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Pathology of *Mycoplasma gallisepticum* infection in naturally infected layer birds

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ABSTRACT

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Sixty five commercial layer farms with lesions suggestive of respiratory problem were investigated during the study period from May 2014 to April 2015 to evaluate the pathology of *Mycoplasma gallisepticum* infection in laying chicken. *Mycoplasma gallisepticum* was confirmed through PCR in 36 out of 65 farms. The positive farms were examined for gross and histopathological changes in tissues like trachea, lungs, air sacs and liver. The highest incidence of MG infection occurred during the winter season. Grossly, MG affected birds showed catarrhal inflammation of trachea and bronchi, slight pneumonic changes in lungs, cloudiness, air sacculitis and caseous exudate in thoracic air sacs, congestion and haemorrhages in lungs, caseous mass in both thoracic and abdominal air sacs. Histopathologically, focal destruction of superficial cells, goblet cell formation, hyperplasia of mucosal epithelium, submucosal infiltration of lymphocytes, macrophages along with haemorrhagic areas were observed in the trachea. Lungs revealed interstitial haemorrhages, parabronchiolar oedema, mucosal hyperplasia of secondary bronchiole and interstitial pneumonia characterized by thickening of interstitium. Air sacs showed epithelial destruction and increased thickening due to proliferation of submucosal connective tissue with congested blood vessels and neovascularization. This study proved that MG infection occurs severely among laying chicken causing more economic loss to the farmers.

Keywords: Layer birds; *Mycoplasma gallisepticum*; pathology

INTRODUCTION

Mycoplasma gallisepticum (MG) is an avian pathogen within the genus *Mycoplasma* (class Mollicutes) which includes approximately 100 other species infecting animals (including humans), insects or plants. Mollicutes are eubacteria without cell wall and the smallest self-replicating (can be grown on artificial cell-free media) prokaryotes[1].

M. gallisepticum is more concerned with respiratory diseases of poultry owing to the special feature of motility and tight adherence to host cells with its terminal tip structure, which give them a flask like shape. They contain fewer cellular organelles particularly those required for metabolism and reproduction[2]. MG can cause significant downgrading of carcasses, also responsible for decreased growth and egg production and reduced hatchability rates[3,4]. The occurrence of other respiratory viral or bacterial agents, immunosuppression and /or adverse climate and housing condition might significantly worsen the clinical expression of the disease. Transmission can occur through bird to bird by contact, exhaled respiratory droplets either as aerosols or on equipment, people and surroundings. Birds recovered from MG might remain shedders of the organism[5].

The present study was designed to confirm *Mycoplasma gallisepticum* in naturally infected layer birds through PCR and to explore the gross and histopathological changes produced by *Mycoplasma gallisepticum.*

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MATERIALS AND METHODS

During the period of 12 months (May 2014 to April 2015), sixty five commercial layer farms with lesions suggestive of *Mycoplasma gallisepticum* were investigated. Samples such as choanal cleft swab and tracheal swab were collected from suspected ailing birds and necropsy was carried out in dead birds. Tissue samples like trachea, lungs, air sacs and liver were collected from dead birds and fixed in neutral buffered formalin.

Polymerase chain reaction

These samples were inoculated aseptically into Frey's broth medium and incubated at 37°C with 90% relative humidity and kept for 5-7 days and the DNA was extracted by using Genomic DNA purification kit. The MG was detected by using the specific primer pair that successfully amplified 185bp of 16S rRNA gene as per the protocol OIE[6].

PCR was performed with initial denaturation at 95°C for 4 minutes, followed by 35 cycles at different temperature segments (Table 1) corresponding to the target DNA denaturation, primer annealing and primer extension respectively. The final extension step was 72°C for 10 minutes. The PCR amplified products were separated at the constant voltage (80 V for 1 hour) by using 2% agarose gel electrophoresis stained with

ethidium bromide in 1X TAE buffer and DNA fragments were documented.

Histopathology

The PCR confirmed farm samples were subjected to routine histopathological evaluation. The tissues were dehydrated, cleared and embedded in paraffin by routine manual tissue processing[7]. Tissues were cut at 3 to 4 μm thickness, which was taken on the glass slides and these were stained with Haematoxylin and Eosin by routine staining procedure. These were mounted with DPX mountant solution and covered with coverslips for histopathological examination.

RESULTS

The birds affected with MG showed respiratory distress, tracheal rales, increased lachrymation, nasal discharge, coughing and sneezing. In the present study the mortality rate of exclusive MG affected birds was about 6.75 per cent. Birds in complicated disease with other organisms were having relatively high morbidity and mortality. The highest incidence was occurred during the winter season.

Polymerase chain reaction

All samples from sixty five commercial layer farms were subjected to PCR for confirmation of MG in layer birds. Out of these, 36 farms were positive for *Mycoplasma gallisepticum* by using a specific primer pair corresponding to the 16S rRNA gene designated for the detection of MG at 185 bp (Fig. 1).

