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Sero-epidemiology of bovine tuberculosis in dairy cattle in Chattogram, Bangladesh

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ABSTRACT

Objective: Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* both in wild and domesticated animals including cattle, and is a significant public health concern due to its cross-species transmissibility. We conducted this study on the dairy farms in Chattogram district of Bangladesh to estimate the seroprevalence and potential risk factors at both animal and farm levels associated with the occurrence of bTB. We targeted to illustrate a complete picture of bTB to the farmers, policymakers and dairy practitioners.

Materials and Methods: Based on the highest density of intensive dairy cattle farms, we recruited three subdistricts namely Double Mooring, Shikolbaha, and Raozan of Chattogram for this cross-sectional study. We sampled a total of 538 animals from randomly selected 37 farms of the selected subdistricts. We collected blood samples from the animals for performing ELISA in the laboratory and used a pretested questionnaire for data collection and epidemiological analysis.

Results: We estimated the overall seroprevalence of bTB was 38.2% and 7.5% at the farm level and animal level respectively. Random effect logistic regression model estimated the low to moderate stocking density (OR=19.6, p=0.02) as the significant risk factor of bTB at the farm level whereas, farms own stock (OR= 3.4, p=<0.01) has been calculated as significant risk factors at individual animal level.

Conclusion: For a dairy-intensified area of any developing country like Bangladesh, a coordinated effort of both veterinarians and local public health officials is critical for implementing an efficient TB control program. A comprehensive survey is always recommended for early detection and control of the zoonotic spillover events of any organisms. Therefore, these research findings will aid in the prevention and control of bTB in the studied region and will prompt removal and good farm management practices. Overall, this study will make dairy farmers and policy planners aware of the necessity of continuous surveillance to eradicate TB from the farm levels in any developing and underdeveloped nations across the world.

Keywords: *Tuberculin, bTB, Prevalence, Dairy farming, Risk factor, ELISA*

INTRODUCTION

Mycobacterium bovis causes bovine tuberculosis (bTB), which is a major zoonotic disease worldwide (Proano-Perez et al., 2006). The disease is most commonly found in cattle, although it can also be found in other domestic animals, wildlife, and humans (Filia et al., 2016). Humans are infected mostly through inhalation of aerosols created by infected animals, as well as intake of raw, unpasteurized milk (Thakur et al., 2012). The dairy

business is a top priority for emerging Asian, African, Latin American, and Caribbean countries, including Bangladesh. Due to a lack of adequate management techniques, the dairy industry's intensification is promoting bTB transmission (Proano-Perez et al., 2006). The disease is rising in many parts of the world especially in Asia and Africa(Collins, 1993; Ameni et al., 2003). This is due to lack of organized and practicable test methods for mass screening (Asiak et al., 2007). Due to its substantial economic impact on animal production and zoonotic nature, bTB has been a serious public health concern for the past three decades.

Seroprevalence of bovine tuberculosis at various levels (cattle and farm) varies widely over the world. The reported prevalence of bTB seroprevalence ranged from 5.9% to 30% (Rahman and Samad, 2008; Mahmud et al., 2014; Mondal et al., 2014; Chakraborty et al., 2015). Many research have been conducted around the world to establish the risk variables related with bTB seropositivity. BCS (poor) (OR=4.4), parity (4 calving) (OR=2.3), history of coughing (OR=6.7), and bigger herd size (OR=5.9) were found to be significant risk factors for bTB in cattle in a prior study in Bangladesh (Mondal et al., 2014; Chakraborty et al., 2015). Rapid removal of infected animals is critical for limiting transmission, and tuberculin skin tests can effectively diagnose early *Mycobacterium bovis* infection in cattle(Buddle et al., 2009). Infected animals are occasionally anergic to the skin test in the late stages of disease due to increased humoral antibodies (Lilenbaum et al., 1999). Bovine tuberculosis diagnosis by antibody based tests are using for many years (Pollock et al., 2001)and it is used to identify anergic cows (Janeiro, 2006). The antibodies are usually found only in later stages of disease and recently infected animals will not react to antibody based test (Wahlström, 2004).The use of ELISA as a beneficial supplemental tool in field conditions for the control of bovine tuberculosis has been demonstrated in practice (Janeiro, 2006).The diagnosis of bovine tuberculosis is a complicated process that relies on a range of laboratory techniques, including serological assays. Traditional ELISA is used for serological testing (Sensitivity and Specificity are 83.2-93.1% and 86.5-98.4%respectively) (Lilenbaum et al., 1999; Lilenbaum et al., 2001; Whelan et al., 2008; Souza et al., 2012) are most frequently used.

