Explor Anim Med Res.
Vol. 14, Issue 1, 2024
DOI: 10.52635/eamr/14.1.154-157

ISSN 2277-470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Short Communication

NON-INVASIVE SAMPLING BASED SCREENING OF DAIRY CATTLE HERDS FOR *MYCOBACTERIUM* SPS

Vinod Kumar Singh[1*], Suman Sagar[1], Chayanika Das[2], Ruchi Tiwari[1], Amit Kumar[3], Sharad Kumar Yadav[1]

Received 21 October 2023, revised 08 January 2024

ABSTRACT: Current diagnostic methods for Bovine tuberculosis (TB) and Paratuberculosis (pTB) involve invasive sampling methods that require handling and restraining causing stress and pain to the animals along with days required for the interpretation of test results. By the 3Rs ethical principle and animal welfare, an alternative non-invasive sampling method to screen herds could be a better option. Milk may be a major source for the large-scale spread of tuberculosis and to a considerable extent of paratuberculosis bacilli in humans. Keeping this in view, milk samples were utilized for the screening of herds employing acid-fast staining microscopic examination to determine the presence of *Mycobacterium* and other acid-fast bacilli (AFB). For the study, a total of 150 milk samples collected randomly from dairy herds of five major gaushalas of the Vrindavan and Mathura region of Uttar Pradesh, India were screened. Microscopic examination reveals the presence of AFB in 18 samples from 3 out of the 5 herds under study. In conclusion, the presence of the AFB in the milk being used for feeding calves and human consumption is alarming for both animal and human health aspects and requires confirmation on a large scale to control the further spread. Also, the study indicates the importance of milk samples microscopic examination for AFB as an alternative non-invasive procedure for initial screening of dairy herds for TB and pTB.

Keywords: Acid-fast bacilli, *Mycobacterium*, TB, pTB, Herd screening, Non-invasive sampling, Testing of milk.

Infection from *Mycobacterium* sp. has a variety of clinical presentations in both humans and animals. Further, mycobacterial diseases with zoonotic potential play a role in establishing a considerable impact on maintaining the health of the nation in terms of healthy human and animal resources. Complete eradication requires a comprehensive approach from both the human and animal health sectors, and the core requirement for a successful control program is the continuous collection of disease data. The screening of the herds for TB and pTB is commonly done by skin testing and/or IFN-γ assay and culture, which involve invasive procedures requiring handling and restraining, accompanied by stress and pain to the animals. Also, both of these test procedures are cumbersome, require animal ethics permission from competent authorities, and take days for the interpretation of test results [1]. Usually, in TB-affected animals, the shedding of the bacteria to the external environment occurs through nasal secretions, feces, and milk, while in the case of pTB, feces and milk are the major routes of MAP shedding. Microbial contamination of milk can pose a risk of spreading infection among animals as well as milk-borne zoonosis [1]. The milk may act as a source of MTB and MAP infection in young animals and humans who consume raw milk and play an important role in the spread and maintenance of the infection [2, 3].

[1]*Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India.*
[2]*Division of Microbiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India.*
[3]*College of Biotechnology, Sardar Vallbhbhai Patel University of Agriculture and Technology, Meerut,Uttar Pradesh, India.*
**Corresponding author. e-mail: vet.vinodsingh@gmail.com*

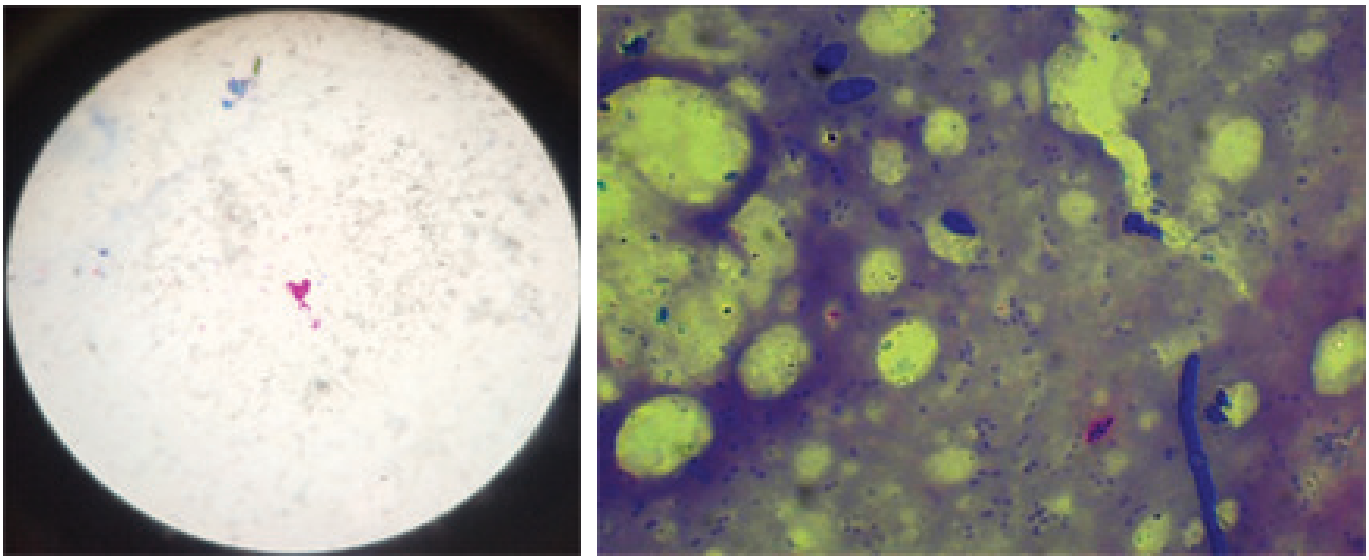

Fig. 1. Representative positive milk sample smear showing AFB in bunch. [(A)15-20 cells and (B) 5-10 cells along with co-infection of non-acid fast pathogens (1000x)].

