

## Prevalence of tuberculosis in men and animals: Confirmation by cultural examinations, tuberculin tests and PCR technique

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### Abstract

The aim of this study was to know the prevalence of tuberculosis in men, animals and environment of dairy farms, primary health centres and Veterinary Hospitals in and around Anand (Gujarat) using cultural examinations, intra-dermal tuberculin (purified protein derivative - PPD) tests and polymerase chain reaction (PCR) technique. *Mycobacterium tuberculosis* were isolated on Lowenstein-Jensen (LJ) media/slants with glycerol after 3-4 weeks of incubation at 37 °C from 13 out of 150 (8.67%) specimens of human, viz. throat swab (4.17%; 1/24), nose swab (9.68%; 6/62) and sputum (9.38%; 6/64). Similarly *Mycobacterium bovis* were isolated on LJ media/slants with sodium pyruvate after 4-6 weeks of incubation at 37 °C from 58 out of 400 (14.50%) specimens of cattle, viz., milk (12.16%, 18/148) and nose swabs (15.87%, 40/252). All the 50 environmental (soil and water) samples were found negative for *Mycobacteria*. All the 71 clinical isolates of *Mycobacteria* obtained were subjected to Ziehl-Neelsen (ZN) staining, biochemical tests, viz. catalase test, niacin detection, nitrate reduction, pyrazinamidase activity and T2CH (thiophene-2-carboxylic acid hydrazide) and PCR for confirmation. Intra-dermal tuberculin testing of 50 human and 260 cattle revealed prevalence of TB in 0.00 and 16.45% cases, respectively. However, on cultural examination of throat swabs of all these 50 human patients, Mycobacterial isolates were obtained in 13 (26.00%) patients, while among 42 cattle positive for tuberculin PPD tests, nasal swab culture gave Mycobacterial isolates in 37 (88.10%) animals. Not only from tuberculin positive cases, but from 218 tuberculin negative cattle also 21 yielded Mycobacterial isolates (9.63%), suggesting the rate of false negative and false positive results of intra-dermal tuberculin test.

**Keyword:** Prevalence, tuberculosis, men and animals, cultural examination, intra-dermal tuberculin test, polymerase chain reaction.

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### Introduction

Tuberculosis is an important, global, highly infectious bacterial zoonotic disease, that most commonly affects the lungs (pulmonary TB) but can also affect the other body systems (extra-pulmonary TB). It is principally caused by *Mycobacterium tuberculosis complex* (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, and *M. canetti*) (Pal, 1997; Bannalika, 2007). The disease occurs in sporadic and epidemic forms and has been reported in men and in a wide variety of animals and birds. Human tuberculosis is mainly spread by aerosol droplets from people with the active disease of the lungs (Cole and Cook, 1998).

The disease is worldwide in distribution and is reported from many countries of the world including India. It is estimated that 3,00,000 people die from TB each year in India (TB India, 2012). Majority of cases occur in old age persons and in immune-compromised and HIV patients (Raviglione, 1995). Bovine tuberculosis is spread primarily through the exchange of respiratory secretions between infected and uninfected animals. Thus, animal density plays a major factor in the transmission of *M. bovis*. The fetus of an infected mother may contract TB by inhaling or swallowing the bacilli in the amniotic fluid. Consumption of milk contaminated by *M. bovis* has long been regarded as

the principal mode of TB transmission from animals to humans (Acha and Szyfres, 1987), as tuberculous mastitis is the predominant clinical manifestation of *M. bovis* in dairy cows (Grange *et al.*, 1996). Tuberculosis in humans caused by either *M. bovis* or *M. tuberculosis* is clinically hard to distinguish (Etchehoury *et al.*, 2010), though considerably fewer cases of TB are caused by *M. bovis* than *M. tuberculosis* (Pérez-Lago *et al.*, 2013).

The diagnosis is confirmed by direct microscopic demonstration of very small red beaded rods (2-4 µm long and 0.2-0.5 µm wide) (acid fast bacilli) in clinical specimen by Ziehl-Neelsen (ZN) staining technique. *Mycobacteria* are intracellular pathogens; survive both in aerobic and anaerobic conditions, Gram +ve, non-motile, non-spore forming, strongly acid-fast rods containing mycolic acid cell wall (Cox, 2004). *Mycobacterium tuberculosis* and *M. bovis* show eugonic and dysgonic growth on LJ medium with glycerol and sodium pyruvate (0.5%), respectively, when incubated at 37°C (Niemann *et al.*, 2000<sup>a</sup>).