Bangladesh is one of the world's most densely inhabited countries, with people living in close proximity to their animals. As a result, the transmission of bTB infection from animals to humans is quite likely. Because of the lack of a monitoring program, limited diagnostic facilities, and the lack of veterinary inspection in slaughterhouses, the status of bTB in Bangladesh remains unclear. On this background this study aimed to estimate the animal and farm level seroprevalence of bTB based on enzyme linked immunosorbent assay and to identify the associated risk factors in order to develop effective bTB prevention and control techniques.

MATERIALS and METHODS

This study was approved by the ethics committee of Chattogram Veterinary and Animal Sciences University and with the decision number CVASU/Dir (R&E) EC/2023/500 (6).

Description of the study areas

For this study, three key dairy cattle areas in Chattogram were purposefully chosen. They were: 1) Double Mooring (Urban), 2) Shikalbaha (Peri-urban), and 3) Raozan (Peri-urban) (Rural). Between 22°18' and 22°21' N latitudes and 91°48' and 91°51' E longitudes, Double Mooring is an important portion of the Chattogram metropolitan region, located alongside the Bay of Bengal. In comparison to other metropolitan areas, it has the highest number of intensive dairy farms with high yielding cross breeds (N=415) (DLO, DLS of Chattogram, Personal Communication, 2018). It is the city's main source of milk. Shikalbaha has the most dairy cattle farms (N=400) of all the peri-urban communities in the Chattogram metropolitan area (DLO, DLS of Chattogram, Personal Communication, 2018). It lies between 22'11' and 22'24' N latitudes and 91'48' and 91'52' E longitudes, alongside the river Karnaphuli (Anon, 2022). Because this location has excellent road and water connections to various parts of Chattogram, a variety of companies have sprung up, including a Dairy Milk Processing Plant. Agriculture is also a significant source of income for the residents of this area. Raozan is 32 kilometers from the Chattogram metropolitan region, with latitudes of 22°25' and 22°40' N and longitudes of 91°51' and 91°59' E. (Anon, 2022). It is bordered on the south by the Karnaphuli River and on the west by the Halda River. Road and water communication are excellent. It has a large number of intensive dairy farms (N=150) with exotic breeds that produce excellent yields (DLO, DLS of Chattogram, Personal Communication, 2018). In Figure 1, we depicted the

study area's geographical position using ArcGIS software 10.8 (Sayeed et al., 2020a).

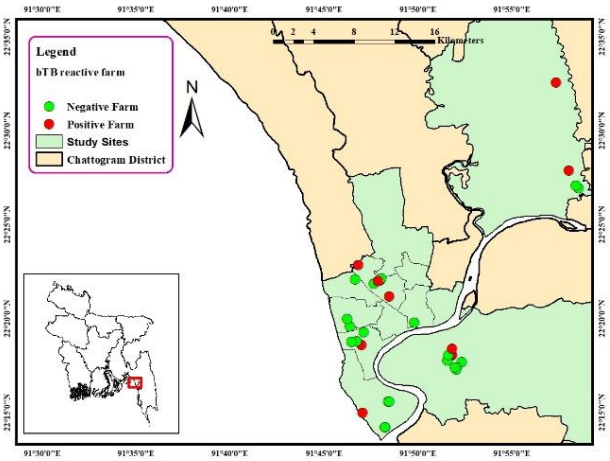

Figure 1. Map bTB reactive study farm.

Study population, duration and study design

A bTB sero-survey was conducted in dairy cattle of high yielding exotic breed in Chattogram during May 2014 to July 2014.Commercial dairy farms and dairy cattle of Chattogram district were considered as a reference population. Dairy farms and dairy cattle of three study areas of Chattogram were considered as a source population. A farm consisting of at least 15 exotic cattle breed was defined as the smallest sampling unit. Accordingly, a total of 96 farms were enlisted among which 65, 21 and 10 farms were belonged to Double Mooring, Shikolbaha and Raozan, respectively.

