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## PREVALENCE OF BOVINE AND AVIAN TUBERCULOSIS IN CALVES OF CENTRAL CATTLE BREEDING AND DAIRY FARM, SAVAR, DHAKA

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### ABSTRACT

Hasan MK, Rahman MM, Kisku JJ, Rahman MB, Rahman MM, Rahman MM (2016) Prevalence of bovine and avian tuberculosis in calves of central cattle breeding and dairy farm, Savar, Dhaka. *J. Innov. Dev. Strategy*. 10(2), 5-12.

An attempt was undertaken to investigate the occurrence of bovine and avian tuberculosis in calves at Central Cattle Breeding and Dairy Farm, Savar, Dhaka. A total of 183 calves were screened for bovine and avian tuberculosis by caudal fold tuberculin (CFT) and comparative cervical tuberculin (CCT) tests. The percentages of responders to CFT test in calves were 12.02%. The overall percentages of reactors to CCT test in calves were 10.92%. In CCT test the reactors were 6.56% for bovine tuberculosis, .0546% for avian tuberculosis and 3.83% for mixed type respectively. Among the 183 calves with a percentage of 13.89 were found as positive in the age group between 7 months to 12 months, with a percentage of 8.62 were found as positive in the age group between 13 months to 18 months and in 19 months to 24 months no calves were found positive by CCT test in this study. The male calves (12.698%) showed more positive to tuberculosis than the female calves (11.666%) of the same area in Caudal Fold Test (CFT) and in Comparative cervical tuberculin (CCT) test the male calves (11.11%) again showed somewhat higher percentage than female calves (10.83%). The study indicates that bovine and avian tuberculosis may cause dangerous effects on human health as well as livestock of Bangladesh so its prevention and eradication steps must be taken without any delay.

**Key words:** tuberculosis, mycobacteria, cattle farm, Bangladesh

### INTRODUCTION

Bovine tuberculosis is a chronic contagious respiratory disease of cattle and buffaloes transmitted horizontally, within and between species, by the aerosol and ingestion (O'Reilly and Daborn, 1996). Tuberculosis plays a central role in public health and animal health because of its severity in humans, in addition to the economic losses related to affected herds (Rodriguez *et al.* 1999). It has been recognized from 176 countries as one of the important bovine diseases causing great economic loss (Hines *et al.* 1995; Samad 2008). *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium avium* all the three species are capable of causing disease in humans although *M. tuberculosis* is the most common in humans with continues to be a major cause of morbidity and mortality throughout the world. Bovine tuberculosis (bTB) has been significantly widely distributed throughout the world and it has been a cause for great economic loss in animal production. In developed countries, bTB in animals is a rarity with occasional severe occurrences in small groups of herds. In developing countries, however, such as in 46% of African, 44% of Asian and 35% of the South American and the Caribbean countries, sporadic occurrences and (particularly in Africa 11%) enzootic occurrences of bTB have been reported (Cosivi *et al.* 1998).

Tuberculosis can be transmitted between cattle percutaneously, by oral ingestion, venereally and through the teat canal (Coetzer and Tustin, 2005). Ingestion of *M. bovis* directly, e.g. from swallowing infected milk or from contaminated pastures, water or fomites is considered secondary to respiratory spread (Menzies and Neill, 2000). However the main route is through inhalation of infected droplets from another animal or dried secretion in dust. Genital transmission occurs if the reproductive organs are infected, but this remains extremely rare (Neill *et al.* 1994), as are congenital and vertical transmitted infections. The congenital route is also important and calves may be born with bovine tuberculosis. Humans with open tuberculosis caused by *M. bovis* can transmit it to cattle by the aerogenous route, spitting, coughing or urinating (Coetzer and Tustin, 2005).

