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Establishment of a polymerase chain reaction assay for monitoring mycoplasma infection in laboratory mice colony

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Abstract
This study presents the development of an in-house polymerase chain reaction (PCR) assay designed for detecting Mycoplasma infections in laboratory mouse colonies. The 16S rRNA gene, conserved among four Mycoplasma species commonly found in mice, was targeted to ensure broad detection. The assay demonstrated high sensitivity, with a detection limit of 0.1 pg, and excellent specificity, as no amplification was observed with genomic DNA from other organisms, including Mus musculus and various bacterial pathogens. Screening 30 lung tissue samples from laboratory mice confirmed the absence of Mycoplasma infection, indicating the assay's potential utility in routine health monitoring of animal colonies. This PCR method offers an efficient, specific, and sensitive alternative for pathogen surveillance, supporting improved animal welfare and research reliability.

Keywords: Mycoplasma, polymerase chain reaction (PCR), laboratory mice

1. Introduction
Health monitoring is crucial for maintaining animal welfare and ensuring reliable research results in laboratory mouse facilities. Polymerase chain reaction (PCR) has emerged as a powerful tool for detecting and identifying various pathogens in mouse colonies (Kim *et al*., 2022) [5]. PCR allows for the amplification of specific DNA sequences, enabling the detection of even low numbers of organisms present in pathological lesions or body fluids (Lebech, 1994) [6].

PCR-based assays have revolutionized laboratory diagnostics over the past two decades, offering advantages such as high sensitivity, specificity, and the ability to detect multiple targets simultaneously (Dale & Dragon, 1994; Yu *et al*., 2019) [2, 10]. In the context of mouse health monitoring, PCR has proven particularly valuable for screening prevalent infectious pathogens like mouse parvoviruses (Wang *et al*., 2013) [9]. The technique can be adapted to detect both DNA and RNA pathogens through variations such as reverse transcription PCR (Cárdenas & Alby, 2016; Jalali *et al*., 2017) [1, 4].

The application of PCR in mouse health monitoring programs has led to improved efficiency and reliability in pathogen detection. For instance, the exhaust air dust (EAD) PCR method has been shown to be a reliable complementary approach to traditional soiled bedding sentinel (SBS) monitoring, especially for pathogens that may not be efficiently transmitted in individually ventilated cage (IVC) systems (Kim *et al*., 2022) [5]. Furthermore, PCR-based methods can reduce the number of animals used in health monitoring, aligning with the 3R principles of animal research (Kim *et al*., 2022) [5].

Mycoplasma infection in laboratory mice poses a significant challenge to biomedical research, affecting the reliability and reproducibility of experimental results. These small, wall-less bacteria can colonize various tissues, with Mycoplasma pulmonis being a common respiratory pathogen (Schoeb, 2007) [7]. The susceptibility to mycoplasma infection can be influenced by factors such as estrogen levels, as demonstrated in studies with Mycoplasma hominis in BALB/c mice (Shkarupeta *et al*., 2004) [8]. Cross-reactivity between different mycoplasma

species in serological tests can complicate diagnosis, as observed with *M. arthritidis* and *M. pulmonis* (Davidson *et al*., 1983) [3]. The impact of mycoplasma infection extends beyond the immediate health of the mice, potentially altering immune responses and experimental outcomes.

In this study we have developed an in-house PCR assay to detect the presence of mycoplasma infection in laboratory mouse and validated using a panel of samples collected from a laboratory mouse colony.

2. Materials and Methods

2.1 Samples and Cultures

All mice lung tissue samples were obtained from the Laboratory animal medicine unit of Tamil Nadu Veterinary and Animal Sciences University. These were collected during routine necropsy examinations. The pure cultures of mycoplasma were a kind gift from Dr. S.G. Ramachandra, Indian Institute of Science. DNA from *Staphylococcus aureus*, *Streptococcuss pyogenes, Klebsiella pneumonia*, *Escherichia coli* and *Camphylobacter jejuni* were a kind gift from Prof. Shrikrishna Isloor, Bangalore Veterinary College, India

2.2 DNA extraction and Polymerase chain reaction

DNA was extracted from tissue samples, environmental swabs and mycoplasma cultures by DNA isolation reagent (TAKARA, India) following manufacturers instructions. The final DNA was reconstituted in nuclease free water and stored at -20C until use. PCR was performed in 25 µl volumes using GoTaq DNA Mastermix (Promega). The final reaction mix consisted of 1× mastermix, 1.5 pmoles each of forward (5'-ACACCATGGGAGCTGGTAAT-3') and reverse primers (5'-CCCACGTTCTCGTAGGGATA -3'). PCR thermal cycling condition was 95 °C for 2 min followed by 40 cycles of95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. Products were separated by electrophoresis in 2%TAE agarose gel.

2.3 Determination of sensitivity and specificity

For determining the specificity, the PCR reaction mix was spiked with a predetermined quantity of DNA from bacteria species. PCR amplification was done as detailed above. For determination of specificity, dilutions of Mycoplasma genomic DNA were subjected to PCR reaction as above.

3. Results and Discussion

Conventional methods such as gross pathology, parasitology and bacteriology continue to be used in monitoring the health of laboratory rodents. However these methods are time consuming and often needs separate laboratories and separate technical personnel to conduct the test. The other widely used method, serology looks for antibodies developed against the pathogen. This method is also suffers from the downside of giving false positives owing to the past infections and false negatives in immune-compromised animals. Polymerase chain reaction (PCR), has been widely used as a sensitive and specific method to detect infection on laboratory animals.