Gross pathology

Moderately affected birds showed catarrhal inflammation of trachea and bronchi. The lungs showed slight pneumonic changes. Air sac showed cloudiness, air sacculitis and caseous exudates in thoracic air sacs (Fig. 2). Severely affected birds showed caseous mass in the trachea, lungs revealed dark red colour and showed congestion and haemorrhages. The liver showed congestion and petechial haemorrhages.

Histopathology

In acute cases, trachea showed thickening of tracheal mucous membrane due to hyperactivity of the mucous glands (Fig. 3). Surface epithelium revealed disorientation with loss of cilia, single cell necrosis and congested blood vessels. In some cases, the trachea showed focal destruction of superficial cells, goblet cell formation of superficial epithelium and hyperplasia of mucosal epithelium. In chronic cases, the trachea showed complete destruction of superficial epithelium and submucosal infiltration of more number of lymphocytes, macrophages along with haemorrhagic areas (Fig. 4). In lungs moderately affected birds showed interstitial haemorrhage and parabronchiolar oedema. In some cases, secondary bronchiole showed mucosal hyperplasia. Severely affected birds showed organized exudates occluding in the parabronchial lumen were also noticed (Fig. 5). In air sacs the moderately affected birds showed increased thickness due to proliferation of submucosal connective tissue (Fig. 6). Severely affected birds showed destruction of superficial epithelium, more

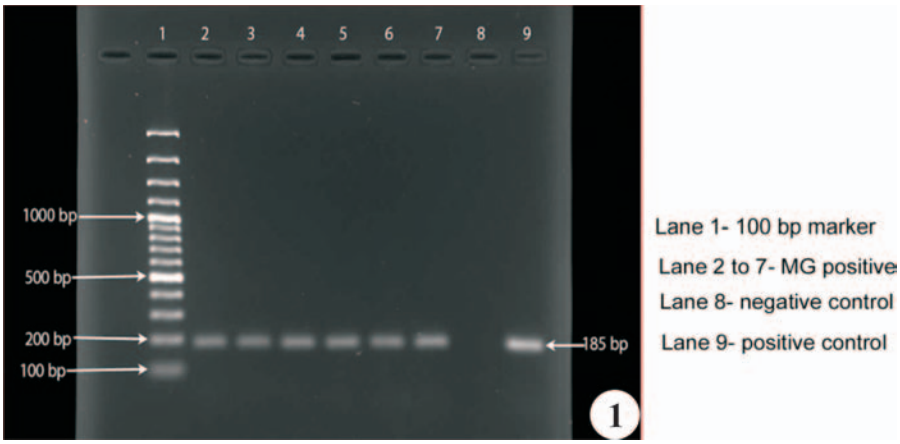
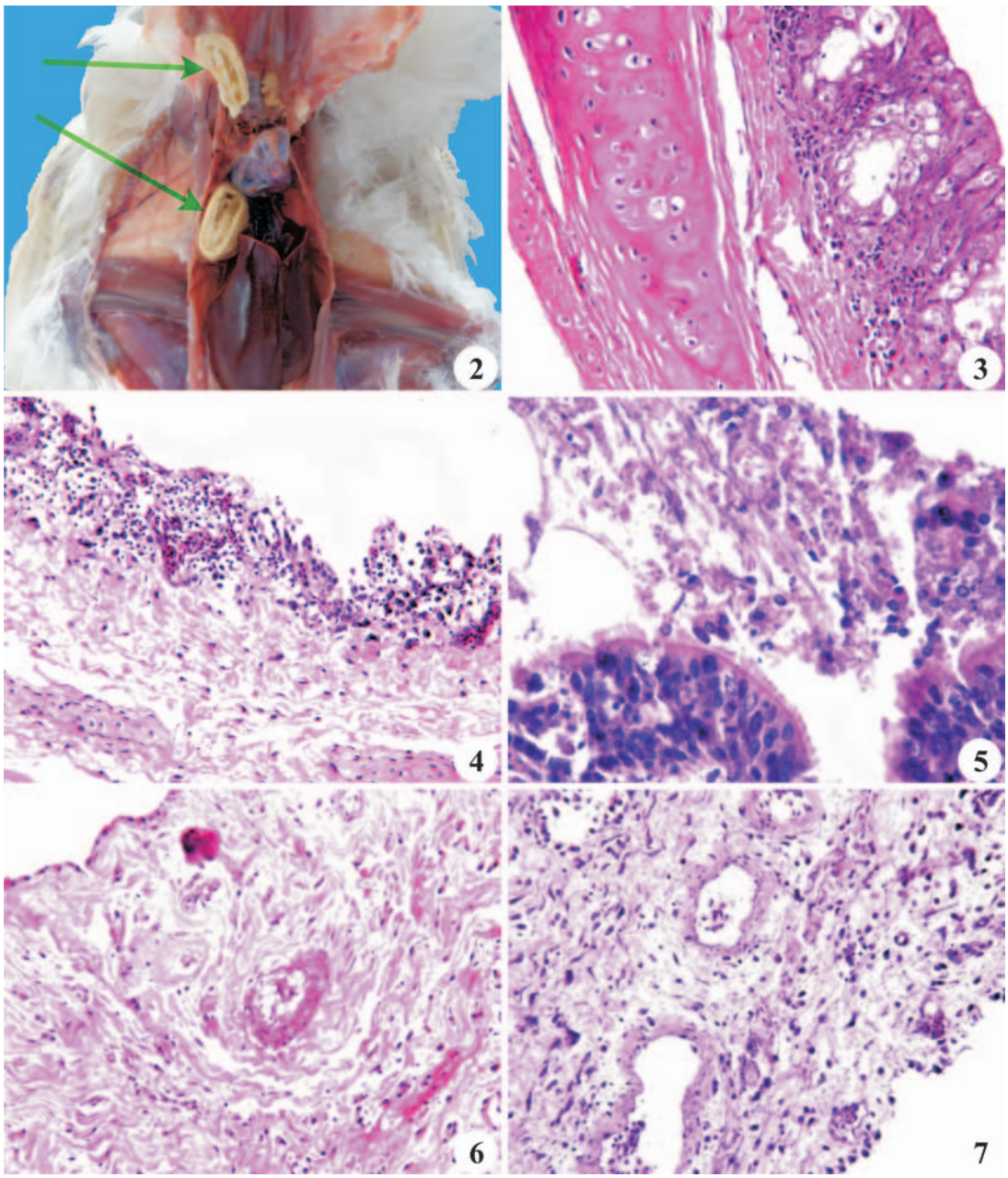