Intra-dermal tuberculin test is also helpful to establish the diagnosis. Biochemical tests namely niacin production, nitrate reduction, catalase activity, pyrazinamidase and thiophene-2- carboxylic hydrazide test (T2CH) are used extensively for differentiation of *M. bovis* and *M. tuberculosis*. Recently ELISA, PCR and gamma-interferon assays are very useful in the diagnosis of tuberculosis (Grange *et al.*, 1996; Ryan *et al.*, 2000; Coad *et al.*, 2007). PCR has been widely evaluated for the detection of *M. tuberculosis* complex in clinical samples (mainly sputum) in human patients and has recently been used for the diagnosis of tuberculosis in animals (OIE Terrestrial Manual, 2009). Unfortunately the efforts to develop vaccine for bovine TB have not been successful so far and there is no effective treatment available for bovine tuberculosis. Moreover, attempts to differentiate cattle infected with *M. bovis* and those vaccinated with BCG using PCR and ELISA on 35 tuberculin positive, 15 tuberculin negative and 15 tuberculin negative but BCG vaccinated cows were not conclusive using single antigen (Sabry and Elkerdasy, 2014). Bovine tuberculosis can be eliminated from a country or region only by implementing official eradication campaigns based on a test and slaughter policy using above diagnostic assays (Cousins and Williams, 1995; WHO, 2006; Bezos *et al.*, 2014).

The present study was aimed to know the prevalence of *M. tuberculosis* and *M. bovis* infection through skin test and cultural examinations and PCR technique on isolates of specimens from men, animals and environment of Anand district in Gujarat, India.

## Material and Methods

This work was carried out at the Department of Veterinary Public Health of the Veterinary College in Anand, India. In all 150 samples from human patients, 400 samples from animals, and 50 from environment (water and soil) were obtained from the different localities of Anand district of Gujarat state. These were inoculated on LJ media for isolation and identification of micro-organisms, particularly *Mycobacteria tuberculosis*, *Mycobacterium bovis* and non-tubercular *Mycobacteria*.

## Sample Collection

The sterile cotton swabs (Hi-Media, India) were used to collect clinical samples (swabs) in duplicate from the throat and nostrils of human (n=86) and animal body (n=252). The sterile clinicols (Hi-Media, India) were used to collect sputum (n=24) from human.

Milk samples (n=148) were collected in sterile wide mouth glass stopper bottles from healthy and diseased/mastitic cows of University livestock farms, Anand and other cows of individual farmers of Navali and Vadod village. Before sampling the udder and teats were cleaned properly with potassium permanganate solution (1:1000). The first streams of milk were discarded prior to sample collection.

Soil samples (n=25) were collected in sterile containers from different sheds of University livestock farms for detection of presence of *Mycobacteria*. Two gram soil from 5-25 cm below surface was obtained. The surface layer was not collected because this layer is subjected to rapid environmental changes including sterilization by solar radiation (Kamala *et al.*, 1994). All samples from the farms were placed into the sterile plastic bags.

Water samples (n=25) approximately 50 ml were collected in sterile wide mouth glass vials from water troughs of different sheds of livestock farms. Water cultures were obtained by centrifugation of samples at 2000 rpm for 15 min. The supernatant was decanted and each pellet was suspended in the residual liquid and plated on media (Kamala *et al.*, 1994).

## Tuberculin Testing in Men and Animals

Mantoux test was carried out in 50 human patients of either sex and of different age group. The PPD tuberculin-10 Tu (purified protein derivative of tubercle bacilli prepared from *Mycobacterium bovis* BCG strain) was obtained from Beacon, India for this test.

Single intra-dermal tuberculin test was carried out in 260 cattle of either sex of exotic (HF) and zebu breeds and of varying age group from different farms of the University and of individual farmers of different villages of Anand district. The PPD tuberculin (purified

protein derivative of tubercle bacilli prepared from *Mycobacterium bovis* AN5 strain) was obtained from Indian Veterinary Research Institute, Izatnagar, Bareilly, India.

The test detects a delayed hypersensitivity through cutaneous reaction to a purified protein derivative of *M. tuberculosis* / *M. bovis* – also called “tuberculoprotein”. The PPD was injected intradermally in the forearm of human and neck fold of animals. The reaction was read at 48-72 hours later. An increase in thickness of skin less than 2 mm without clinical signs such as oedema, pain was taken as negative. Thickness of skin between 2-4 mm without clinical signs was considered as doubtful and thickness of skin 4 mm and above with clinical signs as positive. The patients/animals exhibiting doubtful result were subjected to re-testing after an interval of 42 days. In general, greater the degree of erythema and induration, more likely the patient has TB disease (Lisbet, 2006).

### Cultural Examination

Swabs were inoculated on LJ (Lowenstein-Jensen) culture media with and without glycerol and/or sodium pyruvate. Sputum samples were inoculated on LJ media slants after decontamination by modified Petroff's method with 4% sodium hydroxide (Vestal, 1977). The inoculated tubes/media were incubated at 37°C for 3-6 weeks for microbial growth (3-4 weeks for *M. tuberculosis* and 4-6 weeks for *M. bovis*).

A heavy loopful of the milk sample was directly inoculated on sterile LJ media slants and kept at 37°C for incubation. The tubes were examined after 4-6 weeks for mycobacterial growth (*Mycobacterium bovis*). Similarly, a heavy loopfuls of the soil and water specimen were directly inoculated on sterile slants of LJ medium with 5 % NaCl after decontamination by filtering. Slants were examined after 4-6 weeks for Tuberculous and Non-tuberculous *Mycobacteria* (NTM) (*Mycobacterium fortuitum* group) (Kestle et al., 1967).