Sample size calculation and sampling

A total of 41 farms were required assuming 7% farm level bTB prevalence (Noorrahim et al., 2015) with ±5% precision, 90% confidence interval and 1.0% design effect (http://www.openepi.com/Menu/OE_Menu.htm). However, employing a proportionate chance of random sampling with some variance, 37 farms (90% response rate) were recruited. As a result, 22 farms in Double Mooring (eligible=538, total animal=959), 10 in Shikolbaha (eligible=200, total animal=307), and 5 in Raozan (eligible=108, total animal=204) were distributed. Individual animals aged 6 months or longer were regarded suitable for this study's bTB investigation. The chance of an individual animal exposing bTB increased with age, according to past research carried out in Tanzania and Uganda (Cleaveland et al., 2007; Inangolet et al., 2008), and in Bangladesh, cases of bTB in young animals (under 6 months old) were not being reported (Mahmud et al., 2014b) hence we considered those animals for sampling that were older than 6 months As a result, all animals that satisfied the selection criteria were included in the study, with a total of 538 animals in Double Mooring, 200 in Shikolbaha, and 108 in Raozan.

Collection of blood samples

ELISA was performed on 52% of first tuberculin test (CFTT) cattle, with typical collection ranges of 25-93% of CFTT treated animals across farms. We also performed the ELISA on 34 of the 37 CFTT farms. Due to farmer non-cooperation and technical difficulties, three farms were overlooked. We collected a total of 442 blood samples from 34 farms. Five (5) mL blood was extracted aseptically from the jugular vein of each animal and transferred to a sterile vacutainer (without anticoagulant) labeled with a unique identifying number. Within 2-3 hours of collection, the samples were transported to Chattogram Veterinary and Animal Sciences University's Physiology and Biochemistry laboratory. The whole blood sample was spun at 3000 RPM for 30 minutes to separate the serum. The samples were then kept in the laboratory at -20°C until they were tested at the Poultry Research and Training Centre's laboratory (PRTC).

Recording of data

Face-to-face questionnaire interviews and physical observations were used to collect baseline and risk factor information at the farm and animal level. Each interview and observation lasted around an hour, and each farmer's verbal consent was obtained prior to the commencement of the interview. Farm-level data is made of farmer and farm address, farm coordinates, farmer's education, farmer's knowledge about bTB, farm establishment date, farm size, farm house type, stocking density, floor type, ventilation status, farm sanitary condition, farm biosecurity, mixed with other animals and feeding system. Animal level information consisted of breed, sex, age, parity, body condition score (BCS), lactation status, milk production, pregnancy status and source of animal.

Variable measurement

Farm coordinate information was taken using GIS machine (Model no: eTrex 10 and Company: Garmin, China). Risk factors were classified based on standard essential facilities of a dairy farm. Potential risk factors both in animal related and farm related were recorded by an organized questionnaire and data entry sheet. Collected animal related risk factors were age, breed, sex, parity, body weight, BCS, lactation and pregnancy status, sources of animals etc. and farm establishment date, total population, cattle

movement, previous history about PPD, source of semen, farm management system etc. were collected both by asking the owner and direct observations. Personal information on TB was also gathered by asking owners about their educational level, understanding of bTB (specifically, how it is transmitted, symptoms, etc.), and history of TB in farm workers or family members, as well as contact with animals.

Laboratory evaluation

This study used the AniGen BTB Ab ELISA test kit from BioNote, Inc., 2-9, Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea (445-170). The test kit was kept between 2 and 8 degrees Celsius until it was used. The PRTC Laboratory at Chattogram Veterinary and Animal Sciences University examined serum samples for ELISA.

Data entry and statistical evaluation

Data generated from the study were entered into the spreadsheets of Microsoft Excel 2007 program. Data were then cleaned, coded, and checked for integrity before exporting to STATA-IC13 (Stata Crop, 4905, Lakeway Drive, College station, Texas 77845, USA) for epidemiological analysis.

Descriptive analysis

We calculated the bTB seroprevalence based on ELISA results. A bTB sero-positive farm was considered as a positive farm when a farm had at least one animal tested reactive to ELISA. Bovine tuberculosis sero-positive and negative farms were displayed according to farms and areas using the respective coordinate information and ArcGIS software Version 10.2.1 (Sayeed et al., 2020a). We computed the seroprevalence of bTB at animal level using the total number of ELISA reactive animals divided by the total number of animals tested. The Fisher's exact test and Chi-Square test was performed between categories of the selected factors and a binary response variable for bTB ELISA result at farm/animal level (Yes/No) to assess differences in the proportion of bTB sero-positives between categories of each factor. The results were expressed in a frequency number and a percentage along with 95% confidence interval. The level of significance was set at 0.05 or less.

