



Original Full Paper

## Synergistic Pathological Effect of *Mycoplasma gallisepticum* with other Infectious Organisms in Layer Chickens

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### Abstract

Synergistic pathological effect of *Mycoplasma gallisepticum* with other infectious organisms in layer chickens was evaluated in 70 commercial layer chicken farms. Newcastle disease virus and infectious bronchitis virus were confirmed by haemagglutination inhibition test (HI), and *Escherichia coli*, *Pasteurella multocida*, *Haemophilus paragallinarum* and *Ornithobacterium rhinotracheale* were confirmed by their growth characteristics on agar media and Gram's staining. PCR technique was used for the confirmation of *Mycoplasma gallisepticum*. The mortality rates observed in the occurrence of individual diseases were synergistically increased when they combined with chronic respiratory disease (CRD), which was clearly supported by the gross and histopathological alterations.

**Key Words:** chronic respiratory disease, mixed respiratory infectious diseases, avian diseases.

### Introduction

*Mycoplasma gallisepticum* causing chronic respiratory disease (CRD) has a very small genome and evolved to this minimalist status by losing non-essential genes, including those involved in cell wall synthesis (3). It affects all age groups of chickens, although very young birds are seldom affected (4). The severity of the diseases was exacerbated as the result of mixed infections with other respiratory pathogens (12). Severe outbreaks with high morbidity and mortality observed in chickens were frequently due to concurrent infections (9). This report deals with the synergistic pathological effect of *Mycoplasma gallisepticum* in layer chickens in association with other infectious agents.

### Materials and Methods

This study was conducted during a three years period, in which 70 commercial layer flocks (strength varied from 10,000 to 50,000 birds) with the history and symptoms of mycoplasmosis were investigated. Necropsy was carried out on recently died chickens. Samples such as

trachea, lungs, air sacs, and swabs of infraorbital sinus exudates, heart blood and liver were collected. These samples were utilised for the confirmation of etiological agents. Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) were confirmed by conducting haemagglutination (HA) and haemagglutination inhibition (HI) tests based on the procedure by Alexander (1). Bacteria like *Escherichia coli* (colibacillosis, CB), *Pasteurella multocida* (fowl cholera, FC), *Haemophilus paragallinarum* (infectious coryza, IC) and *Ornithobacterium rhinotracheale* (ORT) by their growth characteristics on agar media and Gram's staining (10). Trachea and air sac pieces collected in Frey's medium were used in PCR for the confirmation of *Mycoplasma gallisepticum* (MG).

### Diagnosis of *Mycoplasma gallisepticum*

#### Collection and culturing of samples

Pieces of trachea and air sacs from suspected birds were collected aseptically in Frey's *Mycoplasma* medium and incubated at 37 °C for 5 - 7 days.

**DNA extraction**

One ml of sample cultured in Frey’s medium was centrifuged at 10,000 g for 20 min twice and the pellet was washed with 70 per cent ethanol. The pellet was resuspended with 50 µl of Tris EDTA buffer and boiled for 3 - 5 minutes to release the DNA. The extracted DNA was stored at – 20 °C until use.

**Polymerase Chain Reaction**

**Primers**

The following forward and reverse primers were used for the amplification of target sequence of “16S rRNA gene” (530 bp) of *M. gallisepticum*. Forward primer: 5’- AAC ACC AGA GGC GAA GGC GAG G - 3’; Reverse primer: 5’ - ACG GAT TTG CAA CTG TTT GTA TTG G – 3’.

The following mixture of materials was subjected to PCR in a thermal cycler (Eppendorff) as per the procedure by Kiss et al. (9) and the amplified product was separated on 1.5 per cent agarose gel. Master Mix : 25 µl (dNTPs, Taq polymerase and PCR buffer); Forward primer: 1 µl (40 picomols); Reverse primer: 1 µl (40 picomols); DNA template: 2 µl; DNase free water to make up to 50 µl.

**Pathology**

After recording the gross lesions, a transverse section of tissue approximately 0.5 cm in thickness was taken from infraorbital sinus, trachea, lungs, liver and kidneys of birds. Air sacs were as such removed. Tissue pieces were fixed in 10 per cent buffered neutral formalin and trimmed to a thickness of about 3 mm. The tissues were dehydrated, cleared and embedded in paraffin in a routine manual processing. Tissues were cut at 5 µm thicknesses, mounted on glass slides, stained with haematoxylin and eosin and covered with coverslips for histopathological examinations (2).