### Identification and Confirmation of Bacterial Isolates

#### Morphologic Examination of Colonies

The colonies that appeared granular light yellow, dry and rough with no pigmentation and more heaped up, sometimes resembling bread crumb (eugonic growth) on LJ media with glycerol when incubated at 37°C for 3-4 weeks were considered of *Mycobacterium tuberculosis* (Niemann et al., 2000<sup>a</sup>), while those appeared buff to yellow, moist, slightly rough and friable with no pigmentation (dysgonic growth) on LJ media with sodium pyruvate (0.5%) after 4-6 weeks of incubation at 37°C were taken as

*Mycobacterium bovis*. Such colonies were subjected to subculturing on microbial media slants (Niemann et al., 2000<sup>b</sup>).

### Microscopic Examination of Isolates

*Mycobacteria* were identified by the most widely used ZN staining, which detects acid fast bacilli. The stained smears were examined under the microscope (100X oil immersion lens and 10X eye piece). The bacilli that appeared as red, beaded rods 2-4 µm long and 0.2-0.5 µm wide with strongly acid fast nature were *Tuberculous Mycobacteria*, while weekly acid fast bacilli were taken as *Non-tuberculous Mycobacteria* (WHO, 2004) (Fig 1).

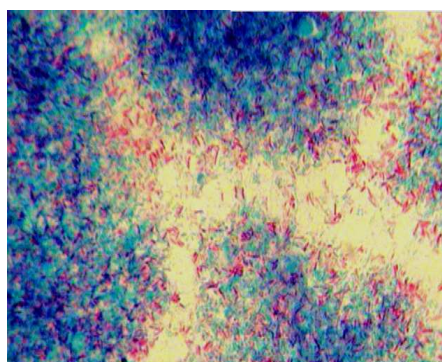


Fig 1: Photomicrograph showing red bacilli of *Mycobacterium tuberculosis* in the smear prepared from cultural isolate of the sputum obtained from a 52 years old man. Z-N stain (x 1200)

### Biochemical Tests

Positive mycobacterial isolates were identified as of *M. Tuberculosis* or *M. Bovis* by standard biochemical tests, viz., niacin production, nitrate reduction, catalase activity, pyrazinamidase and thiophene-2-carboxylic hydrazide test (T2CH) as per CDC manual (Vestal, 1977).

### Molecular Characterization of Isolates by PCR Technique

All the 71 isolates were first screened for polymerase chain reaction (PCR) by using specific primer (p34 gene that codes for 34 kDa) for the detection of *M. tuberculosis* complex and then identification and differentiation of *M. tuberculosis* and *M. bovis* by using another specific primer (*hupB* gene that codes for histone like protein) as per the methods described by Coetsier et al. (2000) and Prabhakar et al. (2004) with suitable modifications. Both the specific primers were procured from Sigma-Aldrich, USA. Standardization of PCR was done by using standard strains of *M. tuberculosis* and *M. bovis* obtained from

Tuberculosis Diagnosis and Training Centre, B.J. Medical College, Ahmedabad, Gujarat, India.

## Results

### Prevalence of TB in Human Beings

One hundred and fifty samples (64 sputum samples, 24 throat swabs and 62 nose swabs) of persons working either in livestock farm, Anand, or attending the OPD of Navali and Vadod primary health centre (PHC), Darbar Gopaldas TB Clinic, Anand and Tuberculosis Diagnosis and Training Centre, Ahmedabad were examined for the presence of *Mycobacteria*. Cultural isolation on LJ media revealed the overall prevalence of *M. tuberculosis* in 8.67 (13/150) per cent samples with the isolation rate of 9.38 (6/64) per cent in sputum, 4.17 (1/24) per cent in throat swabs and 9.68 (6/62) per cent in nose swabs (Table 1, Fig 1).

Out of total 50 human beings tested for Mantoux test, none was found positive for TB. However, total 13 isolates of *Mycobacteria* were obtained on cultural examination of throat swabs from them. The isolates showed typical eugenic colony characteristics and were positive for all the five biochemical tests confirming *M. tuberculosis* (Table 2).

High prevalence (20.00%) of TB was observed in age group of 20-30 years, 14.29 per cent in age group of 40-50 years and 12.5 per cent in age group of 50-60 years. According to sex males were found more susceptible to TB than females (9.38 vs. 0%, respectively).

Table 2: Growth pattern and biochemical characteristics of *M. tuberculosis* and *M. bovis*

| Character                               | <i>M. tuberculosis</i> | <i>M. bovis</i> |
|---|------------------------|-----------------|
| Colony morphology (growth)              | Eugonic                | Dysgonic        |
| Growth in presence of T <sub>2</sub> CH | +                      | -               |
| Pyrazinamidase                          | +                      | -               |
| Nitrate reduction                       | +                      | -               |
| Niacin accumulation                     | +                      | -               |
| Catalase activity                       | +                      | -               |

### Prevalence of TB in Animals and Environment

Under this study 400 samples of animal origin comprising 148 milk samples and 252 nose swabs were examined for the presence of *Mycobacteria* through cultural examination on LJ media. The study revealed 14.50 (58/400) per cent overall isolation rate in animals: 12.16 (18/148) per cent from milk samples and 15.87 (40/252) per cent from nose swabs. All the environmental samples including soil and water

samples were found negative for *Mycobacteria* (Table 3).