Mycobacteria are aerobic, acid-fast, rod-shaped, non-motile bacteria. Historically, they were considered as unencapsulated organisms but it is now known that pathogenic mycobacteria contain a "capsular structure" that protects the bacteria from microbicidal activities of the macrophages and also contributes to the permeability barrier of the mycobacteria cell envelope. Mycobacteria belonging to the *M. tuberculosis* complex are intracellular pathogens and can grow inside phagosomes and phagolysosomes (Rastogi *et al.* 2001). The cell envelope (bacterial cytoplasmic membrane, the cell wall and the mycobacterial capsule) is important to enable mycobacteria to survive and grow intracellularly. It is also important for the ability of mycobacteria to modulate the immune response in the host (Rastogi *et al.* 2001). Due to the high content of lipids in the cell wall, the bacterium is robust and has a long survival in the environment (Anonymous 2003). The bacteria resist drying but are killed by sunlight, ultraviolet radiation and pasteurization (Hirsch and Zee, 1999). In vitro, most mycobacteria belonging to the *M. tuberculosis* complex or the *M. avium* complex are slow growers, having a mean division time of 12-24 hours. A culture thereby requires about 15 to 28 days. The rare pathogens, including the saprophytes, are fast growers with a culture available within two to seven days (Rastogi *et al.*

2001). *M. bovis* may have a long survival in the environment (Kelly and Collins, 1978; Morris *et al.* 1994; O'Reilly and Daborn, 1995; Scanlon and Quinn, 2000). Several factors influence survival, such as the initial number of bacteria present, the organic matter, pH, temperature, sunlight, humidity and possible interactions with other microorganisms (Scanlon and Quinn, 2000). The survival of pathogenic bacteria is greater in soil or sub-soil than in the soil-surface or on herbage. *M. bovis* can be expected to survive up to two years in sub-soil or in slurry-treated soil (Kelly and Collins, 1978; Morris *et al.* 1994), in faeces up to five months in winter (in England) and shorter during warmer periods, less than two months in summer (Stenhouse and Hoy, 1930). However, Menzies and Neill (2000) conclude that under natural conditions it appears that survival in the environment (in Northern Ireland) is only a few weeks.

The primary sources for this zoonotic infection in most situations are consumption of unpasteurized milk and the close association between humans and animals (Daborn and Grange, 1993). Most rural inhabitants and some urban dwellers of Africa still consume unpasteurized, soured milk, potentially infected with *M. bovis*. Milk-borne infection is the main cause of nonpulmonary tuberculosis in areas where bovine tuberculosis is common and uncontrolled (Daborn *et al.* 1996).

Many methods are available for diagnosis of tuberculosis in infected animals however the single comparative intradermal tuberculin test (SCITT) is most widely used for diagnosis and eradication of Bovine tuberculosis (OIE 2008). In Bangladesh so far the single intradermal (SID) skin test with purified protein derivative (PPD) has been used to detect the prevalence of BTB, and an overall 5.9% cattle in the district of Pabna (Pharo *et al.* 1981), 3.05% cattle in the district of Mymensingh (Samad and Rahman, 1986) and 27.5% breeding bulls (Islam *et al.* 2007) showed positive reaction to the tuberculin test. Serodiagnostic tests, ICGA as Antigen Rapid Bovine TB Ab Test Kit has been used for the first time in Bangladesh to detect the prevalence of BTB and its effect on milk production in lactating cows (Samad and Rahman, 2008).

As evident from the preceding discussion, the prevalence of the disease varies from region to region and even from one farm to another in same locality. Countries known to be disease-free have spent years working hard through continuous monitoring and test and slaughter policies. However, unfortunately, bovine TB has never been taken seriously in Bangladesh. Sufficient information regarding its incidence in bovine calves and its economic impact on livestock industry is not available. There is little empirical data regarding how the prevalence of infection changes over time in large, free-ranging mammals. No serious efforts are underway to estimate the gravity of risk and its threat to the public health. The existing situation calls for a comprehensive program to address TB in livestock species including buffaloes in order to improve the lot of livestock and to safeguard the human population from this menace. Considering the above fact, the present study was designed to find out the prevalence and risk factors of bovine and avian tuberculosis in bovine calves in Central Cattle Breeding and Dairy Farm.

