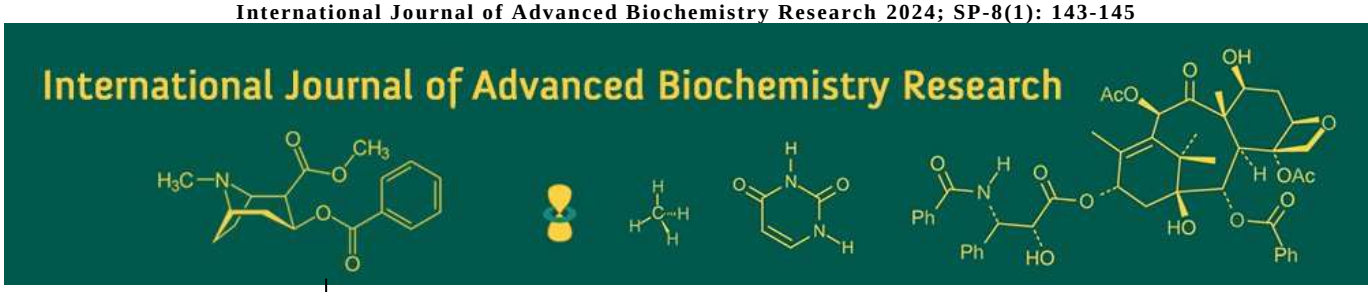

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(1): 143-145
www.biochemjournal.com
Received: 28-09-2023
Accepted: 25-11-2023

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Detection of Maedi Visna Virus (MVV) in milk samples of Indian goats

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DOI: https://doi.org/10.33545/26174693.2024.v8.i1Sc.356

Abstract
Background: Maedi is a retroviral illness with a protracted incubation period that afflicts the lungs of sheep and goats. Maedi is lymphoproliferative inflammatory disease condition affecting multiple organs including lungs, and caused by lentivirus.
Aim: The aim of present study was molecular detection of MVV in milk samples.
Methods: 75 goat milk samples were collected and PCR assay was performed to detect the nucleic acid of MVV in the milk samples.
Results: MVV nucleic acid was detected in 2.66% (2/75) of samples.
Conclusion: Milk/colostrums and infectious droplets are important modes of transmission for SRLV to susceptible animal population. Detection of MVV nucleic acid in the milk samples of Indian goats was reported first time in country.

Keywords: Maedi, MVV, milk, goat

Introduction
Maedi visna (MV) is a retroviral illness that affects small ruminants. MV is progressive inflammatory multi-systemic disease disorder. Farmers suffer productivity losses as a result of these illnesses, which are typically chronic and progressive in nature (Jubb *et al*., 2015). The causative agent of maedi, Maedi Visna Virus (MVV) is a monocyte/macrophage-tropic, non-oncogenic virus that infects sheep persistently. It is a member of the Lentivirus genus (Pepin *et al*., 1998) [11]. The onset of multisystemic disease problems, such as interstitial pneumonia, arthritis, chronic mastitis, etc., causes significant economic losses associated with maedi illness (Kumar *et al.*, 2022) [9].
The prevalence is substantially higher in farms where the practice of feeding lambs or kids pool of colostrum or milk from the tank (Gomez-Lucia *et al*., 2018) [7]. Spread of the infection to the susceptible population usually occurs through aerosol and milk or colostrums but transmission through placenta and sperm is also reported (Blacklaws, 2012) [2]. Milk/colostrums and infectious droplets are important modes of transmission for SRLV to susceptible animal population (Peterhans, *et al*., 2004) [12]. So, in present study detection of MVV nucleic acid was done in milk.

Materials and Methods
Sampling
Milk samples were collected from Rajasthan. Samples were collected as in ice and for further storage kept at -20 °C for molecular biology work. Total 75 goat milk samples were collected and processed for molecular detection of MVV nucleic acid by PCR.