Fig.1. *Mycoplasma gallisepticum* specific 185 bp PCR products (16SrRNA gene) on agar gel electrophoresis.

Table 1. Primers and PCR conditions applied for detection of *Mycoplasma gallisepticum* at 185 bp.

MG	Primer	Sequence (5′- 3′)	PCR conditions			Reference
			Denaturation	Annealing	Extension	
	MG-14F	GAG CTA ATC TGT AAA GTT GGT C	94°C/	54°C/	72°C/	OIE, 2008
	MG-13R	GCT TCC TTG CGG TTA GCA AC	30sec	30sec	60sec	



; **Fig.2.** Caseous exudates in thoracic air sacs; **Fig.3.** Trachea showing hyperactivity of mucous glands. H&E ×400; **Fig.4.** Trachea showing complete destruction of epithelium and submucosal infiltration of more number of lymphocytes & superficial macrophages. H&E ×400; **Fig.5.** Lungs showing organized exudates in the parabronchiolar lumen. H&E ×1000; **Fig.6.** Air sacs showing increased thickness due to proliferation of submucosal connective tissue. H&E ×400; **Fig.7.** Air sacs showing destruction of superficial epithelium, connective tissue proliferation and dilated blood vessels. H&E ×400.

connective tissue proliferation and enlarged blood vessels in air sacs (Fig. 7). Liver revealed coagulative necrosis, dissociation of hepatic cords due to massive necrosis, sinusoidal and central vein congestion.

DISCUSSION

The *M. gallisepticum* affected birds showed severe respiratory signs and high mortality rate in the complicated conditions were in accordance with many early reports[8-11]. The results obtained by PCR were in agreement with early workers[6,10] who used a primer pair complementary to 16S rRNA gene designated for the detection of MG at 185 bp. Grossly, the MG affected birds showed the lesions in trachea, bronchi, lungs and air sacs in moderate infection were in accordance with earlier workers[10,12,13] and the lesions observed in severe infection like caseous mass in the trachea, congestion and haemorrhages of lungs were in agreement with early reports[14,10].

Histopathologically, trachea showed thickening of mucous membrane due to hyperactivity of the mucous glands were supported by[13,15] in acute conditions. Similar findings like disorientation with loss of cilia, single cell necrosis and congested blood vessels[10,12] and focal destruction of superficial cells, goblet cell formation and hyperplasia of mucosal epithelium[9] were also reported. In chronic cases, the tracheal lesions with complete destruction of superficial epithelium and submucosal infiltration of more number of lymphocytes, macrophages along with haemorrhagic areas were in accordance with early report[10]. In lungs, the lesions noticed in moderate infections were observed by earlier worker[13] and severely affected birds with organized exudates occluding in the parabronchial lumen[13,16] were reported.

Air sacs are affected mostly due to the physiology of the avian respiratory system, in which part of the inspired air goes first through the bronchi to this serosa and afterwards to the lungs[17]. In moderate infection, increased thickness due to proliferation of submucosal connective tissue in air sacs were in accordance with the early research works[18,19]. The proliferating connective tissues were evidenced by the presence of more number of young fibroblasts which are not usually observed in normal air sacs. Increased thickness of connective tissue and congested blood vessels were in accordance with early report[16]. More number of blood vessels and neovascularization in the connective tissue area could be explained for the increased blood supply to the proliferating connective tissue.

In the present study, wide spread prevalence of MG infection among laying chicken is proved beyond doubt. PCR provided rapid diagnosis for MG infection in field level. The incidence was highest during the winter season. Severe lesions noticed in respiratory organs indicates that *Mycoplasma* weakens the body defences by producing chronic and debilitating condition that could pave way for other opportunistic pathogen diseases.

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