Risk factors analysis

A set of demographic and management related variables recorded using questionnaire were considered for the risk factor analysis of bTB sero-prevalence. Risk factor analysis for bTB seroprevalence at farm level and animal level were done by random effect logistic regression analysis (Sayeed et al., 2017) where "Farm ID was accounted as a cluster variable". In animal level, significant factors ($p \leq 0.30$) at the univariate *Chi-Square* test were moved to multivariate analysis and in farm level; significant factors ($p \leq 0.30$) at the univariate Fisher's exact test were moved to multivariate analysis. We have used the standard model building procedure along with the checking of confounding and interaction variables, and independency among independent variables as well as validity of the model was explained as described by (Sayeed et al., 2020b). We presented the results as odds ratio (OR), Standard Error (SE), 95% Confidence Interval (CI), and p value.

RESULTS

Seroprevalence estimates of bTB

The seroprevalence of bTB was 38.2% (95% CI: 21.1-52.4) at farm level and 7.5% (95% CI: 4.4-12.3%) at animal level (Table 1).

Table 1. Overall seroprevalence of bovine tuberculosis (*Mycobacterium bovis*) in dairy cattle of Chattogram district (N=442).

Level	No of units tested	No of positive units (%)	95% CI
Farm	34	13 (38.2)	21.1-52.4
Individual level (Farm as cluster)	442	33 (7.5)	4.4-12.3
Individual level (Ignoring farm as cluster)	442	33 (7.5)	5.3-10.3

Risk factors of bTB

Univariate association between factors and bTB seroprevalence at farm level

The farm level bTB seroprevalence was significantly higher ($p=0.03$) in case of larger farm size (N=33-160) than the smaller farms size (N=1-32) (Table 2). Farms belonging to Double Mooring, older farms, face-in housing system, low or moderately populated farms, less frequent contact between animals and humans and farms of poor biosecurity standard, irrespective of kind of prevalence, had greater bTB prevalence, but the association was statistically insignificant ($p<0.05$) (Table 2).

Univariate association between factors and bTB seroprevalence at animal level

Animal level bTB seroprevalence significantly higher in case of own stock (*P*=0.001) than the purchased stock. Others factors including BCS, parity, lactation status, pregnancy etc. are statistically insignificant (Table 3).

Risk factors analysis

Farm level risk factors analysis (random effect regression model)

Farm size with larger stocking (N=33-160) has significantly higher (OR=26.2) risk for bTB than the smaller farm size (N=15-32). Beside this, farms with low/moderate stocking density has significantly higher (OR=19.6) bTB infection than the optimum/high stocking density (Table 4).

Table 2. Univariate association between factors and bTB seroprevalence at farm level in Chattogram, Bangladesh (Fisher's Exact Test)

Factor	Category	ELISA		
		+ n (%)	-n	p
Area	Urban	8 (40.0)	12	1.08
	Peri-urban	5 (35.7)	9	
Farm size/population size	Min-32	3 (17.7)	14	**0.03**
	33-max	10 (58.8)	7	
Establishment year	1980-2000	8 (47.1)	9	0.48
	2001-2013	5 (29.4)	12	
Housing	Face in	9 (52.9)	8	0.16
	Face out	4 (23.5)	13	
Floor type	Concrete	10 (38.5)	16	1.30
	Herring bone	3 (37.5)	5	
Ventilation	Poor/Fair	6 (33.30)	12	0.79
	Good	7 (43.8)	9	
Stocking density	Low/Moderate	10 (41.7)	11	0.29
	Optimum/High	3 (23.1)	10	
Sanitary Condition	Poor/fair	10 (43.5)	13	0.60
	Good	3 (27.3)	8	
Contract between human and other animal	Minimum	7 (41.2)	10	1.11
	Moderate/Intimate	6 (37.5)	10	
Mixed with other animals	No, not all	10 (37.1)	17	1.10
	Yes, (sometimes with other animal species of the farmers)	3 (42.9)	4	
Feeding	Separate	11 (36.7)	19	1.00
	Common/both	2 (50.0)	2	
Biosecurity	Poor	5 (50.0)	5	0.60
	Fair/Good	8 (33.3)	16	
Education of the farmer	Illiterate to HSC	5 (33.3)	10	0.87
	Graduate and or more	8 (42.1)	11	
Knowledge about TB	Yes	8 (30.8)	18	0.40
	No	4 (57.1)	3	

Table 3. Univariate association between factors and sero-bTB at animal level in Chattogram, Bangladesh (*Chi-Square* Test).