## MATERIALS AND METHODS

The study was carried out in calves in Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka. Bovine calves on the farms were screened for the presence of bovine tuberculosis (bTB) and avian tuberculosis (aTB) during the period of February to August 2010. A total of 183 calves on the farms were tested by caudal fold tuberculin (CFT) test using bovine purified protein derivative (bPPD). Among the 183 calves, the responders were further subjected to single comparative cervical tuberculin (CCT) test using both bovine PPD (bPPD) and avian PPD (aPPD). Both the PPDs were obtained from a licensed laboratory in Italy (Istituto Zooprofilattico sperimentale dell'Umbria e delle Marche, Perugia).

Bovine PPD was injected at the lower site and the avian PPD was injected at the upper site of the neck region. The difference in skin thickness after 72 hours of inoculation was measured by slide calipers. The results were recorded and analyzed to determine the prevalence of bTB and aTB. Additionally, evaluations of risk factors associated with the occurrence of tuberculosis in bovine calves were also performed.

### Selection and grouping of animals

Of the 183 calves, 63 were male and 120 were female calves. These calves were grouped into three as group-1 (age within 6-12 months), group-2 (age within 13-18 months) and group-3 (age within 19-24 months). Date of birth, age, breed and other managemental information were recorded.

### Tuberculin Test

#### Caudal Fold Tuberculin (CFT) Test

This is the primary screening test to identify animal potentially infected with bovine TB. The test measures the immune response to *Mycobacterium bovis*, the causative agent of bTB. The test involves the intradermal injection of 0.1 ml bPPD with a hypodermic syringe in the skin of the caudal fold (the fold of skin at the base of the tail). If the animal has been exposed to mycobacteria, the immune system responds with inflammatory cells at the injection site to cause swelling and/or discoloration of the skin. After 72 hours inspects and palpates the injection site to evaluate for a response. Marked edematous swelling, reddening at the injection site classifies the

animal as a responder. If no response is noted, the animal is classified as CFT test-negative. Responder animals were further tested with CCT test for confirmation.

### Comparative Cervical Tuberculin (CCT) Test

This test is a confirmatory skin test to determine if a responder's positive CFT test is more likely due to *M. bovis* or *M. avium*. Shaving of two sites in the middle third of the neck on one side (right or left hand side was used in this test), one above the other. At each site, a fold of skin should be measured using a calipers and the measurement recorded in millimeters. bPPD 0.1 ml and aPPD 0.2 ml (Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy) injected intradermally. The upper site is used for the avian PPD and the lower site for the bPPD. The skin thickness was measured at 72 hours post injection and the differences in skin thickness were recorded. The differences in pre and post test skin thickness determine the test results. Results were then classified as negative or suspect or reactor depending on the thickness of skin.

The results were interpreted according to OIE standards (OIE 2004):

- If the reaction is  $\geq 4.0$  mm greater than the test is considered reactor.
- If the reaction is between 3.0 and  $< 4.0$  mm than the test is considered suspicious.
- If the reaction is  $< 3.0$  mm than the test is considered negative.

Table 1. Schedule and procedure of inoculations

Test	Reagent	Site	Route	Dose	Time of observation
CFT test	bPPD	Caudal fold	i/d	0.1 ml	72 hours
CCT test	aPPD	Neck region (upper)	i/d	0.2 ml	72 hours
	bPPD	Neck region (lower)	i/d	0.1 ml	72 hours

aPPD = Avian purified protein derivative, bPPD = Bovine purified protein derivative

### Reading of the results of inoculations

In CFT test the reading was taken 72 hours after the inoculation. The positive tuberculin reaction was evident from an inflammation of sensitive nature at the point of inoculation. The area of the characteristic swelling ranged from the size of a small pea to that of an orange. The swelling was either soft and edematous or somewhat hard in nature. The swelling was estimated by palpation at the site of inoculation, while the animal showed the sign of pain. In CCT test two injection were made intradermally; bPPD at upper site and aPPD at lower site at neck region. Reading was taken after 72 hours with slide calipers. The difference in thickness before and 72 hours after injection was the measurement of reading.