**Results and Discussion**

The MG positive samples produced 530 bp products corresponding to their 16S rRNA gene (Fig. 1), which confirmed the presence of *M. gallisepticum* in the samples.

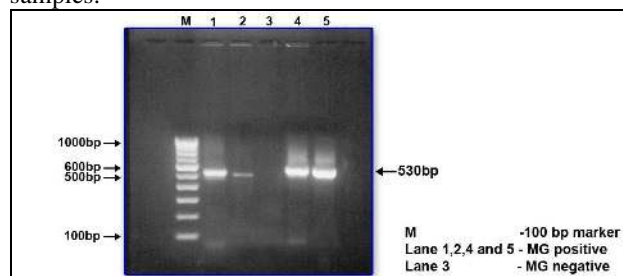


Figure 1. *M. gallisepticum* – 530 bp PCR product on agarose electrophoresis.

**Disease distribution**

The mortality rates of various diseases individually and in combinations with CRD are presented in the table (Table 1) below.

Table 1. Mortality rates of various diseases individually and in combinations with CRD.

S.No	Diseases	Average mortality
01	ND	03.00
02	IB	02.00
03	CB	04.43
04	FC	01.00
05	IC	02.75
06	ORT	--
07	CRD	07.80
08	ND + CRD	13.00*
09	ND + CRD + CB	14.00***
10	ND + CRD + ORT	15.00***
11	CRD + CB	14.80***
12	CRD + CB +FC	17.33***
13	CRD + FC	16.00***

\*represents the value is significant (p<0.05); \*\*\*represents the values are highly significant (p<0.001); ND: Newcastle disease; IB: Infectious bronchitis; CB: colibacillosis; FC: fowl cholera; IC: Infectious coryza; ORT: ornithobacteriosis; CRD:chronic respiratory disease

The mortality rates observed in the occurrence of individual diseases were synergistically increased when they combined with CRD, which was clearly supported by the below mentioned gross and histopathological evidences.

**Gross lesions**

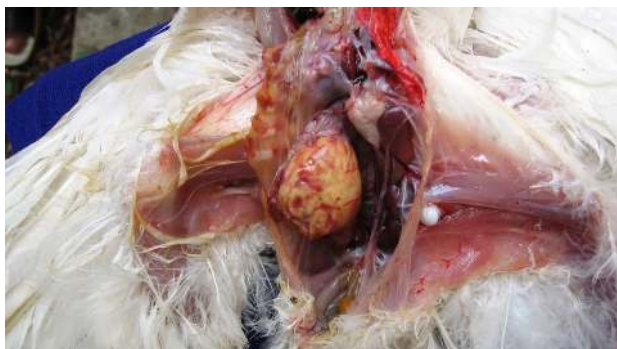
Severe catarrhal sinusitis, tracheitis, bronchitis and pneumonic changes of congestion and consolidation of the lungs noticed in this study might be due to the combined infection of ND. Exclusively CRD affected birds did not reveal much pathological changes in upper respiratory tract and lung except catarrhal tracheitis. Keratoconjunctivitis and marked edema in the facial subcutis and eyelids were observed in combined infection with ORT (Fig. 2).



Figure 2. Severe edema in the facial subcutis with closure of eyelids.

Large masses of caseous exudate in the air sacs, predominantly in the abdominal ones (Fig. 3) and egg peritonitis were observed in combined infection with CB. These observations were in accordance with the earlier findings of Gross (7), and Fabricant and Levine (5), who reported severe airsacculitis in combined infections with CB. These severe lesions might be due to the synergistic effect of MG. In few combined cases, thoracic air sacs

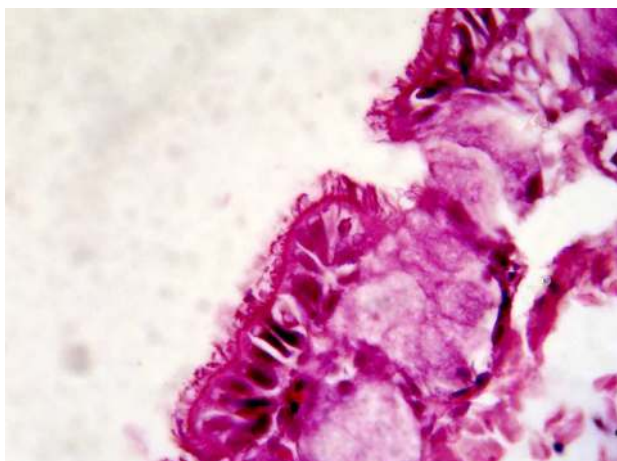
also showed large masses of caseous exudate. Bilateral thoracic as well as abdominal airsacculitis with caseous exudate were observed in severely affected combined cases. In uncomplicated cases, airsacculitis with small masses of caseous exudate was noticed.



