Isolation of *Mycobacterium bovis* & *M. tuberculosis* from cattle of some farms in north India - Possible relevance in human health


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**Background & objectives:** Infection due to *Mycobacterium bovis* typically occurs in cattle and animals transmit infection to each other. The choice of appropriate clinical specimen is very important for isolation of *M. bovis* and *M. tuberculosis* from cattle. The present study reports the isolation of *M. tuberculosis* and *M. bovis* from different types of specimens from cattle suspected to be suffering from tuberculosis in certain organized cattle farms in north India.

**Method:** A total of 768 specimens (heparinized or EDTA containing blood (162), fine needle aspirates from prescapular lymph gland (PSLG, 160), milk (154), pharyngeal swab (PhS, 98), rectal pinch (RP, 97) and faecal sample (97) from 161 cattle of organized cattle farms in north India suspected to be suffering from tuberculosis were analyzed. After decontamination by modified Petroff's method isolation of *M. tuberculosis* complex was done on Lowenstein-Jensen medium (with and without pyruvate). The culture isolates were identified as *M. tuberculosis* and *M. bovis* on the basis of biochemical tests.

**Results:** A total of 54 *M. tuberculosis* complex isolates were obtained, of them 40 were identified as *M. bovis* and 14 as *M. tuberculosis*. *M. bovis* were isolated from 12 of 38 animals in group A (Tuberculin +ve with signs of tuberculosis), 7 of 37 animals in group B (Tuberculin +ve and apparently healthy), 9 of 21 group C animals in (Tuberculin -ve with clinical signs of tuberculosis), 4 of 26 animals in group D (Tuberculin -ve and apparently healthy), 4 of 27 group E animals (having non-mycobacterial infection) and 4 of 12 animals in group F (having clinical signs such as debilitated condition, cough, decreasing milk production, etc). Maximum number of *M. bovis* (19/40, 47.5%) and *M. tuberculosis* (5/14, 35.7%) isolates were grown from prescapular lymph gland biopsy (PSLG) followed by blood from which 9/40 (22.5%) *M. bovis* and 4/14 (28.5%) *M. tuberculosis* were isolated. *M. bovis* [6/40(15%)] and *M. tuberculosis* [4/14(28.5%)] were also isolated from milk. Only 3/40 (7.5%) isolates of *M. bovis* could be isolated from 97 rectal pinch followed by 98 pharyngeal swab 2/40 (5%) and 97 fecal samples 1/40 (2.5%) while 1/14 (7.1%) *M. tuberculosis* isolates were obtained from pharyngeal swab.

**Interpretation & conclusions:** Among the samples analyzed, PSLG was found to be most suitable specimen for isolation of *M. tuberculosis* complex from cattle and is thus of diagnostic importance. *M. bovis* in milk indicates the need to investigate the transmission to human in such settings. Isolation of *M. bovis* and/or *M. tuberculosis* from apparently healthy cattle indicates sub-clinical infection in the herd. Further, isolation of a significant number of *M. tuberculosis* from cattle suggests possible human-to-cattle transmission which need to be confirmed by prospective studies including tools like DNA fingerprinting.

**Key words** Bovine tuberculosis - *Mycobacterium bovis* - *Mycobacterium tuberculosis* - specimen
Infection due to *Mycobacterium bovis* typically occurs in cattle but has been reported in other animals including dogs, cats, swine, rabbits, birds and man. While several investigators from western countries have stressed the possible zoonotic importance of bovine tuberculosis very limited data on this aspect are available from Asian countries including India. Based on studies of slaughtered animals the incidence of bovine tuberculosis in cattle and buffalo in Pakistan has been reported to be varying from 2.25 per cent in 1969 to 7.3 per cent in 1989. All these studies only suggest but do not provide clear evidence about the transmission chain including the zoonotic importance.

Animals transmit infection to each other through ingestion of urine, faeces and lymph, wound discharge, infected milk along with food and water. In the spread of disease from adults to the young stock of animals, milk as a source has been reported. Some investigation have pointed out the risk of human infection through unpasteurised, untreated consumption of milk or using raw milk for producing cream, butter or dahi (curd) among cattle owners and herdsman in community.

