



Research Paper

Comparative diagnosis of *Mycobacterium bovis* by Polymerase chain reaction and Ziel- Neilson staining technique using Milk and nasal washing

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ABSTRACT:- The present study elucidated the status of Bovine tuberculosis (BTB) diagnosis by acid-fast staining and polymerase chain reaction using milk and nasal washing samples to compare their efficacy in the diagnosis of bovine tuberculosis. The study was carried out in unorganized dairy sector and cows attending Madras Veterinary College LAC (OP) hospital. Ninety two nasal washings and eighty two milk samples were collected and subjected to acid fast staining and polymerase chain reaction (PCR). Of the 92 smears examined, 2 (2.17 percent) were found positive for acid-fast mycobacteria. Milk sample smears from 82 animals were subjected to acid-fast staining of which, 2(2.44 percent) were positive for acid-fast mycobacteria.

DNA was extracted from 92 nasal washing and 82 milk samples were subjected to Polymerase chain reaction (PCR). Nine (9.8 per cent) animals were positive for nasal washing PCR and 10 animals (12.2) were positive for milk PCR. Seven animals positive for nasal washing are also positive for milk PCR. Two animals positive for nasal washing were dry animals and three pleuriparous animals positive for milk PCR were not positive for nasal washing.

PCR have high sensitivity (100 per cent) and specificity (97.6 per cent) of detecting *M.bovis* in nasal washings. Similarly for milk PCR have high sensitivity (100 per cent) and specificity (87.6 per cent).

Keywords: Bovine Tuberculosis, Acid fast staining, Polymerase chain reaction, milk and nasal washings

I. INTRODUCTION

Bovine tuberculosis is becoming increasingly important due to the susceptibility of humans to the disease caused by *M. bovis* and there is increasing evidence that *M. bovis* infections may be much more significant than generally considered. The current increasing incidence of tuberculosis in humans, particularly in immunocompromised persons, has given rise to a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries (Radostits et al., 2008). The direct correlation between *M.bovis* infection in cattle and the disease in the human population has been well documented in developed countries, whereas scanty information is available from developing countries. This lack of data, relates to its perception as an animal disease, with the health problems relating to the HIV/AIDS and human tuberculosis given a greater priority. Tuberculosis in cattle is a human health issue. The knowledge about the implication of bovine tuberculosis in the human cases has to be developed and disseminated for effective control. The role of the different commodity chains (milk and meat) has to be evaluated. Wildlife, farm animals, pets, food and milk all pose a potential threat to public health.

II. MATERIALS AND METHODS

The present study was carried out in and around Chennai where dairy animals (100) kept for milking and ailing animals attending Madras Veterinary College teaching hospital.

a) MILK

10ml of milk from 82 cattle was collected in a sterile container and stored at -20°C.

b) NASAL WASHINGS

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The nasal washing tubes from 92 cattle was collected in sterile test tubes and stored at -20°C.

III. METHODS

a. Acid fast Staining(AFS)

The acids fast staining was performed according to the procedures detailed in “A Manual On Veterinary Preventive Medicine”, published by ICAR (1996).

- 1) Smears made form nasal mucus and milk (centrifuged at 4000 rpm for 5 min and sediment used) were air-dried and heat-fixed.
- 2) The slides were covered with filtered Carbol Fuchsin and heated with a spirit lamp until steam raised up (the stain was not allowed to evaporate).The smears were allowed to stand for 5 minutes.
- 3) The slides were washed in running tap water and were covered with 20 percent sulphuric acid. After a minute, the slides were washed in running water, and more acid was poured and the process was repeated several times until the decolourisation was completed. The slides were washed in running water and covered with 95 percent alcohol for 2 minutes and washed in running water.
- 4) The slides were washed in running tap water and then counter-stained with methylene blue solution for 2 minutes, washed in running water, blotted, dried and examine under oil immersion objective.

b. Polymerase chain reaction

A reliable diagnostic test elutes the detection of tuberculous herds. In spite of the large number of ancillary tests available, PCR was recently employed for BTB diagnosis offers potential advantages of sensitivity, flexibility and speed (OIE,2008). DNA from milk and nasal washing samples were extracted using EZ-genomic spin column DNA blood and Tissue Kit (Biobasic, Canada) as per the manufacturer’s instructions.