A total of 260 cattle (including indigenous and exotic cattle of different age group) were tested by single intra-dermal tuberculin test in the neck fold. The result revealed 16.15 (42/260) per cent positive reactors, while the rests 218 animals were found tuberculin negative. Out of 42 tuberculin positive cattle, *Mycobacteria* were isolated from 37 animals on cultural examination of milk or nasal swabs and from 218 tuberculin negative cattle *Mycobacteria* were isolated from 21 animals (Table 4, Fig 2-3). These cattle isolates from milk or nose swabs showed typical dysgonic colony characteristics and were negative for all biochemical tests, thus were identified as *M. bovis* (Table 2).

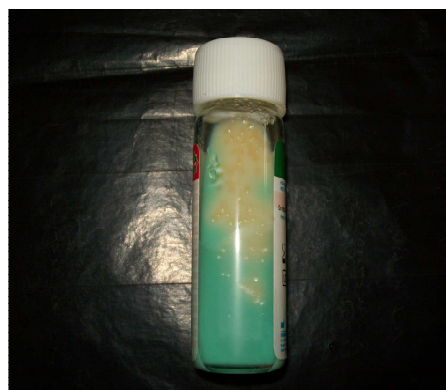


Fig 2: Isolation of *Mycobacterium tuberculosis* in pure and luxuriant growth from the nose swab obtained from a 25 years old man on LJ medium at 37°C after 3 weeks



Fig 3: Four weeks old growth of *Mycobacterium bovis* on LJ medium with 0.5 % sodium pyruvate incubated at 37°C, isolated from milk of a 8 years old Kankrej cow

The prevalence of bovine TB was found to be higher in exotic (37.29 % in HF) than indigenous (1.41% in Kankrej) cattle (Table 5). The highest -

Table 1: Cultural isolation of *Mycobacteria* (*M. bovis*; *M. tuberculosis*) from various clinical specimens of human beings

| Type/ Source of specimen | No. of patients | Cultural isolation     |                 |           | Proportion of humans with <i>M. tuberculosis</i> (%) |
|--------------------------|-----------------|------------------------|-----------------|-----------|--|
|                          |                 | <i>M. tuberculosis</i> | <i>M. bovis</i> | No growth |  |
| Sputum                   | 64              | 6                      | 0               | 58        | 9.38   |
| Throat swab              | 24              | 1                      | 0               | 23        | 4.17   |
| Nose swab                | 62              | 6                      | 0               | 56        | 9.68   |
| Total                    | 150             | 13                     | 0               | 137       | 8.67   |

Table 3: Isolation of *Mycobacteria* from Animals and Environment

| Source of sample | No. of sample examined | Isolation of <i>Mycobacteria</i> | No growth/ sterile samples | Isolation rate (%) |
|------------------|------------------------|----------------------------------|----------------------------|--------------------|
| Milk             | 148                    | 18                               | 130                        | 12.16              |
| Nose Swab        | 252                    | 40                               | 212                        | 15.87              |
| Total            | 400                    | 58                               | 342                        | 14.50              |
| Environment      | 50                     | 00                               | 50                         | 00.00              |

Table 4: Isolation of *M. bovis* from tuberculin tested cattle

| Tuberculin testing  | No. of samples | Cultural isolation of <i>M. bovis</i> from |           | No. of Sample negative for <i>M. bovis</i> |
|---------------------|----------------|--|-----------|--|
|                     |                | Milk                                       | Nose swab |  |
| Tuberculin positive | 42             | 13   | 24        | 5  |
| Tuberculin negative | 218            | 5  | 16        | 197  |
| Total               | 260            | 18   | 40        | 202  |

Table 5: Breed wise prevalence of Tuberculosis in cattle

| Breed   | No. of animals tested | No. of positive reactor | Per cent |
|---------|-----------------------|-------------------------|----------|
| Kankrej | 142                   | 2                       | 1.41     |
| HF      | 118                   | 44                      | 37.29    |
| Total   | 260                   | 46                      | 17.69    |

prevalence (70%) of TB in HF cattle was seen in age group of 0-1 year which was followed by comparatively low prevalence (40% and 17.86%) in HF animals of 1-5 and 5-10 years age group, respectively, whereas only 3.28 per cent prevalence of TB was observed in Kankrej cattle (2 case) in the age group of 5-10 years. Females were found more susceptible to TB; almost all *Mycobacteria* were isolated from female animals (in Kankrej animals 1.67% and in HF animals 46.32% prevalence).

#### Detection of p34 and *hupB* gene by PCR

All the 71 isolates of *mycobacteria* from different samples of men and animals were subjected to detection of gene p34 by PCR. They all yielded desired amplified product of approximately 363 bp, similar to that of reference strain of *M. tuberculosis* complex (*M. bovis* and *M. tuberculosis*). These isolates when subjected to detection of gene *hupB*, yielded desired amplified product of approximately 336 bp and 309 bp in case of *M. tuberculosis* and *M. bovis*, respectively (Fig 4-5).

