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Prevalence of bovine and avian tuberculosis in sheep and goat population of Bangladesh

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A R T I C L E   I N F O

A B S T R A C T

An attempt was undertaken to investigate the occurrence of bovine and avian tuberculosis in sheep and goat population in Bangladesh. A total of 273 sheep and 155 goats were screened for bovine and avian tuberculosis by caudal fold tuberculin (CFT) and comparative cervical tuberculin (CCT) tests. The percentage of responders to CFT test in sheep and goat were 9.15% and 1.29% respectively. The overall percentage of reactors to CCT test in sheep and goats were 1.46% and 1.29% respectively. In CCT test the reactors were 0.36%, 1.29% for bovine tuberculosis, 1.09%, 1.29% for avian tuberculosis and 0.00%, 1.29% for mixed type for the sheep and goats respectively. In sheep of 3-4 years of age the reactors were 2.39 times higher than sheep below 3 years of age. In goats of 4.1-6 years of age the reactors were 2.40 times higher than goats below 3 years of age. The present prevalent study showed significant (*P*<0.05) association for sexual variation with tuberculosis in goats and insignificant (*P*>0.05) association in sheep. In sheep the percentages of reactors were 1.24 times higher in male (1.72%) than female (1.39%). Jamunapari (2.85%) breed of goat had 3.5 times higher percentage of reactors as compared to Black Bengal breed (0.83%). 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.

1. Introduction

Tuberculosis is an important disease in many countries including Bangladesh. Tuberculosis in cattle and other domestic animals is caused by two members of *Mycobacterium tuberculosis* complex (*MTC*): *M. bovis* and *M. caprae* (Pavlik et al., 2002; Prodinger et al., 2002; Erler et al., 2004). However, *M. bovis* is the most universal pathogen among mycobacteria and affects many vertebrate animals of all age groups including humans although, cattle, goats and pigs are found to be most susceptible, while sheep and horses are showing a high natural resistance (Radostits *et al.,* 2000; Thoen et al., 2006). Mycobacteria are aerobic, acid-fast, rod-shaped, non-motile bacteria. Historically, they were considered as unencapsuled organisms but it is now known that pathogenic mycobacteria contain a "capsular structure" that protects the bacteria from microbiocidal activities of the macrophages and also contributes to the permeability barrier of the mycobacteria cell envelope. 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).

Goat rearing is the main source of meat and milk for poor people and goat is popularly known as "poor man's cow". Rearing of sheep is mainly for meat purposes. Due to economic reasons and prevalence of certain diseases, sheep and goats rearing could not develop into farming system. The infectious diseases are a serious threat, especially during the winter season. Tuberculosis is one of the infectious diseases in sheep and goats.

M. avium and *M. tuberculosis* can also infect sheep and goat and cause a positive reaction to tuberculin (Francis et al., 1978). However, localized lesions can develop and may persist for a number of months. The importance of sheep and goat infection with *M. avium* lies in the development of a sensitisation to bovine tuberculin. If a single skin test is performed, the reactions in sheep and goat, infected with *M. avium*, can be interpreted as false positive or para-allergic. In the comparative skin test, reactions to avian tuberculin are usually stronger than the reactions to bovine tuberculin.

Therefore the study was conducted to determine the prevalence and risk factors associated with the occurrence of tuberculosis in sheep and goats through tuberculin test by PPDs. The tuberculin test has been used for more than 100 years and this test, applied in various formats, remains the mainstay of eradication programs for *M. bovis.* Control programs based on the tuberculin test have resulted in freedom from BTB in many countries. The test is based on the delayed type hypersensitivity (DTH) reaction, a component of the cell-mediated immune CMI response (Wood *et al.*, 2001) which is the main immunological reaction observed in *M. bovis* infection (Thorns and Morris, 1983). So, it is with this view that the present study has been undertaken to find out the prevalence of tuberculosis through tuberculin testing in sheep and goat population in Bangladesh.