Tuberculin testing has traditionally been used to determine the prevalence of infection in animals and human. This has been used as a mean to identify and cast all tuberculin positive animals, which could be an overestimate of true active infection. The standard single intradermal comparative tuberculin test (SICTT) using purified protein derivative (PPD) of *M. bovis* has been used to detect cattle infected with *M. bovis* and prevalence of disease in cow twice as high as compared to heifers and bulls has been reported. In India, higher incidence of tuberculosis in buffaloes as compared to cattle has been estimated on the basis of tuberculin incidence of tuberculosis in buffaloes as compared to cattle has been estimated on the basis of tuberculin testing. There are many reports from India showing the presence of *M. bovis* in milk and in cervical lymph gland. A couple of years ago reports of increasing number of cases of bovine tuberculosis in Kerala (Kerala) have attracted attention. It was reported that 60 per cent of the total 520,000 cattle in the Kerala State were partially or fully affected by the tuberculosis disease and almost all crossbred cattle were suffering with tuberculosis. However, accuracy of these data needs to be established. In India, there is little information available on the transmission of bovine tuberculosis and its impact on human health. There is general impression that it leads to significant economic losses due to morbidity and mortality in animals. The exact magnitude of the problem can be known only by well-conducted studies. We report the isolation of *M. tuberculosis* and *M. bovis* from different types of specimens from cattle with varying clinical presentations and suspected to be suffering from tuberculosis in some organized cattle farms of north India.

**Material & Methods**

**Samples:** A total of 768 specimens from 161 cattle belonging to various clinical groups were procured during 1999 to 2001 from the cattle farm of Central Military Veterinary Laboratory (CMVL), Meerut Cantonment, Uttar Pradesh, for bacteriological analysis. These groups were: A- Tuberculin +ve and showing signs of tuberculosis, B-Tuberculin +ve and apparently healthy animals, C - Tuberculin -ve and showing clinical signs of tuberculosis, D - Tuberculin -ve and apparently healthy individual, E - Animal infected with non-mycobacterial infection (mastitis, enteritis, chronic mastitis, and pyrexia of unknown origin), F- Animal having clinical sign of debilitated condition, cough, decreasing milk production and laboured respiration. Sample analyzed were heparinized as well as EDTA containing blood (n=162), fine needle aspirates from prescapular lymph gland (PSLG n=160), milk (n=154), pharyngeal swab (PhS n=98), rectal pinch (RP n=97) and faecal sample (n=97).

**Methods:** The samples were processed for isolation of mycobacteria following standard procedures for homogenization, suspension, centrifugation and decontamination with 4 per cent NaOH (modified Petroff’s method). Rectal pinch (50 mg) was homogenised in 1 ml sterile normal saline, faecal samples (aprox. 100 mg) were mixed in 1 ml of normal saline and pharyngeal swabs were suspended and washed in 1 ml of normal saline. Milk (2 ml), fine needle aspirates from prescapular lymph gland (PSLG 0.5 ml), heparinized as well as EDTA blood (0.5 ml) and all above samples were mixed with equal volume of 4 per cent sodium hydroxide and processed by modified Petroff’s method of washing with water rather than neutralization recommended in the original method. Fine needle aspirates from prescapular lymph gland and heparinised blood were also decontaminated, in animal samples as an extra precaution, as there are chances of contamination during samples collection. A few drops (about 50 µl) of processed sample were inoculated on Lowenstein-Jensen (L-J) media with and without pyruvate and incubated at 37°C in a BOD incubator for culture for a maximum period up to 8 wk.
Identification: Species level identification of growth of acid fast bacilli (AFB) positive mycobacterial isolates was done by standard biochemical tests [niacin production, nitrate reduction, catalase activity at 68°C and at room temperature, tween hydrolysis, arylsulphatase and thiophen-2 carboxylic acid hydrazide (TCH) sensitivity, etc.] as per CDC Manual\(^2\). A combination of positive activity for niacin, nitrate reduction, TCH and negative activity for catalase at 68°C and arylsulphatase were considered as characteristics of *M. tuberculosis* while negative activity for niacin, nitrate reduction, catalase at 68°C, tween hydrolysis, arylsulphatase and TCH were considered as characteristics of *M. bovis*\(^2\).