In this study we developed an in house PCR assay to monitor the Mycoplasma infection in laboratory mice. The primers were designed on the 16S rRNA gene and is conserved in four species of Mycoplasma (*M. pulmonis, M. arthritidis, M. neurolyticum*, and *M. collis*) that commonly affect mice. The performance of the assay was evaluated by using the Mycoplasma genomic DNA as template in PCR reaction. The positive PCR amplification produced 147 bp amplification (fig.1). The limit of detection of the assay was assessed by using serial dilution of the Mycoplasma genomic DNA in the PCR reaction. The limit of detection of the assay was found to be 0.1 pg (fig 2).

Fig 1: Polymerase chain reaction of partial 16S rRNA region of Mycoplasma DNA produced an 147 bp amplification visualized after electrophoresis in a 2% agarose gel.

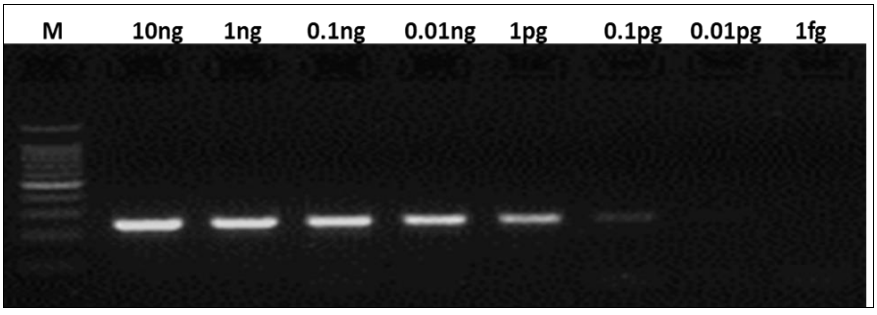

Fig 2: Limit of detection of polymerase chain reaction assay of the 16S rRNA region of Mycoplasma DNA. A visible band was detected until 0.1 pg of template.

The specificity of the assay was assessed by performing PCR reactions using genomic DNA templates of Mus musculus (Balb/c strain; genomic DNA) *Staphylococcus aureus*, *Streptococcuss pyogenes, Klebsiella pneumonia, Escherichia coli* and *Camphylobacter jejuni.* The PCR did not produce any amplification demonstrating the specificity of the assay.

The optimized PCR assay was used to screen the lung tissue samples (Table -1). Of the thirty samples tested none showed amplification specific for Mycoplasma, indicating that the colony is free of mycoplasma infection

Table 1: Mouse lung tissue samples used for screening of Mycoplasma infection by polymerase chain reaction.

Sample ID	Sex	Age	PCR result
RS-1	Male	<2 month	negative
RS-2	Male	<2 month	negative
RS-3	Female	<2 month	negative
RS-4	Female	<2 month	negative
RS-5	Female	<2 month	negative
RS-6	Female	<2 month	negative
RS-7	Female	<2 month	negative
RS-8	Female	<2 month	negative
RS-9	Female	<2 month	negative
RS-10	Female	<2 month	negative
RS-11	Female	<2 month	negative
RS-12	Female	<2 month	negative
RS-13	Male	<2 month	negative
RS-14	Male	<2 month	negative
RS-15	Female	<2 month	negative
RS-16	Male	<1 month	negative
RS-17	Male	<1 month	negative
RS-18	Female	<1 month	negative
RS-19	Female	<1 month	negative
RS-20	Female	<1 month	negative
RS-21	Female	<1 month	negative
RS-22	Female	<1 month	negative
RS-23	Male	<1 month	negative
RS-24	Male	<1 month	negative
RS-25	Female	<1 month	negative
RS-26	Female	<1 month	negative
RS-27	Female	<1 month	negative
RS-28	Female	<1 month	negative
RS-29	Female	<1 month	negative
RS-30	Male	<1 month	negative

The developed in-house PCR assay for monitoring Mycoplasma infection in laboratory mice demonstrates promising results in terms of sensitivity and specificity. The assay's design, targeting the conserved 16S rRNA gene across four common Mycoplasma species affecting mice, provides a broad detection range for potential infections. The limit of detection of 0.1 pg indicates high sensitivity, which is crucial for early detection of Mycoplasma infections in laboratory mice. The specificity of the assay is a notable strength, as demonstrated by the lack of amplification when testing against genomic DNA from various other organisms, including the host species (Mus musculus) and common bacterial pathogens. This high specificity reduces the likelihood of false-positive results, which is essential for accurate monitoring of Mycoplasma infections in mouse colonies.

The application of the optimized PCR assay to screen lung tissue samples from 30 mice yielded negative results for all samples. This outcome suggests that the tested colony is currently free of Mycoplasma infection. However, it is important to note that this result is based on a limited sample size and may not be representative of the entire colony or facility. Implementing regular screening of the mouse colony over time would help establish the assay's reliability for ongoing Mycoplasma surveillance.

In conclusion, the developed in-house PCR assay shows potential as a valuable tool for monitoring Mycoplasma infections in laboratory mice. Its high sensitivity and specificity make it a promising candidate for routine screening in animal facilities. However, further validation and optimization may be necessary to ensure its reliability and effectiveness in diverse research settings.

4. Conflict of Interest
Not available

5. Financial Support
Not available

6. References
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