Primers used in this study were designed by liebana *et al.*(1995) which amplify target region of repeat sequence(IS 6110) which is encoded in the *mycobacterium bovis* genomic DNA.

a) Oligonucleotide primers(IS 6110) used for PCR:

Forward Primer 5’ CCT GCG AGC GTA GGC GTC GG 3’

Reverse Primer 5’ TCA GCC GCG TCC ACG CCG CCA 3’

b) PCR reaction(25µl)

DNA sample	4µl
Forward primer	1 µl
Reverse primer	1 µl
Red dye master mix	12.5µl
DNase free water to make up to	25µl

c) PCR cycle condition

Initial Denaturation	94°C	2 min
Followed by 30 cycles of Denaturation	94°C	2min
Annealing	68°C	2min
Extension	72°C	2min
Final Extension	72°C	10min

The PCR was performed in thermal cycler (Eppendorf,Germany). The PCR product was subjected to electrophoresis in 1.5% agarose gel with 5µg/ml of ethidium bromide and observed under UV transilluminator for the presence of amplicon of desired product.

IV. RESULT AND DISCUSSION

Nasal washing smear from the 92 animals were subjected to acid-fast staining. Of the 92 smears examined, 2 (2.17 percent) were found positive for acid-fast mycobacteria. Milk sample smears from 82 animals were subjected to acid-fast staining of which, 2(2.44 percent) were positive for acid-fast mycobacteria. The one pleuriparous animal which was found to be positive by acid-fast staining using nasal washing and milk was same. The other animal less than two years of age positive for nasal washing and a pleuriparous animal was positive for milk sample not for nasal washing sample.

The detection of acid fast bacilli(AFB) in this study depends on excretion of the organism at different points of time. Similar type of variability in the excretory pattern of the organism was also observed by Neil *et al.*(1994). The excretory pattern could explain the lower number of positivity for AFB in the milk and nasal washing. Radostits (2006) opined that organisms are excreted in the exhaled air, in sputum, feces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes. Cattle in the early stages of the disease, before any lesions are visible, may also excrete viable mycobacteria in nasal and tracheal mucus (McIlroy, 1986).

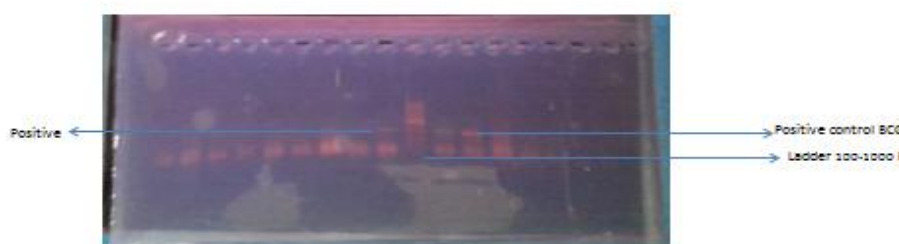
DNA elements that are unique to tuberculosis complex particularly in human and bovine targeted in the PCR detection system for the diagnosis of tuberculosis infection in bovines and humans. This study also utilized the IS6110 sequence for PCR based diagnosis of BTB. DNA was extracted from 92 nasal washing and 82 milk samples were subjected to Polymerase chain reaction (PCR). Nine (9.8 per cent) animals were positive for nasal washing PCR and 10 animals (12.2) were positive for milk PCR. Seven animals positive for nasal washing were also positive for milk PCR. Two animals positive for nasal washing were dry animals and three pleuriparous animals positive for milk PCR were not positive for nasal washing. The four samples which were positive by acid fast staining also positive by PCR.

Skuce et al., (2011) study found that *M. bovis* was not isolated from any of the nasal mucus samples, and subsequent studies indicated that detectable nasal mucus excretion in naturally-infected animals was very low or sporadic.

PCR have high sensitivity (100 per cent) and specificity (97.6 per cent) of detecting *M.bovis* in nasal washings. Similarly for milk PCR have high sensitivity (100 per cent) and specificity (87.6 per cent). The five animals positive for acid fast staining were positive by both nasal washing and milk PCR. PCR could be an ideal choice for confirmatory diagnosis of BTB, as conventional diagnostic procedures are often time consuming and have low sensitivity and or specificity (Thierry et al.1990;Collins et al.1993;Kox et al.1994;Sreedevi and krishnappa,2004).

V. CONCLUSION

Diagnosis of bovine tuberculosis using nasal washing and milk samples carried out. There is no significant difference in detection of bTB using nasal washings and milk samples. The excretory pattern of bTB plays a key role in the diagnosis using different clinical samples. The sensitivity is less for acid fast whereas for PCR it is 100 per cent and also specific in detection.



Samples showing positivity for Tuberculosis using PCR

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