MOLECULAR CHARACTERIZATION OF *Mycoplasma gallisepticum* INFECTION IN JAPANESE QUAILS

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ABSTRACT

The present study describes the identification of *Mycoplasma gallisepticum* in a flock of Japanese quails from a private farm in North Kerala. Heavy mortality was reported among the birds aged four to five weeks. Post mortem examination revealed lesions, suggestive of chronic respiratory disease (CRD). The tissue samples were subjected to cultural examination and polymerase chain reaction (PCR) targeting *Mycoplasma gallisepticum*. No bacterial organism of pathogenic significance could be isolated on culture. But, the tissue samples were found to be positive for *Mycoplasma gallisepticum* species specific PCR, which revealed a product size of about 186 bp.

Keywords: Kerala, *Mycoplasma gallisepticum*, Japanese quail, polymerase chain reaction, chronic respiratory disease

INTRODUCTION

Mycoplasma gallisepticum is the most common pathogenic etiological agent involved in the polymicrobial chronic respiratory disease (CRD) in chicken and infectious sinusitis in turkey, causing serious economic loss in commercial and non-commercial flocks of poultry worldwide (Ley and Yoder, 1997). CRD is also reported in pheasants, pigeons, quails, ducks, geese and psittacine birds. Transmission is either vertical via transovarian route or horizontal through aerosols, contaminated feed and water, fomites, etc. The organism may remain latent in some birds for days to months. Clinical manifestation of the disease and subsequent spread occurs when the birds are stressed. Cold weather, overcrowding, poor air quality and concurrent infections facilitate the infection to spread. Once infected, birds may remain carriers for life.

Clinical signs in birds vary from apparent to mild respiratory distress with slight to marked rales, dyspnoea, coughing or sneezing. Nasal discharge and conjunctivitis with swollen infra-orbital sinus may occasionally be present. Concurrent infections with *Escherichia coli* may occur resulting in severe air sac thickening and cloudiness, exudative accumulation, pericarditis and perihepatitis.

In uncomplicated cases, morbidity is high and mortality is low. Present study documented the detection of *M. gallisepticum* outbreak in a flock of Japanese quails from a private farm in Northern Kerala using polymerase chain reaction (PCR).

MATERIALS AND METHODS

Three number of live Japanese quails aged six weeks, were brought to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur for disease investigation from a private farm at Peravoor, Kannur district, North Kerala. The owner reported a heavy mortality rate among the flocks and was not responding to the commonly used antibiotics in the field conditions. The birds were sacrificed and post mortem examination was conducted. Samples collected aseptically from liver, lungs and spleen, were inoculated onto Blood agar (BA) and Sabouraud's Dextrose Agar (SDA). The BA plates were incubated at 37°C and the duplicate samples on SDA plates were incubated at 37°C and at room temperature.

For conducting PCR, samples were obtained aseptically from lung and air sacs in sterile specimen vials and were transferred to a sterile mortar and pestle, and 5ml of 0.8 per cent saline was added. Tissues were triturated and pelleted down by centrifugation. The supernatant was discarded and pellet was subjected to further lysis and DNA extraction using HiPurA Multi-Sample DNA Purification Kit (Himedia). Polymerase chain reaction was conducted using *M. gallisepticum* species specific primers as per OIE, 2008. A 12.5 µl reaction mixture was set up for the single PCR reaction consisting of

10X PCR master mix	6.25µL
Forward Primer	1µL
Reverse Primer	1µL
Template DNA	3µL
Nuclease Free Water	1.25µL

Template DNA from *M. gallisepticum* vaccine (Nobilis Intervet, India) was kept as positive control. The PCR tubes were placed in a thermal cycler (Eppendorf) and reaction was run as per the following protocol:

Denaturation	94°C for 30 sec	40 cycles
Annealing	55°C for 30 sec	
Elongation	72°C for 60 sec	
Final extension	72°C for 5 min	

Identification of the PCR product was done in a submarine agarose gel electrophoresis system using one per cent agarose stained with ethidium bromide, and Tris Borate EDTA buffer was used as the matrix at a voltage of 50V. The gel was visualised under a UV transilluminator and results were documented on gel doc system (Bio-rad).