Statistical analysis: Data were analyzed by using statistical package STATA-7 State Corporation, Texas, USA. Chi square at 5 per cent level of significance was used to assess statistical significance of difference among the following data sets - (i) Isolation rates of *M. tuberculosis* complex (*M. tuberculosis, M. bovis*) among different clinical groups of animals (A, B, C, D, E & F). (ii) Isolation of *M. tuberculosis* complex among different type of clinical samples (blood, fine needle aspirates from PSLG, milk, pharyngeal swab, rectal pinch and faecal sample).

Results

In present study, 54 *M. tuberculosis* complex isolates were obtained on Lowenstein-Jensen/pyruvate media out of 768 specimens, 40 of these isolates were identified as *M. bovis* and 14 as *M. tuberculosis*. Of the 40 *M. bovis* isolates, 12 were grown from 38 animals of group A (Tuberculin +ve and showing signs of tuberculosis), 7 from 37 animals belong to group B (Tuberculin +ve and apparently healthy animals), 9 from 21 animals of group C (Tuberculin -ve and showing clinical signs of tuberculosis), and 4 isolates each from 27 animals of group E (infected with non-mycobacterial infection (mastitis, enteritis, chronic mastitis, and pyrexia of unknown origin-PUO), 4 of 26 animals of group D (Tuberculin -ve and apparently healthy individual) and 4 isolate from 12 animals of group F (animal having clinical sign of debilitated condition, cough, decreasing milk production laboured respiration) (Table). Out of 14 *M. tuberculosis* isolates, eight were grown from 38 animals of group A, 2 each from 37 animals of group B and 27 animals of group E and 1 isolate each from 21 animals of group C and 26 animals from group D. When these clinical groups were compared with each other for isolation of *M. tuberculosis* complex (*M. bovis* and *M. tuberculosis*) no statistically significant difference was found.

Among the samples analyzed PSLG appeared to be most suitable specimen for isolation of *M. tuberculosis* complex as 19 of 40 (47.5%) isolates of *M.bovis* and 5 of 14 (35.7%) of *M. tuberculosis* were isolated from these biopsies followed by heparinized as well as EDTA blood [9 of 40 (22.5%) *M. bovis* and 4

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<th>Diagnosis</th>
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<tr>
<td></td>
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<td>(Faecal-1)</td>
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<td><em>M. tuberculosis</em></td>
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<td>Total- 8</td>
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*Clinical symptoms: A, Tuberculin +ve and showing signs of tuberculosis; B, Tuberculin +ve and apparently healthy animals; C, Tuberculin -ve and showing clinical signs of tuberculosis; D, Tuberculin -ve and apparently healthy individual; E, Animal infected with non-mycobacterial infection (mastitis, enteritis, chronic mastitis, and pyrexia of unknown origin); F, Animal having clinical sign of debilitated condition, cough, decreasing milk production and laboured respiration. PSLG, prescapular lymph gland; Ph.S, pharyngeal swab
of 14 (28.5%) *M. tuberculosis*. *M. bovis* [6/40 (15%)] and *M. tuberculosis* [4/14(28.5%)] could be isolated from some of the milk samples. Only 3 of 40 (7.5%) isolates of *M. bovis* could be isolated from rectal pinch followed by pharyngeal swab [2/40(5%)] and faecal specimens [1/40 (2.5%)]. Maximum numbers of *M. tuberculosis* were isolated from PSLG [5/14 (35.7 %)] followed by blood and milk [4/14 (28.6%) and 1/14 (7.1%) from pharyngeal swabs. There was no *M. tuberculosis* isolate from rectal pinch and faecal samples. The per cent of *M. bovis* isolates from different groups was 1.03 percentages (1/97) in fecal samples, 2.04 per cent (2/98) in pharyngeal swab, 3.09 per cent (3/97) in rectal pinch, 3.90 per cent (6/154) in milk, 5.56 per cent (9/162) in blood and 11.88 per cent (19/ 160) in PSLG. There was significant difference in percentage of *M. bovis* isolates among the different groups (faecal samples, pharyngeal swabs, rectal pinch, milk and blood, *P*<.001). However, if PSLG group was excluded from statistical analysis, there was no difference in the remaining groups. Thus PSLG was the only sample which was significantly different than others in terms of isolation rates and can thus be considered as appropriate type of sample for diagnosis of tuberculosis in cattle. Percentage of *M. tuberculosis* among different groups ranged from 1.02 per cent (1/98) in pharyngeal swab, 2.60 per cent (4/154) in milk, 2.47 per cent (4/162) in blood, none in rectal pinch as well as faecal specimens and 3.13 per cent (5/160) in PSLG. This difference in percentage of *M. tuberculosis* isolation was not found statistically significant among different types of samples.

