

Original Research Article

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Molecular Diagnosis of Chronic Respiratory Disease Associated with *Mycoplasma gallisepticum* in Ducks

N. Aiswarya¹, Surya Sankar¹, Roshin M. Reji¹, P. S. Reshma¹,
S. Reshma¹, A. Aneesh¹, K. Krithiga² and M. Mini¹

¹Department of Veterinary Microbiology, ²Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, India

*Corresponding author

ABSTRACT

Keywords

Chronic respiratory disease (CRD), *Mycoplasma gallisepticum*, Ducks, Polymerase Chain Reaction (PCR)

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Three birds aged six weeks belonging to a private farm in Thrissur district were brought to the department of Veterinary Microbiology, college of Veterinary and Animal sciences, Mannuthy for disease investigation. The owner reported that a total of about 50 animals in the team experienced severe respiratory distress. The clinical signs observed in the ducks characterized by coryza, conjunctivitis, respiratory rales and sneezing. The post mortem lesions in lungs were suggestive of chronic respiratory disease (CRD). On culturing the clinical samples, no bacteria could be isolated. Lung tissue was processed and subjected to polymerase chain reaction (PCR) targeting *Mycoplasma gallisepticum* species. Samples revealed amplicon size of 183 bp.

Introduction

Chronic respiratory disease is considered as one of the most economically significant diseases of poultry, caused by several pathogens. Among them, *M.gallisepticum* is the most important pathogen causing CRD in chickens (Ley and Yoder, 1997) and infectious sinusitis in turkey. Chronic Respiratory Disease affects mainly chicken, but turkey, pheasants, geese, pigeons, psittacine birds and rarely ducks are also

getting infected. The disease is characterized by coryza, conjunctivitis, respiratory rales and sneezing in chicken. The present study describes the molecular detection of *M.gallisepticum* among ducks from a private farm in Thrissur district. The owner reported that the birds have been treated with antibiotics like Co-trimoxazole, Amoxicillin-clavulanic acid and Enrofloxacin and were not responding to the therapy. The birds were submitted to the department of Veterinary Pathology, COVAS, Mannuthy for

postmortem examination. The samples were collected aseptically and send to the department of Veterinary Microbiology for detailed investigation.

Materials and Methods

On postmortem, lesions in lungs were suggestive of CRD. The samples were inoculated on to Blood agar (BA) and Sabouraud's dextrose agar (SDA). Blood agar plates were incubated at 37°C for 24-48 h and duplicate samples on SDA plates were incubated at 37°C and at room temperature. Since no specific medium for *Mycoplasma* was readily available, we proceed for the molecular detection.

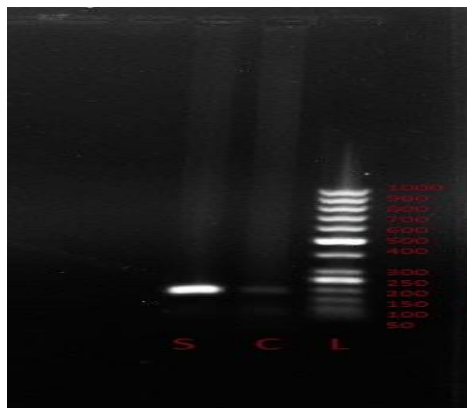
For conducting *M.gallisepticum* species specific Polymerase Chain Reaction (PCR), lung tissues were transferred to a sterile mortar and pestle and 5mL of 0.8% phosphate buffered saline was added. Tissues were triturated and pelleted by centrifugation at

8000 rpm for 10 min. The supernatant was discarded and pellet was subjected to further lysis and DNA extraction using HiPurA multi sample DNA purification kit. Polymerase chain reaction was conducted using *M. gallisepticum* species specific PCR as per OIE, 2008. Template DNA from *M.gallisepticum* vaccine (NobilisIntervet, India) was kept as positive control.

Results and Discussion

In the present study, no bacterial organisms of pathogenic significance could be isolated on BA even after incubation up to 72 h. No growth could be observed on SDA even after incubation at room temperature for 7 days. It may be attributed to the antibiotic therapy already started for the flock. The PCR was found to be positive for *M.gallisepticum* which revealed an amplicon size of 183 bp in electrophoresed gel under UV transillumination (Fig. 1).

Fig.1 Polymerase chain reaction assay depicting the detection of *M. gallisepticum* from ducks



Diagnosis of avian mycoplasmosis can be done by various methods, but the gold standard test for confirmation of diagnosis is isolation and identification of the organism (Ley, 2008).It takes at least two weeks for cultural isolation, and the procedure is laborious too. Molecular techniques such as

PCR are found to be highly sensitive and specific for the detection of *Mycoplasma* (Abdelwhab *et al.*, 2011). Polymerase chain reaction assays offer a rapid and a sensitive method for the detection and identification of *M.gallisepticum* directly from the clinical samples (Nascimento *et al.*, 1991; Callison *et*

al., 2006, Hess *et al.*, 2007, Sankar *et al.*, 2012). According to Evans and Leigh (2008), PCR technique alone can be used for the confirmative diagnosis of *M.gallisepticum*. In the present study, positive results were obtained within 24 h with PCR, which enables an accurate and timely diagnosis.

The present study deals with the molecular detection of *M.gallisepticum* infection in ducks using polymerase chain reaction.

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