Predisposing effect of E.*coli* to M*ycoplasma gallisepticum* infection in layer chicken

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Abstract : *Predisposing pathological effect of E.coli to Mycoplasma gallisepticum in layer chicken was evaluated in 70 commercial layer chicken farms. E.coli was confirmed by their growth characteristics on Eosin Methylene Blue (EMB) agar media. Polymerase Chain Reaction (PCR) technique was used for the confirmation of Mycoplasma gallisepticum. The average mortality rates observed in the occurrence of CRD as an individual disease was 8 per cent, whereas E.coli combined outbreaks recorded 15 per cent. Severe airsacculitis characterised by appearance of large masses of caseous exudate in the air sacs and egg peritonitis were observed in combined infection with E.coli , whereas uncomplicated CRD affected birds showed milder airsacculitis. Microscopically, air sacs revealed more intensity of epithelial hyperplasia, subepithelial infiltration of heterophils and macrophages, and thickening of connective tissue in complicated CRD with E.coli. The predisposing effect of E.coli to the outbreaks of CRD is proved well by the gross and histopathological observations.*

Keywords: *Mycoplasma gallisepticum; E.coli; predisposing effect; chicken*

INTRODUCTION

Chronic respiratory disease (CRD) caused by *Mycoplasma gallisepticum* (MG) in chicken is characterised by airsacculitis. Economic losses occur due to mortality, condemnations of down graded carcasses, reduced feed and egg production efficiency and increased medication costs (Ley, 2003). The severity of the diseases was exacerbated as the result of mixed infections with other respiratory pathogens. Presence of *E.coli* appeared to assist the MG to invade the lower respiratory tract and conversely MG assisted dissemination of the *E.coli* organisms (Murakami *et al*., 2002). This report deals with the pathological evidence to the predisposing effect of *E.coli* to enhance the severity of CRD with more mortality rates.

MATERIALS AND METHODS

This study was conducted for 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 chicken carcasses and ailing birds. Samples such as trachea, lungs, air sacs, and swab of heart blood were collected.

Heart blood swab was utilised for the confirmation of *E. coli* based on the growth characteristics on Eosin-Methylene-Blue (EMB) agar media. Trachea and air sac pieces collected in Frey's medium were used in PCR for the confirmation of MG.

One ml of sample cultured in Frey's medium at 37 °C for 5 -7 days was centrifuged at 10,000 x 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. The following forward and reverse primers were used for the amplification of target sequence (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'

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The following mixture of materials was subjected to PCR in a thermal cycler (Eppendorff) as per the procedure of Kiss *et al*. (1997).

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

The reaction consisted of initial denaturation for 5 min at 95 ºC followed by 35 cycles of denaturation at 94 ºC for 30 seconds, annealing at 55 ºC for 30 seconds and extension at 72 ºC for 30 seconds with final extension at 72 ºC for 10 min. The PCR products were separated on 1.5 per cent agarose gel in 1X TAE buffer containing ethidium bromide 50 µg per ml at 100 volts for 45 minutes to one hour. After recording the gross lesions, a transverse section of tissue approximately 0.5 cm in thickness was taken from the organs collected. 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 (Bancroft and Stevens, 1996).

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.

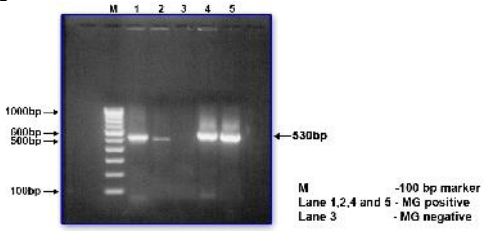

Fig. 1 PCR pattern of MG positive samples

The *E.coli* organisms were identified based on black metallic sheen colonies on EMB agar.

The average mortality rates observed in the occurrence of CRD as an individual disease was 8 per cent, whereas *E.coli* combined outbreaks recorded 15 per cent.

CRD affected birds did not reveal much pathological changes in upper respiratory tract and lung except catarrhal tracheitis. Airsacculitis with small masses of caseous exudate was noticed.

Large masses of caseous exudate in the air sacs and egg peritonitis were observed in combined infection with *E.coli*. These observations were in accordance with the earlier findings of Fabricant and Levine (1962), who reported severe airsacculitis in combined infections with *E.coli*. In few combined cases, thoracic air sacs also showed large masses of caseous exudate. Bilateral thoracic (Fig. 2) as well as abdominal airsacculitis with caseous exudate were observed in severely affected combined cases.

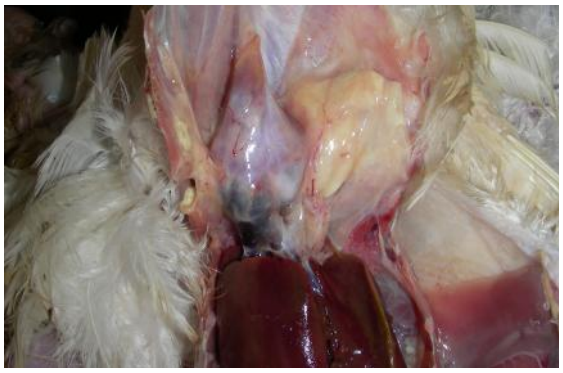

Fig.2 Caseous Exudate and bilaterally affected thorax

In uncomplicated cases of CRD, thickening of the tracheal mucous membrane due to hyperactivity of the mucous glands was noticed and lung did not reveal much microscopic alterations except mild infiltration of heterophils in the Interstitium. Organised exudate composing of RBCs, lymphocytes and fibrin occluding the parabronchial lumen was also noticed in *E.coli* combined outbreaks.

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 *E.coli* (Fig. 3).

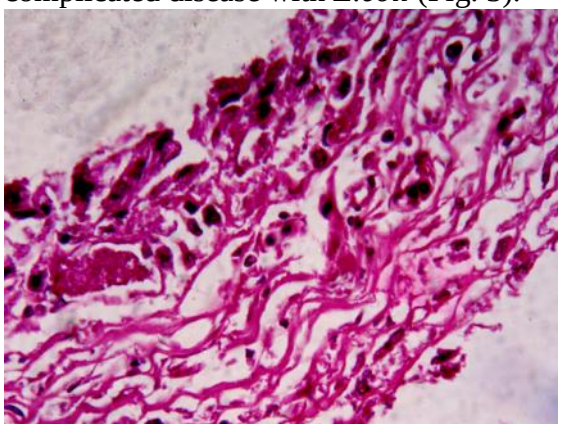

Fig 3. Histopathological examination revealing epithelial hyperplasia and subepithelial massive infiltration of heterophils

These findings were in accordance with the earlier observations of Nakamura *et al*. (1985) and Kumar *et al*. (1996). The severe microscopic lesions of air sacs correlated well with the gross lesions observed in the combined cases.

In conclusion, the predisposing effect of *E.coli* to the outbreaks of CRD is proved well by the gross and histopathological observations.

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