**Discussion**

Our study showed that prescapular lymph gland was the most appropriate specimens for isolation of *M. bovis* from infected cattle. There have been other studies showing relatively higher isolation of *M. bovis* from lymph glands and pus as compared to other specimens like liver and lung9, 23, 24. Swollen lymph node especially of the head, and discharging lymph node abscesses were important clinical signs of tuberculosis infection in animals35. Thus lymph glands could be recommended as a preferred specimen for confirmation of diagnosis of bovine tuberculosis. Faeces and milk are considered as important media for transmission of bovine tuberculosis7,24. As is also evident from the data published by others blood could be used as specimen for detection of tuberculosis in cattle26-28. Similar approaches have also been reported to be useful for diagnosis of mycobacterial infection in humans29-32. It should also be kept in mind that detection of presence of mycobacterium in blood is handicapped by intermittent nature of mycobacteraemia30 hence repeated blood samples need to be collected.

Tuberculosis infections in animals transmitted from men have been earlier reported from western countries1,4,11. It would be important to watch animal handlers carefully, who would be the most probable category of suspects to have transmitted these infections to animals. Transmission could have been by aerosols or contamination of fodder due to indiscriminate spitting. Hutching et al33 suggested that the possible route of human to cattle transmission is by inhalation of bacilli from grass contaminated with infected human urine, faeces, or sputum.

Smith et al34 have suggested that there may be a small risk for transmission of tuberculosis carried by *M. bovis* from cattle to human, making continued vigilance particularly necessary. According to Hardie and Watson35, approximately 1 per cent of human tuberculosis cases could be attributed to *M. bovis*35. Milk and meat are one of the most important links between bovine tuberculosis and human beings especially children7,24,36. Cosive et al36 reviewed zoonotic tuberculosis due to *M. bovis* in developing countries and estimated that the proportion of human cases due to *M. bovis* accounted for 3.1 per cent of all forms of tuberculosis. Accurate information about tuberculosis in animals particularly its transmission to humans in India is speculative and studies using classical epidemiological as well as molecular typing should be conducted to determine the exact magnitude and also mode as well as chain of transmission10,12,15-19.

As setting of culture on L-J medium with pyruvate has become standard practice, accurate picture will emerge in future.

The isolation of *M. bovis* from apparently healthy animals shows active transmission in the infected herds and such findings have also been reported earlier2,24,37. Significance of sub -clinical infection in terms of future development of active disease and transmission dynamics is not known and should be focused in future studies.

Conventionally, tuberculin reactivity has been used to identify animal infected with tuberculosis7, 14-16. Similar information has been generated in slightly different manner by gamma interferon assay27,28. Our data and also an earlier report38 show that tuberculin reactivity is not a reliable indicator of active disease and/or tuberculous infection in cattle. Half of isolates
of *M. bovis* and *M. tuberculosis* were obtained from animals that were tuberculin negative. Thus our study showed wider circulation of *M. bovis* in this herd, which did not correlate with clinical findings and/or tuberculin reactivity. It would thus not be realistic to rely on this marker for control of tuberculosis in animals.

However, mere isolation of mycobacteria from animals can not establish the transmission pattern. It is well known that animals including wild ones, do suffer from *M. tuberculosis* infection\(^{25}\). To establish the human sources as the cause of animal infection, *M. tuberculosis* isolates from the farm workers should be typed by molecular methods. The pattern of the animal isolates then must be matched with human isolates to establish transmission from humans to animals. Until this is established, it remains a hypothesis only that animals are infected by *M. tuberculosis* from human sources.

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