Factor	Category	ELISA		P (*Chi-Square* Test)
		+ n (%)	-n	
Age	Min/66	15 (6.5)	216	0.34
	68/max	18 (8.9)	184	
Parity	Min/2	7 (5.2)	128	0.43
	3	10 (9.1)	100	
	4/Max	14 (8.6)	149	
BCS	Min/3	18 (8.5)	195	0.45
	3.5/4	15 (6.6)	214	
Lactation	No	16 (6.6)	228	0.42
	Yes	16 (8.6)	170	
Pregnancy	No	16 (6.6)	228	0.42
	Yes	16 (8.6)	170	
Source	Own stock	17(14.4)	101	**0.001**
	Purchased	16 (5.1)	299	
Milk production	Min/16 litter	16 (6.7)	214	0.56
	17/Max litter	15 (8.5)	161	
Farm area	Urban	8 (40.0)	12	1.08
	Peri-Urban/Rural	5 (35.7)	9	

Table 4. Outputs of random effect model (at farm level).

Factor	Category	OR	95% CI	p value
Farm size/population size	15-32	1.0		
	33-160	26.2	2.2-319.1	0.01
Housing	Face out	1.0		
	Face in	4.5	0.7-30.0	0.123
Stocking density	Optimum/High	1.0		
	Low/Moderate	19.6	1.5-261.5	0.024

Table 5. Outputs of the random effect model (at animal level).

Factor	Category	OR	95% CI	p value
Source	Purchased	1.0		
	Own stock	3.4	1.4-8.1	0.006
Age	Min/66	1.0		
	66/max	1.8	0.8-4.0	0.127

Animal level risk factors analysis (random effect regression model)

Animal from own source has a significantly higher (OR=3.4) bTB infection than the animal from purchased source in the farms (Table 5).

DISCUSSION

Bovine tuberculosis is one of the important zoonotic diseases and a serious public health concern all over the world (Proaño-Perez et al., 2006; Javed et al., 2010; Awah-Ndukum et al., 2012). Bangladesh, in particular, is one of the densely populated countries in the world and peoples live very closely with their domestic animals. Therefore, likely transmission of bTB infection between animals and humans can easily be occurred in Bangladesh context. So, sero-epidemiological exploration of bTB was warranted to identify appropriate strategies to prevent and control bTB. This section discusses the important

findings of the current study on sero-epidemiology bTB and their implications as well as potential limitations.

In the current investigation, farm-level bTB seroprevalence was high (38%) but could not be compared due to a lack of comparable national and international records. Other Bangladeshi research back up the current study's 7.5% animal (cross-bred) level bTB sero-prevalence: 7.9%in a combination Red Chattogram cattle and Holstein Friesian in Chattogram (Chakraborty et al., 2015), 7.9% in Holstein Friesian in Sirajganj (Mahmud et al., 2014), 5.9% in Holstein Friesian in Mymensingh (Mondal et al., 2014) and5.88% in crossbred cattle of Chattogram metropolitan area (Chakraborty and Prodhan, 2020). However, some previous studies have found greater levels of bTB sero-prevalence, such as 30% in Mymensingh's Red Chattogram cattle (Rahman and Samad, 2008). Different countries had varying levels of bTB seroprevalence in cattle. In India, estimates of bTB seroprevalence ranged from 3.2-13.8% (Prakash et al., 2015; Didugu et al., 2016), 1.4% in Albania (Koni et al., 2015), 1.0% in Lao People's Democratic Republic (Vongxay et al., 2012), 10.4% in Ethiopia (Ameni et al., 2010), 37.2% in Cameroon (Awah-Ndukum et al., 2012), 36.3% in Nigeria (Asiak et al., 2007), 3.49% in east Algeria (Djafar et al., 2020) and 34.38% in Pakistan (Leghari et al., 2020).Geographical differences breed types, and management approaches could explain the disparities in bTB seroprevalence in the cited references (Omer et al., 2001; Lilenbaum et al., 2007; Nuru et al., 2015; Endalew et al., 2017).

