISCUSSION

IIn this study, the prevalence of brucellosis was evaluated using MRT, and the KAP levels of dairy farmers were studied. Despite most farms having good attitudes regarding brucellosis, only two thirds of the respondents showed good levels of knowledge and practices. Farm locations, community pasture sharing and history of abortion were identified as risk factors for bovine brucellosis. However, some practices considered to increase the risk of brucellosis in humans, such as drinking unpasteurized milk, inappropriate storage of manure and inappropriate disposal of aborted placenta were still present within the study area. Brucellosis was then identified as an ongoing problem in Hlegu and Taikkyi Townships. Strengthen surveillance system and improvement of knowledge and practices in dairy farmers would be beneficial to successfully control brucellosis in the area.

Our study showed the prevalence of brucellosis was 11.7 % in Hlegu and 29.6 % in Taikkyi Township, that correlated but lower than the study in 2007 that found 20.0% in Hlegu Township and 50.0 % in Taikkyi Township (Tun, 2007). In the study area, some dairy farmers gained knowledge about brucellosis prevention and control from the previous study, and based on the experience of the previous study results, they raised cows with good practices for brucellosis prevention. Therefore, the difference of prevalence may be due to the disease control in Hlegu and Taikkyi Township. The difference was assumed to be due to the milk samples were collected from milk collecting point center in the previous study. But the milk samples were collected from individual farm and mastitis and colostrum milk were excluded in this study, which seems to have contributed to a lower prevalence. Milk from cow with mastitis, colostrum milk and last stage of milk may give false positive reactions with MRT (OIE, 2009). When compared with prevalence studied in other parts of the country, our prevalence was higher than that in Yangon Region (3.83%) (Tun, 2007) and that in Amarapura and Phin Oo Lwin Townships (0.88% (Myint,et al., 2010). However, it was similar to Thin (2007) who reported the prevalence of 18.18% in Mandalay and Kyaukse District. In addition, a number of studies from neighboring countries reported a higher prevalence of brucellosis at a herd level which ranged 21.8% in cattle herd in Malaysia (Anka et al., 2013) and 24.1% in dairy cattle herds in Thailand by using CELISA (Jittapalapong et al., 2008). However, the prevalence in this study was higher than the reports of India

showed that and 12.8% in organized dairy farms by using MRT (Trangadia et al., 2010) and 2.55% in dairy cattle herds by using MRT (Shome et al., 2015). Furthermore, more recent studies conducted around the world showed different prevalence of brucellosis at herd level; 32.5% in Mali (Musallam et al., 2019), 32.9% in India (Holt et al., 2021), 44.4% in cattle herd in Tanzania (Asakura et al., 2018) and 62.0% in Togo (Musallam et al., 2019), 12.6% in Cameroon (Musallam et al., 2019), 14.7% in Burundi (Musallam et al., 2019), 18.1% in Jordan (Musallam et al., 2015), 18.6% in Pakistan (Ali et al., 2017). But in some countries our prevalence was higher than the reported prevalence such as 4.6% in Pakistan (Shafee et al., 2011) and 6.5% in Uganda (Makita et al., 2011). The difference in prevalence between our study and the previous studies may be partly explained by the sample types and the methodology used in the study protocol as well as the difference in sensitivity and specificity of serological tests used for screening and confirmatory test. This factor contributes to the variability of results among researchers (Mangen et al., 2002, McDermott et al., 2002, Nielsen, 2002, Saegerman et al., 2004). The significant variation in the prevalence of brucellosis by MRT is also attributed to the study designs. The sensitivity and specificity of MRT have been explored in several studies. The sensitivity varied from 83.3% to 89.0% (Hunter et al., 1972) of 89.0 % (Hunter et al., 1972). However, Kumar et al. (2018) reported that MRT had 53.6% sensitivity and 98.7% specificity by using mELISA as a gold standard test. Significant disadvantages using MRT have been reported, including false positive results in milk samples collected shortly after parturition, around the end of lactation, or those collected from cows with mastitis or previous vaccination history (MacMillan, 1990). This study was conducted with care by avoiding all these situations.

MRT, first described in Germany by Fleischhauer (1937), is used as a routine test for brucellosis free herds and for identifying infected herds. The test detects IgM and IgA antibodies bound to fat globules, and have wide acceptability as it is cost effective, easy to perform and can cover a large population in a short time (Cadmus et al., 2008). MRT is an inexpensive test for the brucellosis surveillance in dairy herds because milk and whey samples can be obtained easily, and they have been widely used for testing herds or individual animals for antibodies. The stronger MRT reaction is, the more likely that *Brucella abortus* can be isolated by culture (Leech et al., 1964).

In this study, history of abortion, sharing community pasture and location were considered as risk factors of *Brucella spp*. infection in dairy farms. Our findings were different from those previous studies (Thin, 2007, Tun, 2007, Makita et al., 2011, Sanogo et al., 2012,) where a significantly higher brucellosis prevalence was found in larger size farms. It is inferred that the better practice for brucellosis prevention among the large size farms in our study area. A previous study (Tun, 2007) stated that hand milking and artificial insemination was not significantly related with brucellosis, which was in agreement with this study. The use of artificial insemination was not significant protection as suggested by Salman (1987), though using artificial insemination had a potential to reduce the risk for brucellosis. History of abortion was also agreed with the previous study by Thin (2007) and Makita (2011) that identified history of abortion as risk factor for brucellosis.