## Discussion

Bovine tuberculosis is caused by *Mycobacterium bovis*, a mycobacterium highly similar to *M. tuberculosis* that belongs to the *M. tuberculosis* complex. The main host of *M. bovis* is cattle but it also affects many other mammals including humans. Tuberculosis in humans caused by either *M. bovis* or *M. tuberculosis* is clinically hard to distinguish (Etchehoury *et al.*, 2010). In the present study 150 samples (sputum, throat swab and nose swab) from human beings were examined for the presence of *Mycobacteria* by cultural examination. The overall isolation rate revealed the prevalence of 8.67 per cent. These findings are in accordance with Concepcion *et al.* (2001) who isolated 5.8 per cent *Mycobacteria* from sputum. However, Dravid *et al.* (1992) obtained 13.40 per cent *Mycobacteria* from human respiratory secretions and Mawak *et al.* (2009) isolated 12.15 per cent *Mycobacteria* from sputum samples of 329 volunteer patients.

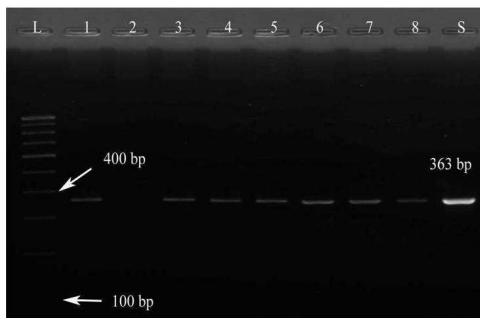


Fig 4: Agarose gel showing PCR amplified product of p34 gene

Lane 1, 3-8: Showing amplified product of *M. tuberculosis* complex (approx 363 bp); Lane 2: Negative sample, L: 100 bp DNA Lader; S: *Mycobacterium tuberculosis* standard strain showing amplified product (approx 363 bp)

A total of 50 clinically tuberculosis positive animal handlers, farm labourers and human patients were administered 0.1 ml PPD intra-dermally on flexor surface of forearm. All these human cases were turned out to be negative for tuberculin test, though 13 of them yielded *Mycobacterial* isolates on cultural examination of their throat swabs. These findings were in contrast with Kathleen *et al.* (1996) who tested tuberculosis positive elephants' handlers (trainers and care takers) and found positive result for tuberculosis by indurations more than 5 mm.

A tuberculin positive test signifies that the individual has been exposed to *Mycobacteria* but does not necessarily mean TB disease. eg. Mantoux test is positive in primary infection and after BCG

immunization. The skin reaction may be negative or reduced in patients with TB disease, if they are immunosuppressed by a wide variety of disorders, e.g. HIV infection, malnutrition and also overwhelming TB. False positive results are obtained due to cross-reacting organism like *M. paratuberculosis* and wild animals are required to get immunized twice (OIE, 2006; Sharma, 2007).

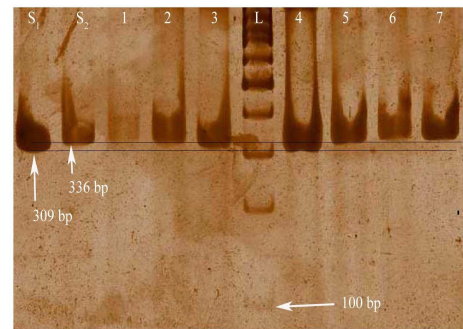


Fig 5: Polyacrylamide gel showing PCR amplified product of *hupB* gene

Lane 3-4: showing amplified product of *Mycobacterium bovis* (approximately 309 bp); Lane 1-2 and 5-7: showing amplified product of *Mycobacterium tuberculosis* (approximately 336 bp); S1: *Mycobacterium bovis* standard strain showing amplified product (approximately 309 bp) S2: *Mycobacterium tuberculosis* standard strain showing amplified product (approximately 336 bp); L: 100 bp DNA Lader

Further, 400 samples (148 milk and 252 nose swabs) from zebu and exotic cattle were examined for the presence of *Mycobacteria* by cultural examination. The overall isolation rate was 14.50 per cent (12.16% in milk and 15.87 % in nose swabs). These findings are in accordance with Appuswami *et al.* (1980), Jalil *et al.* (2004) and Leite *et al.* (2009). They suggested that milk and nasal secretions are considered as important media for transmission of bovine tuberculosis. Appuswami *et al.* (1980) isolated 20.25 per cent *Mycobacteria* from milk and nose swabs of 1000 animals comprising buffaloes and cows. Present study showed that milk and nose swabs were the most appropriate specimens for isolation of *M. bovis* from infected cattle.

Moreover, out of 50 samples from soil and water examined, not a single sample was found positive for isolate of *Non-tuberculous Mycobacteria* - NTM (*Mycobacterium fortuitum*). This was in contrast with Chilima *et al.* (2006) who isolated 75 per cent of NTM isolates from 24 water samples and 51 per cent from 148 soil samples. This difference might be

associated with prevalence rate/severity of the disease and hygienic measures adopted on the farm.