Table 2. Reading of the results of tuberculin test 72 hours after inoculations

Test	Reagent	Time of observation	Reading of the results of inoculations	
CFT Test	bPPD	72 hours	Marked swelling indicates responder	No/ slight swelling indicates negative
CCT test	aPPD	72 hours	Swelling above $\geq 4$ mm from the initial reading indicates reactor	Swelling 3.0- $<4$ mm from the initial reading indicates suspect and $<3$ indicates negative
	bPPD	72 hours	Swelling above $\geq 4$ mm from the initial reading indicates reactor	Swelling 3.0- $<4$ mm from the initial reading indicates suspect and $<3$ mm indicates negative



Fig. 1. A) Swelling at inoculation site of CFT indicated Responder in Calves, B) The calf showing bTB Positive and measuring of skin thickness with slide calipers 72 hours after inoculation of PPD in calves.

## RESULTS AND DISCUSSION

### CFT test using bPPD

In this study 183 Bovine calves of different sex, age and types in Central Cattle Breeding and Dairy Farm, Savar, Dhaka were examined to determine the incidence of tuberculosis by CFT test. Out of 183 calves, 22 calves (12.02%) were reacted to CFT test with bovine PPD which showed marked swelling of the inoculation site at the caudal fold and 2 female calves (1.24%) showed suspicious reaction with the test which showed only slight swelling at the site of inoculation (Figure 1) and the remaining 159 calves (86.88%) did not react or showed no swelling at the site of inoculation.

Table 3. Results of CFT test using Bppd

Nature of reaction	Number of cases (%) by sex		Number of cases (%) by age (Months)		
	Male (63)	Female (120)	7-12 (108)	13-18 (58)	19-24 (17)
Positive	8 (12.698)	14 (11.666)	17 (15.741)	5 (8.62)	0 (0.00)
Suspicious	0 (0.00)	2 (1.66)	0 (0.00)	2 (3.77)	0 (0.00)
Negative	55 (87.301)	104 (86.66)	91 (84.26)	51 (87.93)	17 (100)
Total	183				

Of the 183 calves which were subjected to CFT test, the number of male reactor was 08 (12.698%) while the numbers of female reactors were 14 (11.666%). Of the 183 Bovine calves which were subjected for CFT test, the number of suspicious male reactors was 0 (0.00%) while the numbers of suspicious female reactors were 2 (1.69) in same test.

### Results of CFT test on the basis of age

Among the 183 Bovine calves of different age those were subjected to initial CFT test, highest 15.741% of reactor calves were observed in the age group between 7 to 12 months, 8.62 percentages of positive reactor and also 3.77 suspicious calves were found in the group between 13 to 18 months. No reactor to CFT test was found in Bovine calves more than 19 to 24 months of age.

### Results of CCT test using bPPD and aPPD

All the reactors and suspected Bovine calves at CCBDF farm were subjected CCT test using both aPPD and bPPD at two sites (upper and lower sites, respectively) of neck. The reactors calves showed inflammatory swelling on one side or both (Figure 1). Out of 183 calves 20 calves showed positive response.

Table 4. CCT test using both aPPD and bPPD at neck region

Name of test	Nature of reaction	Number of cases & percentages (%)
CCT test	Positive	20 (10.92)
	Suspicious	0 (0.00)
	Negative	163 (89.07)

### CCT test for bPPD, aPPD and mixed type of reactors

Out of 183 Bovine calves, 12 Bovine calves showed an increase in the thickness of skin (Figure 1) for above 4 mm at the site of inoculation (positive reaction) of bovine PPD on the neck region, 7 calves showed both increase thickness at the site of bPPD and aPPD and only one calves showed increases thickness at the site of aPPD to CCT test (Figure 1).

Table 5. Results of CCT using bPPD and aPPD in Bovine calves

Animal species	Test results		
	No. of bPPD reactor (%)	No. of aPPD reactor (%)	No. of mixed reactor (%)
Calves (n=183)	12 (6.56)	1 (0.546)	7 (3.83)

### Results of CCT test on the basis of sex

Among the 183 Bovine calves under study, 7 male calves with a percentage of 11.11 were confirmed by CCT test as TB infected, on the other hand it was found only in 13 female calves (10.83%).