The test results generally reflect previous findings from serological studies of Kang'ethe (2000) and Kadohira (1997) which indicating a higher farm-level prevalence of brucellosis in extensive and/or communal grazing areas than in small stall-fed herds In this study, pasture sharing was significantly associated with MRT positive which agreed with the previous study (Makita et al., 2011) that reported the dairy herds with pasture sharing practice had a higher risk for brucellosis than a herd with movement restrictions. Pasture sharing has been previously described as a risk factor for disease transmission, presenting a risk for herd-to-herd transmission (Arif et al., 2017). In this study, MRT positive samples were found significantly in Taikkyi Township more than in Hlegu Township. This might be due to the fact that there was a higher proportion of free grazing practice on community pasture in Taikkyi Township.

The KAP analysis revealed that two thirds of the participants did not demonstrate good levels of knowledge and practices regarding brucellosis. Although most participants knew about brucellosis in animals, less than 50% knew that brucellosis is zoonotic disease, and it can cause abortion in cattle. These findings coincides with the previous studies from Tajikistan (Lindahl et al., 2015) and Kenya (Kang'ethe et al., 2007), which showed low disease awareness among farmers. The low level of knowledge and practices regarding brucellosis could be attributed to the lack of health education especially on zoonoses in this dairy production area. In relation to practices posing a risk for brucellosis transmission from animals to humans, consumption of raw milk has been previously described as one the most important risk practices (Young, 1995). In the current study, there was 17.8% of the participants reported that they have consumed unpasteurized milk and its products on a regular basis, suggesting an increased risk of brucellosis transmission. The reason for raw milk consumption in some participants was described as its freshness and better taste. About aborted placenta disposal, one third of dairy farmers reported burying aborted placenta with little personal protective equipment, while more than half of them preferred to eat or give placenta to others. These risky behaviors increase the risk of infection in humans that occurs through breaks in the skin, following direct contact with contaminated materials (Bercovich, 1998). In addition, majority of the farmers reported storing manure in their compound for more than six months and it was accessible by animals in their households. This practice prolongs the survival of the bacteria due to the humid environment and present as a risk for disease transmission (Abubakkar et al., 2011). In this study, 14.4% of the participants reported their feeding practice by pasture sharing which allows their dairy cows to contact with other animals. Pasture sharing has been previously described as a risk for disease transmission and the management of such pastures should be considered when implementing any control measures. Hence, mixing animals from different farm location through community grazing could present a risk for herd-to-herd transmission (Arif et al., 2017). The lack of awareness and these risk practices need to be recognized to control brucellosis in dairy cattle and prevent the disease spread to human.

There was no statistically significant difference between the education level and the knowledge and practices regarding brucellosis. While the education level of majority of the participants was undergraduate degree, but the higher education level has a higher percentage of good practices than the

undergraduate level. The studies conducted in Yemen (Al-Shamahy et al., 2000) and Tajikistan (Lindahl et al., 2015) illustrated that farmers with no or lesser level of education were not likely to have good practices related to brucellosis prevention. Our finding showed that farmers in Taikkyi Township were more likely to have better practices in comparison to those located in Hlegu Township. However, there was no statistical significantly correlation between KAP. Although most of the dairy farmers in this study showed good attitude levels, their lack of knowledge and inadequate practices regarding brucellosis prevention was significant. It is possible that, if dairy farmers were made aware of brucellosis, their knowledge and practices would improve.

In the study, samples were collected from lactating farms. Therefore, male cattle, which may play a role in disease transmission, were not tested for brucellosis. However, most farms did not keep males for breeding. Recall bias may have occurred during the interview. The scoring system was developed and agreed upon by brucellosis experts then the assigned questions' scores may have been impacted by the perceived importance of KAP in the local context.

CONCLUSIONS

Our study showed an overall prevalence of 17.2% for bovine brucellosis in the study area. The KAP survey identified that 35.1% of dairy farmers have good knowledge, 99.4% of good attitude and 31.0% of good practices. There was no correlation between knowledge, attitudes, and practices found in this study. Farm locations and feeding system were identified as the risk factors of having MRT positive. In conclusion, brucellosis remains as an understated public health problem in the study area and encouraging the use of MRT at a farm level would improve the disease detection according to its stable spot-on test and readily feasible for field conditions. However, no conclusion can be drawn about sensitivity of MRT because the exact situation of the animals tested is unknown in the study. Regular and systematic screening of brucellosis within herds is one of the essential components to control brucellosis in dairy farms and enhancing the safety of both farmers and consumers according to its zoonotic importance.

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CONFLICT OF INTEREST

The authors declare that they hold no conflicting interests with regards to the publishing of this manuscript.

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