So, the present study aimed to evaluate the utility of milk or colostrums as an alternative non-invasive sample in screening cattle herds for the presence of acid-fast bacilli (AFB), possibly *Mycobacterium* sps, employing acid-fast staining (AFS) microscopic examination for a simple and economic diagnosis that can easily be performed even in primary laboratories with limited resources.

The study

The study was conducted on dairy cows of goshalas in the Mathura and Vrindavan regions of Uttar Pradesh, India. The study was approved with approval no. IAEC/18/25 by the Institute Animal Ethics Committee, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura, Uttar Pradesh, India registered with Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India, with registration no. 386/PO/ReBi/SL/01/CPCSEA.

For the investigation, a total of 150 healthy lactating cows of different age groups from five gaushalas in Mathura and Vrindavan were sampled, collecting approximately 10 mL of milk pooled from all four quarters. The breed of the cattle, the number of milk samples collected from a herd, and the husbandry practices followed at the farm were recorded (Table 1). 5 mL of milk sample was centrifuged at 5000 rpm for 10 minutes at 4°C. Then, the supernatant was discarded,

Table 1. Details of the AFB positive animals and herds under study.

Location	Study ID for Farm/ Goshala	Breed*	Husbandry System	No of samples	Positive for AFB indistinguishable to MAP	Percent positive
Vrindavan	Panchayati Goshala	HR/ND	Unorganized	36	7	19.44
Vrindavan	Hasanand Goshala	Sahiwal	Organized	45	0	0
Vrindavan	Surabhi Goshala	Sahiwal	Organized	17	2	11.76
Vrindavan	Iskon Goshala	HR/ND	Organized	11	0	0
Mathura	Gurudev Goshala	CB	Organized	41	3	7.31
Total	5			150	12	8.0

*HR=Haryana; ND=Non-descriptive; CB=Crossbred.

retaining the cream, which was then thoroughly mixed with sediment in 2.5 mL of 1x PBS by vortexing for 2 minutes for use in AFS [4]. 20 µL of processed milk sample was used to prepare smears on a clean, grease-free microscopic glass slide for AFS. All the stained slides were examined under 1000x magnification in four steps (random field observation, horizontal and vertical battlefield manner observation, and examining the periphery of the smear) for 20-25 minutes, and the observations were recorded according to the AFB scoring criteria of Fujiki [5], as follows: No AFB in at least 100 microscopic fields was scored as negative, one to nine AFB in 100 microscopic fields was scored as doubtful, and 10 to 99 AFB in 100 microscopic fields was scored as positive. The microscopic examination of the AFS milk sample smears revealed a total of 12 out of 150 cattle samples (Fig. 1), corresponding to an 8% prevalence with the highest percent of positive animals (7/36) in the Panchayati Goshala herd under unorganized husbandry practices. The herd-wise percent-positive animal details are detailed in Table 1.

Discussion

Understanding the epidemiology of *Mycobacterium* diseases is essential for directing control interventions and understanding the pathogen spread in animals and humans. The specific diagnosis of *Mycobacterium* infections in live animals is challenging and currently uses invasive sampling methods for skin sensitivity screening tests and confirmatory immunoassays (IFNγ assay, ELISA). Also, culture from non-invasive sources such as feces and/or milk is challenging due to decontamination methods. AFS microscopy is an easy, rapid, and cost-effective method for screening herds that can be performed even in resource-limited primary diagnostic laboratories and provide information about the shedding pattern and shedding load of AFB. It is very difficult to diagnose an early infection when the disease is subclinical. However, sub-clinically infected animals continue to shed *Mycobacterium* bacilli in their milk and feces, thereby contaminating pastures, the environment, and the food chain for a long time [1, 6, 7]. *Mycobacterium* is a major milk-borne pathogen and has been reported to spread to young animals born to infected mothers as well as human beings [1, 8, 9]. The findings of the study support the utility of milk as a viable, non-invasive, alternate sample for *Mycobacterium* diagnosis employing confirmatory molecular tests [10]. Given animal welfare and the rapid screening of dairy herds with minimal laboratory resources, the results of the study are hopeful, but the major limitation is the small number of samples and the inability to test the non-lactating and dry animals. Studies with a large sample size of non-invasive samples, including milk, animal farm slurry, and/or dropped dung, would help to better elucidate the diagnostic utility of AFS microscopy of milk and other non-invasive samples in TB/JD screening.

ACKNOWLEDGMENT

We thank all the veterinarians, animal farm in-charges, and animal care personnel at various goshalas for their kind help in sample collection for the present study. We are also thankful to the Vice Chancellor, U.P. Veterinary University (DUVASU), Mathura for providing the necessary funds for the study.

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Cite this article as: Singh VK, Sagar S, Das C, Tiwari R, Kumar A, Yadav SK. Non-invasive sampling based screening of dairy cattle herds for *Mycobacterium* sps. Explor Anim Med Res. 2024; 14(1), DOI: 10.52635/eamr/14.1.154-157.