DNA Extraction
The milk samples were centrifuged at 5000 rpm for 10 minutes to obtain milk pellet. This pellet was resuspended in 1ml PBS. Using this suspension, DNA was extracted from milk pellet using body fluids DNA extraction kit (APS labs, India). 100 µl milk pellet suspension was mixed with 20 µl proteinase K and final volume was adjusted to 220 µl with PBS and mixed by vortexing. After addition of 200 µl AL buffer, milk samples were incubated at 56° C for 10 min or until the suspensions were completely lysed and clear.

Then, equal volume of ethanol (96–100%) was added and mixed thoroughly by vortexing. About 700-800 µl of mixture was pipetted out in previously labeled DNeasy mini spin column placed in a 2 ml collection tube and centrifuged @ 8000 rpm for one minute. The flow through was discarded and the process was repeated if the suspension volume was more. DNeasy mini spin column was placed in a new 2 ml collection tube, washed with 500 µl of AW1 buffer by centrifuging @ 8000 rpm for 1 minute and the flow through was discarded. DNeasy mini spin column was washed again with 500 µl AW2 buffer by centrifuging @ 12,000 rpm for 3 minutes. The spin column with collection tube was once again centrifuged at 14,000 rpm for 3 min to remove the traces of any solutions followed by transferring the spin column in new sterile properly labeled 1.5 ml micro centrifuge tubes. AE buffer (50-100 µl) was pipetted directly onto the membrane of the column, incubated for 2-3 min at RT and centrifuged @ 12000 rpm for one min to elute the DNA. Concentration and purity of the isolated DNA was assessed with the aid of Nanodrop 1000 spectrophotometer (Thermo Scientific, USA) at A260/A280. During the entire procedure of DNA extraction, all the reagents, tubes and samples were kept in ice to avoid DNA degradation. The DNA was stored at -20 °C for further use.

Oligonucleotide primers

Published PCR primers (specific LTR region) pairs for MVV external (Fex and Rex) (Fex 5'-TGACACAGCAAATGTAACCGCAAG-3', Rex 5'-CCACGTTGGGCGCCAGCTGCGAGA-3') and MVV internal (Fin and Rin) (Fin: 5'-AAGTCATGTA(G/T)CAGCTGATGCTT-3', Rin: 5'-TTGCACGGAATTAGTAACG-3') were used for the detection of MVV nucleic acid in the milk samples. The primers were expected to amplify the specific viral DNA template of sizes 276 and 199 bp for MVV – Fex & Rex and MVV – Fin & Rin, respectively (Preziuso *et al.*, 2013) [13].

Polymerase chain reaction assay

MVV proviral DNA were detected using PCR in standard 12 µl reaction volumes in 0.2 ml tubes using sets of primers as specified. Green Dream Taq Master mix (Thermo Scientific, USA), was used to create a PCR reaction mix of 12 µl, consisting of 6µl of Green Dream Taq Master mix, 0.3 µl of forward and reverse primer for each, 3.4 µl of nuclease-free water, and 2 µl of DNA template. The contents of the tubes were combined, then they were put in a thermocycler (Q-Cycler 96, Hains Lifesciences, Verti 96 well thermocycler), and they were exposed to the standardized thermal cycling conditions. The thermal cycling conditions were composed of initial denaturation for 5 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C (MVV - Fex & Rex) and 50 °C (MVV - Fin & Rin) for 30 s, with a 40 s extension at 72 °C and single cycle of final elongation of 7 min at 72 °C.

The amplified DNA products were visualized by transillumination under UV light (Geldoc; c150Azure Biosystem) in 1.5% agarose containing ethidium bromide (0.5 g/mL) after 45 minutes of electrophoresis at 90 volts.

Results

Detection of maedi visna virus (MVV)

Total 75 milk samples were screened for the detection MVV nucleic acid by nested PCR assay using specific LTR region. DNA extracted from the samples were subjected for nested PCR, which amplified products of 276bp and 199bp with external (Fex and Rex) and internal (Fin and Rin) sets of primers, respectively (Fig. 1 A&B). MVV nucleic acid was detected in 2.66% (2/75) of samples.