Table 6. Results of CCT test on the basis of sex

		Number of animal	Positive cases & percentages (%)
Sex	Male	63	7 (11.11)
	Female	120	13 (10.83)
Age	7 to 12 months	108	15 (13.89)
	13 to 18 months	58	05 (8.62)
	19 to 24 months	17	0 (0.00)

(Sex: Likelihood-ratio chi-sq. =0.268; P>0.05); (Age: Likelihood-ratio chi-sq. =2.302; P>0.05)

### Results of CCT test on the basis of age

Among the 183 Bovine calves of different age those were further subjected CCT test, 15 calves with a percentage of 13.89 were found as positive in the age group between 7 months to 12 months, 05 calves with a percentage of 8.62 were found as positive in the age group between 13 months to 18 months and in 19 months to 24 months no calves were found positive by CCT test in the current study.

The method of tuberculin testing as described by Grooms and Molesworth, (2000) was followed in both the caudal fold tuberculin (CFT) test and comparative cervical tuberculin (CCT) test in this study. In the caudal fold tuberculin test 12.02% reactions in calves were evident from a clear inflammatory whorl like swelling (Table 3). In CCT testing within 10 days of the initial inoculation, 10.92% cases were proved as reactor in calves. So in case of calves the CFT test gives 1.1% false reaction. These results were found in accordance with the findings of Grooms and Molesworth, (2000) who stated that the response to CFT test may be caused by infection with *Mycobacterium bovis*, exposure to or infection with other closely related bacteria, such as *Mycobacterium avium* (avian tuberculosis) and *Mycobacterium paratuberculosis* (Johne's disease). This is referred to as a false-positive test. It is expected that false-positive results on the CFT test will occur in a normal population approximately 5% of the time. In herds that have increased exposure to *Mycobacterium avium* (avian tuberculosis) or *Mycobacterium paratuberculosis*, the response rate may be greater than 5% (Grooms and Molesworth, 2000).

In accordance with findings from the studies of Ameni *et al.* (2001), this study showed that as the herd size increased, there was a corresponding rise in the prevalence of bovine TB. This could be due to the fact that overcrowding creates conducive condition to the transmission of the bacillus. Animals with zero grazing are at a high risk of infection than those kept on free grazing and mixed grazing. As herd size increased, so did the risk of cattle within the herd showing a positive reaction. This result is also consistent with previous reports (Asseged *et al.* 2000) which could arise from the fact that risk of an individual animal introducing tuberculosis infection in to a negative herd may increase with herd size. A recent report from Ethiopia indicated that herd size is less significance as risk factor (Tschopp *et al.* 2009) but contrary reports are also published (Cleaveland *et al.* 2007). As previously indicated by other researchers (Ameni *et al.* 2003; Doherty *et al.* 1996), similar result was observed that positively reacted animals to tuberculin test increased as the age of the animal increases though it was not hold true on multivariate analyses. In my study area the bovine calves are kept in 2-4 calves in a cage and 55-70 calves in a shed. As the calves are kept in close confined condition, and they are closely contact with each other, are mostly susceptible for being contaminated with *Mycobacterium* bacillus from infected TB to non-infected TB. It has previously been emphasized in a report that reduction in susceptibility to *M. bovis* can be achieved through management. However, it has been suggested that considerable further research is required (Phillips *et al.* 2002).

In the present study, high prevalence of bovine TB was recorded in pure Holstein as compared to Zebu breeds and their crosses, which is consistent with earlier reports (Ameni *et al.* 2001; Kazuwala *et al.* 2001). Majority of previous reports (Doherty *et al.* 1996; Kazuwala *et al.* 2001) support the idea that Europeans breeds are more susceptible to bovine TB due to the predisposing factors related to environment, poor management, and also production stress that reduce the body defense. In my research area most of the calves are crossed with Holstein Friesian.

The result showed that animals under good body condition reacted more to tuberculin test than those of with poor body condition similar to the Turkish study (Barwinek and Taylor, 1996). This could be because of the fact that animal with poor body condition may not react due to immune compromises related to certain stress factors. As a result, animals could fail to respond to the skin tuberculin test, even if they are infected. In the Central Cattle Breeding and Dairy Farm the most of the bovine calves are in healthy condition.