2. Materials and methods

In this study a total of 273 sheep and 155 goats on the farms were tested by caudal fold tuberculin (CFT) test using bovine purified protein derivative (bPPD). Among the 428 sheep and goats, the responders were further subjected to single comparative cervical tuberculin (CCT) test using both bovine PPD and avian PPD. 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 bovine and avian tuberculosis.

2.1. Materials required

Bovine purified protein derivative (bPPD), Avian purified protein derivative (aPPD) were kindly provided by Dr. Monica Cagiola (Director, *Instituto Zooprofilacttico Sperimentale dell' Umbria e delle Marche*, Perugia, Italy).

2.2. Selection and grouping of animals

To determine the prevalence and risk factors associated with bovine and avian tuberculosis of 273 sheep and 155 goats kept at Bangladesh Livestock Research Institute (BLRI).

Sheep and Goat farms, Savar, Dhaka were selected using CFT and CCT test. Out of the 273 sheep, 46 rams, 207 ewes, 20 lambs and of the 155 goats, 59 buck and 96 were doe. Date of birth, age, breed and other managemental information were recorded in a questionnaire.

2.3. Selection of tuberculin and other necessary materials and instruments

For the CFT testing, bovine PPD and for the CCT testing, both the bovine (*M. bovis*) PPD and avian (*M. avium*) PPD were used in this study. Both the PPDs were obtained from a licensed laboratory in Italy (*Instituto Zooprofilattico sperimentale dell'Umbria e delle Marche, Perugia*). All the tuberculin vials were kept in a refrigerator for maintaining the potency and quality. For the purpose of cleaning and disinfection of the inoculation site, sterilized cotton and 70% ethyl alcohol were used. For inoculation, 1 ml tuberculin syringe of 100 graduations, fitted with a short hypodermic needle was utilized.

2.4. 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 bovine TB. 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.

2.5. 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 (Instituto 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 bovine PPD. 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):
a. If the reaction is ≥ 4.0 mm greater than the test is considered reactor.
b. If the reaction is between 3.0 and < 4.0 mm than the test is considered suspect.
c. If the reaction is < 3.0 mm than the test is considered negative.

2.6. Statistical analysis

The collected data was compiled, tabulated and analyzed in accordance with the objectives of the study. The approximate percentage was calculated for each parameter. Finally data were analyzed using the statistical software 'SPSS' by chi-square tests for the relationship of different factors on the occurrence of tuberculosis in buffaloes where P value of ≤ 0.5 was considered statistically significant.

3. Results

In this entire study 273 sheep and 155 goats of different breeds, sexes and ages were examined for the prevalence of bovine and avian tuberculosis by CFT test and CCT test in BLRI sheep and goat farms. Out of 273 sheep, 25 (9.15%) sheep (Fig. 2) and out of 155 goats, 2 (1.29%) goats (Fig. 1) responded to CFT using bovine type tuberculin. In case of CCT the overall percentage of reactor cases were 4 (1.46%) and suspect cases were 7 (2.56%) out of 273 sheep. In goat the overall percentage of reactor cases were 2 (1.29%) and suspect cases were 0 (0.00%) out of 155 goats.

The results revealed that out of 273 sheep and 155 goats of the BLRI sheep and goat farms showed 25 (9.15%), 2 (1.29%) responders and 248 (90.84%), 153 (98.70%) negative to bovine TB (Fig. 3) for the sheep and goats, respectively.

The overall percentages of reactors to CCT were 1.46 in sheep and 1.29 in goats. The overall percentages of suspect cases were 2.56 and 0.00 in sheep and goats, respectively.

In case of CCT the percentage of reactors were 0.36 for bovine TB, 1.09 for avian TB and 0.00 for mixed type in sheep. In goats the percentages of reactors were 1.29 for bovine TB, 1.29 for avian TB and 1.29 for mixed type.

In CCT tuberculin test, the overall percentages of male reactors were 1.72, 3.38 and that of female reactors were 1.39, 0.00 for the sheep and goats, respectively.

The findings indicated that the overall percentage of reactor animals in sheep were 1.33, 3.12, 0.00 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years respectively. On the other hand, the overall percentage of reactor animals in goats were 1.36, 0.00, 3.22 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years respectively.