Single intra-dermal tuberculin test is most widely used for diagnosis of tuberculosis in animals (Dhanda and Lall, 1963). In the present study, 37 isolates of *Mycobacterium bovis* were obtained from 42 PPD tuberculin positive cattle and 21 *Mycobacterium bovis* isolates from 218 tuberculin negative cattle. This means false positive and false negative results are not uncommon with intra-dermal PPD test. Of the total 58 isolates, 18 isolates of *M. bovis* were obtained from 148 cow's milk samples (12.16%) and 40 isolates from nose swabs (15.87%). These findings are in accordance with the report of Appuswami et al. (1980). In their study, out of 815 buffaloes and 185 cows, 57 (6.91 %) buffaloes and 16 (8.64%) cows gave a positive reaction to tuberculin test. *Mycobacterium bovis* was isolated from 28.07 and 12.18 per cent of milk and nasal secretions, respectively, of tuberculin positive buffaloes and 25.00 and 12.55 per cent from tuberculin positive cows. The present findings are also in accordance with Meikle et al. (2007) who isolated *Mycobacteria* from nose swabs by cultural examination from 3 out of 14 tuberculin positive HF heifers.

*Mycobacterium bovis* is both the causative agent of bovine tuberculosis (TB) and a zoonotic pathogen. *M. bovis* isolated from milk (lactating animals) and nose swabs of animals makes it most important for public health. In earlier studies from other countries, *M. bovis* was often considered to be the most common cause of lymph node tuberculosis in humans, mainly acquired through drinking raw milk from tuberculous cattle. *Mycobacterium bovis* also poses human health problems as well because it is suspected that *M. bovis* infection is responsible for approximately 4000 of the approximately 80000 cases of human tuberculosis reported each year in Brazil (WHO, 1993). Although in humans, considerably fewer cases of TB are caused by *M. bovis* than *M. tuberculosis*, diagnostic limitations currently available probably underestimate the true dimension of the problem (Perez-Lago et al., 2013).

In present study in organized farms, the prevalence of Tuberculosis in exotic (HF) and Indigenous (Kankrej) cattle was 37.29 and 1.41 per cent, respectively. This observation suggested that zebu cattle are more resistant to tuberculosis as compared to exotic cattle. The present findings are in accordance with Samad and Rahman (1986) who reported 7.8 per cent prevalence in exotic and crossbred cattle and 2.1 per cent in indigenous cattle. Lall (1969) have documented a prevalence of 2.39 per cent among cows and 6.85 per cent among buffaloes of bovine TB infection in organized farms.

Prevalence of tuberculosis was 70 per cent in HF cattle in young (0-1 year) age group, 40 per cent in

1-5 years age group and 17.86 per cent in 5-10 years age group. These findings are in contrast to the observations of Nair and Ramakrishnan (1969) that the prevalence was higher in adults than young ones. Ameni et al. (2007) also reported that cattle aged 5-9 years had higher risk of bovine TB than those 2 years or below.

The highest incidence of bovine TB is generally observed where intensive dairy production is most common, notably in the milk sheds of larger cities. In developing countries, bovine TB infects a higher proportion of exotic dairy breeds (*Bos taurus*) than crossbred and indigenous zebu (*Bos indicus*) cattle. However, under intensive feedlot conditions, a death rate of 60 per cent and depression of growth have been found in tuberculous zebu cattle. Where extensive management is more common, animal crowding plays a major role in the spread of the disease (Acha and Szyfres, 1987; Perez-Lago et al., 2013).

In the present study, all the 71 isolates of *Mycobacteria* when subjected to PCR for detection of the p34 gene yielded desired amplified product of *M. tuberculosis* complex approximately 363 bp. These findings are in accordance with Coetsier et al. (2000) who also using similar primer pair found that all clinical samples contained p34 gene. Further, all these isolates of *Mycobacteria* when subjected to PCR for the detection of the *hupB* gene using species and genus specific primers yielded desired amplified product of *M. tuberculosis* and *M. bovis* approximately 336 and 309 bp, respectively. These findings are in accordance with Prabhakar et al. (2004) who used similar primer pair and found that all the isolates had *hupB* gene which yielded 645 and 618 bp in *M. tuberculosis* and *M. bovis*, respectively. Recently developed more advanced diagnostic tests like multiplex PCR, ELISA, gamma interferon assay etc are claimed to differentiate *M. bovis* and *M. tuberculosis*, and confirm tuberculin positive and negative reactors, infected and vaccinated animals ((Perez-Lago et al., 2013), we have not employed them in the present study.

## Conclusion

Based on the findings, it was concluded that the prevalence of tuberculosis is high in dairy animals in the organized farms as well as in human beings exposed to cattle. The cultural examinations of specimens from humans and animals on LJ media under specific conditions yielded typical *Mycobacterial* colonies, which could be distinguished as of *M. tuberculosis* or *M. bovis* based on positive and negative results of standard biochemical tests, respectively, and even with molecular characterization using PCR technique with specific primers. The intra-dermal tuberculin test in both men and animals is not always

conclusive, since false negative and false positive results are not an uncommon feature of this test.