Table 1: Details of samples the screened for MVV nucleic acid by PCR

Place of sample collection	Tissues		
	No. of samples screened	No. of positive samples	% Positivity
Rajasthan	75	2	2.66

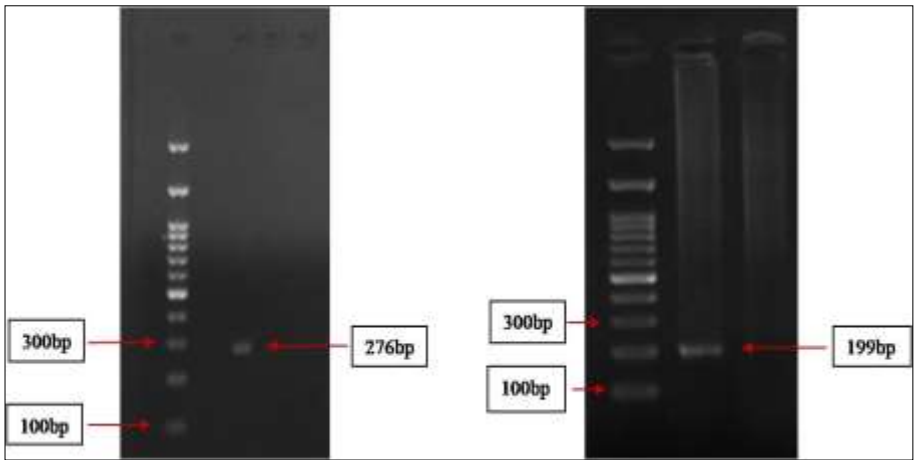

Fig 1: Agarose gel electrophoresis (2%) showing specific PCR amplified products 276bp (A) and 199bp (B) of MVV with external and internal set of primers

Discussion

A non-oncogenic retrovirus is the source of Maedi-Visna illness, which has a protracted incubation period ranging from several months to years (Sakhaee and Khalili, 2010) [15]. The infiltration of mononuclear cells and lymphoid hyperplasia in the central nervous system, lung parenchyma, and other organs are characteristics of the disease's progressive clinical history in sheep (Sayari and Lotfi, 2001) [16]. The viral particles released from alveolar pneumocytes and bronchiolar epithelium may be responsible for aerosol

spread while viral particle associated with MNCs may be responsible for spread through milk or colostrum to young lambs (De Las Heras *et al*., 2021) [4]. SRLVs can be isolated from the biological samples (peripheral blood, milk, nasal swabs) collected from the live animals (Christodoulopoulos 2006; Gil *et al*. 2006; McNeilly *et al*. 2008; Barquero *et al*. 2011) [3, 6, 10, 1]. Transmission can be possible through milk and colostrum (Sharp and DeMartini, 2003) [17]. SRLVs can enter through air or milk/colostrums to respiratory or gastrointestinal systems, respectively, and through antigen-presenting cells and dendritic cells reach to regional lymph nodes. For detecting MV provirus DNA in tissue, milk, and blood samples of infected sheep, Extramiana *et al*. (2002) [5] compared the LTR-PCR methodology to two serological methods, ELISA and AGID and found that the LTR-PCR technique had 100% specificity and 98 percent sensitivity. Reddy *et al*. (1993) [14] used PCR to detect MVV in milk samples, PBMCs and synovial fluid samples from infected goats. Total 75 lung tissue samples were screened for MVV and 2.66% (2/75) of samples showed MVV positivity. Detection of MVV nucleic acid in the milk samples of Indian goats was reported first time in the country.

Acknowledgements
The authors are thankful to the SERB-DST, Govt. of India and Director, ICAR-Indian Veterinary Research Institute, Izatnagar for providing the necessary facilities.

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