As regards the goat breeds, the overall percentages of reactors were 0.83 and 2.85 for the Black Bengal and Jamunapari breed, respectively.
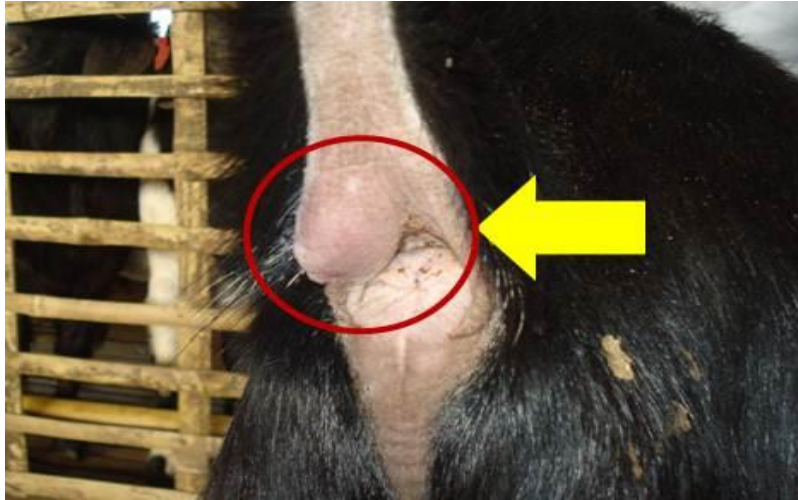

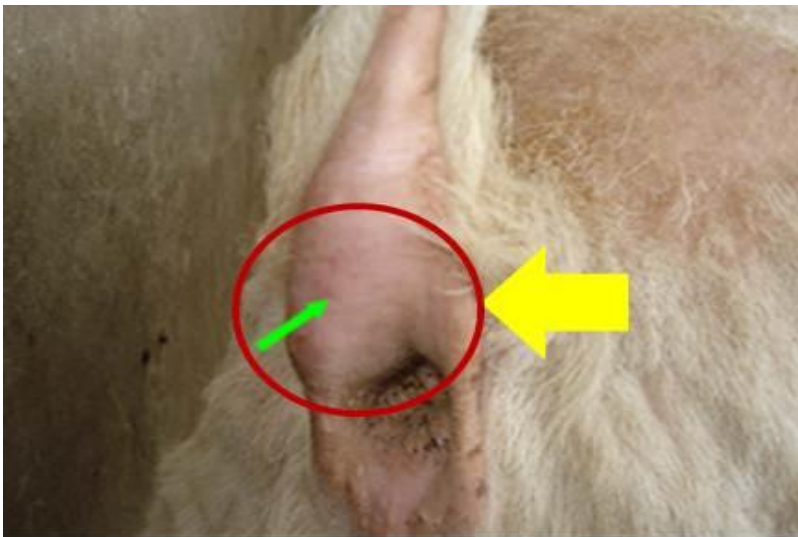**Fig. 1.** Swelling at inoculation site of CFT indicated responder in goat.

Fig. 2. Swelling at inoculation site of CFT indicated responder in sheep.

Fig. 3. Negative Control (No Swelling).

4. Discussion

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 9.15% reactions in sheep and 1.29% reactions in goats were evident from a clear inflammatory whorl like swelling of painful nature (Table 1).

In CCT testing within 10 days of the initial inoculation, 1.46% cases were proved as reactor in sheep and 1.29% reactor in goats so in case of sheep the CFT test gives 7.95% 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 percent 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 percent (Grooms and Molesworth, 2000).

The percentages of reactors to CCT test in sheep were 1.46% and in goats were 1.29% (Table). Ashraf *et al.,* 1986 reported that the prevalence of tuberculosis in sheep was 2% in Pakistan. The overall percentage of reactor animals to SCCIT at farms in sheep and goats were 0.9% and 2.4% respectively (Javed *et. al.,* 2010). Pignata *et al.*, (2009) reported the prevalence of tuberculosis in goats was 0.47% in Brazil. One study reported a prevalence of tuberculosis was 3.5% in domestic goats (Sanson, 1998), while another reported 0.5% in goats (Arellano *et al.,* 1999).