## Acknowledgements

## References

- Acha PN and Szyfres B (1987). Zoonotic tuberculosis. In: Zoonoses and communicable diseases common to man and animals. 2<sup>nd</sup> ed., Washington: Pan American Health Organization / World Health Organization: Scientific Publication No. 503.
- Ameni G, Bonnet P and Tibbo (2007). A cross-sectional study of bovine tuberculosis in selected dairy farms in Ethiopia. *Clinical Vaccine and Immunology*, 14: 1011-1013.
- Appuswami S, Batish VK, Prakash OR and Rangnathan B (1980). Prevalence of *Mycobacteria* in raw milk samples in Karnal, India. *Journal of Food Protection*, 43: 778-781.
- Bannalikal AS (2007). Molecular diagnosis of non-tuberculous *Mycobacteria* (NTM) in animals. *Proc. ICAR winter school on molecular diagnosis of animal tuberculosis with particular reference to recent developments in the Tuberculosis research for its diagnosis and prophylaxis*. pp. 82-88.
- Bezoz J, Casal C, Romero B, Schroeder B, Hardegger R, Raeber AJ, López L, Rueda P and Domínguez L (2014). *Current ante-mortem techniques for diagnosis of bovine tuberculosis*. pii: S0034-5288(14)00100-3. doi: 10.1016/j.rvsc.2014.04.002.
- Chilima BZ, Clark IM, Floyd S, Paul EM and Hirsch PR (2006). Distribution of environmental *Mycobacteria* in Karonga District, Northern Malawi. *Applied Environmental Microbiology*, 72: 2343-2350.
- Coad M, Hewinson RG, Clifford D, Vordermeier HM and Whelan AO (2007). Influence of skin testing and blood storage on interferon-gamma production in cattle affected naturally with *Mycobacterium bovis*. *Veterinary Record*, 160(19): 660-662.
- Cole E and Cook C (1998). Characterization of infectious aerosols in health care facilities: an aid to effective engineering controls and preventive strategies. *American Journal of Infection and Control*, 26(4): 453-464.
- Concepcion F, Myrna T and Mendoza MD (2001). Isolation rates of *Mycobacterium tuberculosis* from smear negative and smear positive sputum specimen using the Ogawa culture technique and standard Lowenstein Jensen culture technique. *Philippines Journal of Microbiology and Infectious Diseases*, 30(2): 37-39.
- Coetsier C, Vannuffel P, Blondeel N, Francois J, Cocito C and Luc Gala J (2000). Duplex PCR for differential identification of *Mycobacterium bovis*, *M. avium*, and *M. avium* subsp. *paratuberculosis* in formalin-fixed paraffin-embedded tissues from cattle. *Journal of Clinical Microbiology*, 38(8): 3048-3054.
- Cousins DV and Williams SN (1995). A study of *Mycobacterium bovis* infection in Australian patients (1970-1994). In: Griffith, F. and de Lisle, G. editors. *Tuberculosis in wildlife and domestic animals*. Otago, New Zealand: University of Otago Press. pp. 260-263.
- Cox R (2004). Quantitative relationships for specific growth rates and macromolecular compositions of *Mycobacterium tuberculosis*, *Streptomyces coelicolor* A3 (2) and *Escherichia coli*: an integrative theoretical approach. *Microbiology*, 150: 1413-1426.
- Dhanda MR and Lall JM (1963). Tuberculosis in man and animals. *Indian Journal of Public Health*, 7: 97-102.
- Dravid MN, Joshi S, Bhardwaj RS and Khare PM (1992). Differential identification of *Mycobacterium tuberculosis* from various clinical specimens from Sassoon General Hospital, Pune. *Indian Journal of Medical Science*, 46 (2): 43-45.
- Etchehoury I, Valencia GE, Morcillo N, Sequeira MD, Imperiale B, López M, Caimi K, Zumárraga MJ, Cataldi A and Romano MI (2010). Molecular typing of *Mycobacterium bovis* isolates in Argentina: first description of a person-to-person transmission case. *Zoonoses and Public Health*, 57 (6): 375-381.
- Grange JM, Yates M and De Kantor I (1996). Guidelines for speciation within the *M. tuberculosis* complex. 2<sup>nd</sup> ed. Geneva: WHO. 96: 104.
- Jalil H, Das P and Suleman A (2004). Bovine tuberculosis in dairy animals at Lahore, threat to public health, metropolitan Corporation Lahore, Pakistan. [http://www.priory.com/vet/bovine\\_tuberculosis.htm](http://www.priory.com/vet/bovine_tuberculosis.htm). (2004).
- Kamala T, Paramasivan D, Herbert P, Venkatesan N and Prabhakar R (1994). Evaluation of procedures for isolation of non-tuberculous *Mycobacteria* from soil and water. *Applied Environmental Microbiology*, 60: 1021-1024.
- Kathleen Michalak, Connie Austin, Sandy Diesel, J. Maichle Bacon, Phil Zimmerman and Joel N Maslow (1996). *Mycobacterium tuberculosis* infection as a zoonotic disease: transmission between humans and elephants. *Veterinary Record*, 155: 171-175.
- Kestle DG, Abott VD and Kubica GP (1967). Differential identification of *Mycobacteria*. II. Subgroups of Groups II and III (Runyon) with different clinical