RESULTS AND DISCUSSION

In the present study, the owner reported a heavy mortality in a flock of 2000 birds. The birds were kept in two sheds built one

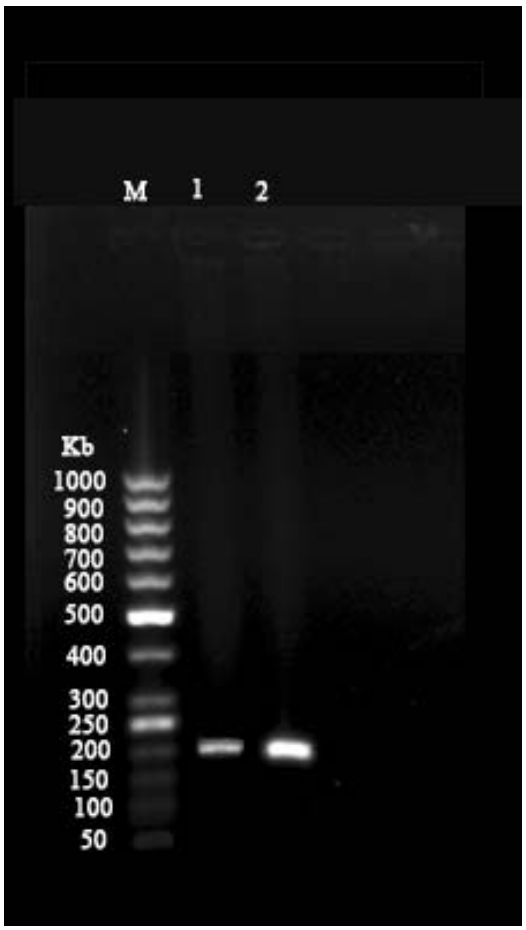
metre apart with 1000 birds per shed, of which mortality was reported from a single shed alone. The birds appeared apparently healthy with mild respiratory distress. The birds were under treatment with levofloxacin to which, no response was exhibited.

Post mortem examination of the sacrificed birds revealed sinusitis, air sacculitis and congested lungs, suggestive of avian mycoplasmosis (Levisohn *et al*., 1986). On BA, no bacterial organisms of pathogenic significance could be isolated. No growth could be obtained on SDA even after 7 days of incubation at room temperature (37°C). It may be attributed to the antibiotic therapy already started for the flock.

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. It takes at least two to three weeks for cultural isolation, and the procedure is laborious. Polymerase chain reaction offers a rapid and a sensitive method for the detection and identification of *M. gallisepticum* directly from clinical samples (Nascimento *et al*., 1991; Callison *et al*., 2006; Hess *et al*., 2007; Sankar *et al*., 2012).

The DNA extracted from clinical samples were subjected to *M. gallisepticum* species specific PCR. According to Evans and Leigh (2008), PCR technique alone can be used for the confirmative diagnosis of *M. gallisepticum*. A positive PCR result was indicated by the presence of a 186 bp (Figure 1) fragment in electrophoresed gel under UV transillumination.

Fig. 1. Agarose gel electrophoresis of *Mycoplasma gallisepticum* species specific PCR products



Lane M: Molecular weight marker

Lane 1: *M. gallisepticum* positive sample

Lane 2: Reference strain from *M. gallisepticum* vaccine (Nobilis Intervet, India)

SUMMARY

The present study was conducted in a flock of Japanese quails exhibiting no

significant clinical symptoms apart from mild respiratory distress, and they were not responding to common antibiotic treatments, which suggest the involvement of a chronic respiratory distress of which *Mycoplasma gallisepticum* is the main etiological agent. The scope of identification with bacteriological culture and isolation is less, because the process is laborious and time consuming. Under these circumstances, PCR offers a rapid and sensitive alternative for the accurate diagnosis of the disease.

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