In the CCT test, the reactor percentage of male animals is 1.72, 3.38 and female animal is 1.39, 0.00 for the sheep and goats respectively (Table). The percentage of reactor animals in relation with sex to CCT in sheep is insignificant ($P > 0.05$) and in goats are significant ($P < 0.05$).

No such type of study in sheep and goats has undertaken as yet, so we correlates our findings with the study on cattle. Our findings are in agreement with the finding of Kazwala *et al.,* 2001, who stated that male cattle were significantly more affected by BTB than female animals. Male cattle are mostly used as oxen, which are kept longer in the herd than females. Due to this particular longevity, it is more probable that they get in contact with infected cattle from other affected herds and in turn get infected. From this point of view it can be said that sheep and goat male are also kept in longer time than females. So due to this particular longevity, the prevalence percentage is high in male than female.

Table 1
Reading of the results of inoculation.

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 ≥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 ≥4 mm from the initial reading indicates reactor	Swelling 3.0-<4 mm from the initial reading indicates suspect and <3mm indicates negative

The findings indicated that the overall percentage of reactor animals in sheep was 1.33, 3.12, 0.00 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years respectively. On the other hand, the overall percentage of reactor animals in goats were 1.36, 0.00, 3.22 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years respectively (Table). The correlation in different age groups for the prevalence of tuberculosis were insignificant in sheep ($P > 0.05$) and goats ($P > 0.05$).This finding correlates with the findings of Javed *et al.,* 2010, who stated that the older animals are more affected than the younger animals. The study strengthens the view that as the age increases the chances of tuberculosis also increases (Guindi *et al.,* 1975 and Siva *et al.,* 1997). The duration of exposure increases with age; older animals are more likely to have been exposed than younger ones, as shown by several cross-sectional studies carried out in Tanzania.

Under the consideration of breed variations the percentage of reactors was 2.85% found in Jamunapari breed and 0.83% in Black Bengal breed in goats. There was an insignificant difference noted in the percentage of reactor animals to CCT test in goat breeds ($P > 0.05$). Jamunapari breed is about 3.50 times higher at risk for the occurrence of tuberculosis than Black Bengal breed. This, however, needs further studies to rule out the genetics, epigenetic/environmental and Mycobacterium interplay of the different breeds of goat. In sheep the avian TB was 1.09% and the bovine TB was 0.36%.This finding indicates that and sheep is highly susceptible to avian TB infection than bovine TB and act as a carrier for spreading avian TB to other animals and birds including human. In goat mixed TB (avian TB and bovine TB) infection was 1.29%. This finding indicates that goats are equally susceptible to both avian and bovine TB infection. The avian TB may be acquired from sheep while grazing in same flock.

5. Conclusion

In the CFT test the percentage of responders were found to be 9.15 and 1.29 in sheep and goat, respectively. The CFT test is the primary screening test used to identify potentially infected with bovine tuberculosis. Since it is a screening test, results are not considered absolute proof that an animal or herd has bovine TB. Therefore, it is used to identify animals and herds that need to undergo further testing with CCT test for bovine TB and avian TB.

The overall percentages of reactor animals in CCT test were 1.46 and 1.29 in sheep and goat respectively. The percentages of reactors were 0.36 for bovine TB, 1.09 for avian TB in sheep. In goat the percentage of avian TB and bovine TB infection was 1.29 %. The overall percentages of male reactors were 1.72, 3.38 and that of female reactors were 1.39, 0.00 in sheep and goat respectively. The overall percentage of reactor animals in sheep were 1.33, 3.12, 0.00 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years respectively. On the other hand, the overall percentage of reactor animals in goats were 1.36, 0.00, 3.22 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years, respectively. The Jamunapari breed showed maximum (2.85) and the Black Bengal breed showed minimum (0.83) percentage of infection for both types of TB infection. In general the female, the younger and the older animals were found to be more infected than the male and adult animals in both sheep and goats.

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