- significance. *American Review of Respiratory Diseases*, 95: 1041-1052.
- Lall JM (1969). Tuberculosis among animals in India. *Veterinary Bulletin*, 39: 385-390.
- Leite A, Carvalho I, Tavares E and Vilarinho A (2009). Tuberculosis disease – statistics of a paediatric department in the 21<sup>st</sup> century. *Review of Port and Pneumology*, 15: 771-782.
- Lisbet S (2006). Immunohistochemistry using a *Mycobacterium tuberculosis complex* specific antibodies for improved diagnosis of tuberculous lymphadenitis. *Modern Pathology*, 19: 1606-1614.
- Mawak J, Gomwalk N, Bello C and Kandakai-Olukemi Y (2009). Human pulmonary infections with bovine and environment (atypical) mycobacteria in Jos, Nigeria. *Ghana Medical Journal*. 40(4): 132-136.
- Meikle V, Schneider M, Azenzo G, Zumarraga M, Magnano G and Cataldi A (2007). Individual animals of a cattle herd infected with the same *Mycobacterium bovis* genotype shows important variations in bacteriological, histopathological and immune response parameters. *Zoonoses and Public Health*, 54: 86-93.
- Nair Chandrasekharan KP and Ramakrishnan R (1969). Bovine tuberculosis due to the human strain *Mycobacterium tuberculosis*. *Indian Journal of Tuberculosis*, 16: 103-112.
- Niemann S, Richter E, Dalgge-Tamm H, Schlesinger H, Graupner D and Knigstein B (2000<sup>a</sup>). Two cases of *Mycobacterium microti* derived tuberculosis in HIV-negative immuno competent patients. *Emerging Infectious Disease*, 6: 539-542.
- Niemann S, Richter E and Sabine Rusch-Gerdes (2000<sup>b</sup>). Differentiation among members of the *Mycobacterium tuberculosis complex* by molecular and biochemical features: Evidence for two pyrazinamide-susceptible subtypes of *M. bovis*. *Journal of Clinical Microbiology*, 38(1): 152-157.
- OIE - Office International des epizootics (2006). Manual of standard for diagnostic tests and vaccines (Paris). <http://www.oie.int>.
- OIE (Office International des epizootics) Terrestrial Manual (2009). Chapter 2.4.7. Bovine Tuberculosis. pp. 1-16.
- Pal M (1997). *Zoonoses*. 1<sup>st</sup> Ed. R.M. Publishers and Distributors, Delhi, India, pp.126-127.
- Pérez-Lago L, Navarro Y, García-de-Viedma D (2013). Current knowledge and pending challenges in zoonosis caused by *Mycobacterium bovis*: A review. *Research in Veterinary Science*, pii: S0034-5288(13)00370-6. doi: 10.1016/j.rvsc.2013.11.008.
- Prabhakar S, Mishra A, Singhal A, Katoch VM, Thakral SS, Tyagi JS and Prasad HK (2004). Use of the *hupB* gene encoding a histone-like protein of *Mycobacterium tuberculosis* as a target for detection and differentiation of *M. tuberculosis* and *M. bovis*. *Journal of Clinical Microbiology*, 42(6): 2724-2732.
- Raviglione MC (1995). Global tuberculosis incidence and mortality during 1990-2000. *Bulletin of World Health Organization*, 72: 213-220.
- Ryan TJ, Buddle BM and De Lisle GW (2000). An evaluation of the gamma interferon test for detecting bovine tuberculosis in cattle 8 to 28 days after tuberculin skin testing. *Research in Veterinary Science*, 69: 57-61.
- Sabry M and Elkerdasy A (2014). A polymerase chain reaction and enzyme linked immunosorbent assay based approach for diagnosis and differentiation between vaccinated and infected cattle with *Mycobacterium bovis*. *Journal of Pharmacology and Bioallied Sciences*, 6 (2): 115-21.
- Samad MA and Rahman MS (1986). Incidence of bovine tuberculosis and its effect on certain blood indices in dairy cattle of Bangladesh. *Indian Journal of Dairy Science*, 39: 231-234.
- Sharma AK (2007). Advances in Pathology in the diagnosis of animal tuberculosis. *Proc. on ICAR sponsored short course on "Molecular Diagnosis of Animal Tuberculosis with particular reference to Recent Developments in the Tuberculosis research for its Diagnosis and prophylaxis"*. pp. 89-94.
- TB India (2012). *Revised National TB Control Programme, Annual Status Report, New Delhi, India*, [www.tbindia.nic.in/documents.html#](http://www.tbindia.nic.in/documents.html#)
- Vestal AL (1977). In: Procedures for isolation and identification of Mycobacteria, U.S. Department of Health, Education and Welfare, CDC Atlanta, Georgia, Publication No CDC-77-8230. pp.15-19.
- WHO-World Health Organization (1993). Report of the World Health Organization meeting on zoonotic tuberculosis (*Mycobacterium bovis*), Geneva. pp. 27.
- WHO-World Health Organization (2004). Global tuberculosis control, surveillance, planning, financing, Geneva.
- WHO-World Health Organization (2006). Tuberculosis Fact sheet - Global and regional incidence. Options for vaccinating cattle against bovine tuberculosis, DEFRA report. [http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/tb/documents/vaccine\\_cattle.pdf](http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/tb/documents/vaccine_cattle.pdf).