One work was found in bovine TB by Carlos *et al.* (2009) they try to estimate the relationship between the occurrence of tuberculosis and the length of the cow-calf contact in dairy herds. A case-control study was performed in 62 dairy herds of the provinces of Santa Fe and Cordoba; 31 herds were cases with more than 2% BT-positive reactors in 2007 and the other 31 herds were controls which were BT-negative between 2005-2007. The Wilcoxon rank sum test and logistic regression were used to analyze the putative factor: age at weaning (in days). There was an association between age at weaning and BT occurrence ( $P = 0.0033$ ). In control herds the length of the cow-calf contact was  $3.2 \pm 2.5$  days, whilst in the case herds it was  $5.3 \pm 2.9$  days. The Odds Ratio was 1.38 for each day of increase of weaning age (95% confidence interval 1.09-1.74). The results indicate that weaning age was associated with the transmission of BT in dairy herds. *M. bovis* may be transmitted from the cow to the calf through ingestion of colostrum and/or milk or by the airborne route, which is considered the most common way of transmission of BT. A short time of cow-calf contact may decrease the transmission of BT in dairy herds in Argentina.

The percentages of reactors to CCT test in calves were 10.92% (Table 4). Debnath *et al.* (1995) reported that a retrospective study of calf losses on the Central Dairy Cattle Breeding Station in Bangladesh was carried out

between January 1980 and July 1992 (both months inclusive). Tuberculosis was responsible for 11.3% of the calves' mortality. Calf mortality rate was significantly higher in male calves and in calves having exotic breeding and lower birth weight. Age at death during the first 12 months of life was described by life table methods. Our findings were more or less similar with the findings of other researchers for the prevalence of tuberculosis in calves however it is impracticable to draw exact conclusion as a whole on the prevalence of tuberculosis in calves. Prevalence of bovine tuberculosis is influenced by many factors such as geographical situation of a country, and its temperature, hygienic status of humans and animals and enforced regulatory laws in Public health and Veterinary Public Health sectors that also affect the prevalence percentage. In the CCT test, the reactor percentage of male animals is 11.11 and female animal is 10.83 respectively (Table 6). The percentage of reactor animals in relation with sex to CCT in calves is insignificant ( $P > 0.05$ ). In our study we found that in male calves the prevalence of tuberculosis is slightly higher than the female calves.

The findings indicated that the overall percentage of reactor animals in calves was 13.89, 8.62, and 0.00 for the age group-1 (7-12 months), group-2 (13-18 months) and group-3 (19-24 months) respectively (Table 6). The correlations in different age groups for the prevalence of tuberculosis were insignificant in calves ( $P > 0.05$ ). The younger groups of calves are mostly reacted to PPD, these may be due to the disease is transmitted through the milk, or it may be due to the lower diseases resistance in early stage of the life of calves or it may be that in my study area there are fewer number of older calves in compared to younger calves. This, however, needs further studies to rule out the genetics, epigenetic/environmental and Mycobacterium interplay of the different age and sex group of calves.

In calves the bovine TB was 6.56%, the avian TB was 0.546% and mixed (bovine and avian TB complex) was 3.83% (Table 5). This finding indicates that calves is highly susceptible to bovine TB infection than avian TB and act as a carrier for spreading of both bovine and avian TB to other animals and birds including human.

## CONCLUSION

In the CFT, the percentage of positive reaction was found 12.02% against 10.92% in the CCT test. The overall percentages of suspicious cases were 1.24 in CFT test while no suspicious cases were found in CCT test. The male calves (11.12%) showed somewhat equally susceptibility to tuberculosis than the female calves (10.83%) of the same area in CCT test while it was somewhat higher (12.698%) in male calves and in female calves (11.666%) with CFT test. Younger calves (7 months – 12 months) were found more susceptible to tuberculosis than older calves. It might be suggested that for obviously complete eradication of tuberculosis from the country, there should be very well coordination in activities among the public health and veterinary public health personnel.

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