Variables at the individual animal and farm level were studied to better understand the disease's epidemiology. By using a random effect model, the source of cattle (own stock: OR=3.4, 95% CI: 1.4-8.1, p=0.006) was found as a possible risk factor at the individual animal level. The potentiated risk factors by random effect model were population size (Larger: OR=26.2, 95% CI: 2.2-319.1, p=0.010) and stocking density (Low or moderate: OR=19.6, 95% CI: 1.5-261.5, p=0.024). International cattle movement or purchase has been recognized as a herd-level risk factor (Tschopp et al., 2009; Singhla et al., 2017). Animals were classified into two categories in this study: own stock and purchase. Surprisingly, purchased animals had a lower prevalence of bTB infection than own-stock cattle in this study. Although, an author reported that purchased cattle (Tschopp et al., 2009a) is a risk factor for bTB on the farm the difference between the earlier findings with our findings might be due to the purchasing of new cattle from low bTB risk areas (Sedighi and Varga, 2021), other factors including the difference includes the husbandry practice and geographical area of the study. The procurement of livestock from non-endemic areas could be one of the explanations (Dejene et al., 2016) or less endemic areas from this study. As an aerosol transmitting disease, close contact between animals is an important risk factor for bovine tuberculosis (Ameni et al., 2006). Unfortunately, in this study, farms with low and moderate animal density had a higher incidence than farms with optimal and high animal density. Because of the small number of reactor farms, some critical risk considerations may be overlooked.

By random effect model, population size is a potential risk factor in this study, which is consistent with some prior studies from various nations including Bangladesh, (Islam et al., 2020; Islam et al., 2021); Ecuador, (Proano-Perez et al., 2006; Proaño Pérez et al., 2009); Eritrea, (Omer et al., 2001); Zambia, (Cook et al., 1996); Tanzania, (Cleaveland et al., 2007); Ethiopia, (Ameni et al., 2003); Nigeria, (Ibrahim et al., 2010); Ethiopia, (Ameni and Erkihun, 2007). In case of larger herd, the risk of introduction of infected animal also become high (Cleaveland et al., 2007; Cadmus et al., 2010). The dairy farms those are not under bTB control measures, one infected cattle can transmit the disease to 2.2 cattle per year and this was calculated from Argentina (De Kantor and Ritacco, 2006). *Mycobacterium bovis* transmission mainly occurs through aerosols (Skuce et al., 2012)which depends on the density of animals (Huang et al., 2013) and especially in intensive farming practices this is more appropriate in larger farms than smaller. Unfortunately, in this investigation, stocking density (low or moderate) was found as a potential risk factor. It could be because there are fewer seropositive farms.

The power of this study was not so high because only 13 farms were found as a reactor farms and some important risk factors in farm level may have been missed. In individual cattle level, eight (8) variables were analyzed but only source of animals (own stock/ purchased) was found as a potential risk factors (p=0.001). Due to minimum number of infected cattle (33), some important risk factors in cattle level were also might be missed. Also, some farm owners and attendants were not fully motivated due to lack of incentive. Due to this some CFTT positive farms and animals were missed from collection of blood. Due to some unavoidable

circumstances collection of blood from all tuberculin tested cattle were not possible. So, blood was collected only from 52.2% of tuberculin tested (CFTT) cattle.

CONCLUSION

The overall seroprevalence of bTB (*Mycobacterium bovis*) was 7.5% in intensively managed commercial dairy farm with high yielding cross breeds. In farm level, identified risk factors by multivariate logistic regression modeling were population size and stocking density. In individual cattle level, identified risk factor was source of animal. The overall high bTB seroprevalence suggest that the percentage of late-stage diseased animals were high in dairy farms of Chattogram region and it was due to absent of bTB control measure. Our study recommends that, face in housing system has more susceptible to bTB transmission thereby we should try to avoid this system and can use face out system where there is less chance to bTB transmission among the susceptible animal. Moreover, high population size in farm has more susceptibility to bTB transmission that lower population size of the farm, so farms with a higher population should managed with extra precaution. Animal should purchase after determined whether the source stock is bTB free. Unknown source of animal should avoid during purchase as they may have bTB and some others infection in the animal. The education and awareness level for bTB among the farmers should increase which may help to prevent bTB transmission between the animal and human as well.

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