**Figure 3.** Large mass of caseous exudate in the abdominal air sac.

### Microscopic lesions

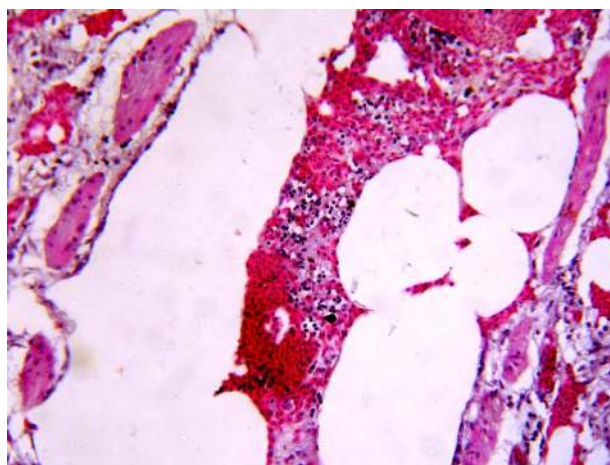
In uncomplicated cases, thickening of the tracheal mucous membrane due to hyperactivity of the mucous glands was noticed (Fig. 4). The lesions found in the trachea in CRD cases were also supported by the earlier experimental work of Gaunson et al. (6). This infers the milder pathological effect of MG in upper respiratory tract. When combined with ND, complete loss of cilia, necrosis and sloughing of both glandular and surface epithelium were noticed.



**Figure 4.** Thachea showing thickening of the mucosa due to hyperactivity of the mucous glands. HE, 1000x.

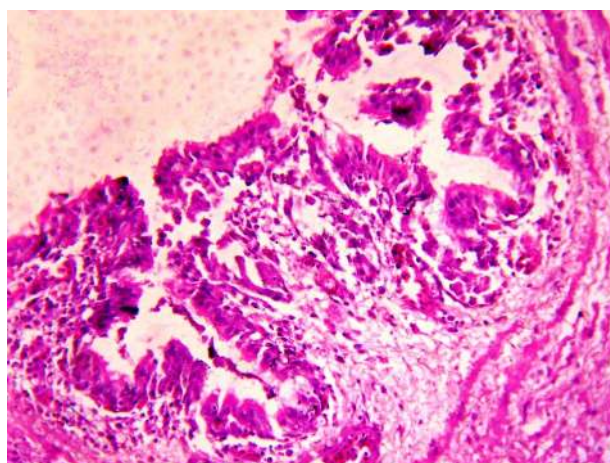
Parabronchial edema and haemorrhage, and secondary bronchial hyperplasia and haemorrhage observed in CRD complicated cases were in agreement with the earlier report of Javed and Siddique (8). Lungs showed interstitial pneumonia characterised by septal thickening due to the deposition of fibrin and infiltration of lymphocytes in FC combined cases. Organised exudate composed of RBCs, lymphocytes and fibrin

occluding the parabronchial lumen was also noticed in CB combined outbreaks (Fig. 5).



**Figure 5.** Lung showing organized exudate comprising RBCs, lymphocytes and fibrin occluding the parabronchial lumen. HE, 400x.

Air sacs revealed moderate epithelial hyperplasia, subepithelial stray infiltration of lymphocytes and macrophages in uncomplicated cases. Epithelial hyperplasia, subepithelial massive infiltration of heterophils and macrophages, and increased thickening of connective tissue were observed in complicated disease with CB (Fig. 6). These findings were in accordance with the earlier observations of Nakamura et al. (13) and Kumar et al. (11). The severe microscopic lesions of air sacs might be due to the synergistic effect of MG, which correlated well with the gross lesions observed in the combined cases.



**Figure 6.** Air sac revealing moderate epithelial hyperplasia and infiltration of heterophils and macrophages. HE, 1000x.

In conclusion, gross and pathological observations described above allowed the confirmation of a synergistic increase of mortality within the flocks when

chronic respiratory disease combined with other pathogens.

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