PATHOLOGICAL STUDIES ON CHICKENS EXPERIMENTALLY INFECTED WITH FIELD STRAIN OF MYCOPLASMA GALLISEPTICUM AND VACCINATED WITH MG VACCINE

By

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SUMMARY

Pathological changes in respiratory organs of chickens experimentally challenged with a field strain of Mycoplasma Gallisepticum (MG) and vaccinated with MG live attenuated vaccine was studied. For this purpose ,forty one day old chicks were used. The chicks were classified into four groups 10 birds for each. Group I (GI), infected by a field strain of M G, Group II (GII) with (F vax MG),vaccinated and challanged, GroupIII (GIII),vaccinated and Group 1V (GIV) used as negative control

The antibody response in sera of vaccinated and infected chickens was detected using Serum Plate Agglutination test (SPA) and Enzyme Linked Immunosorbant Assay (ELISA).MG could be reisolated from chickens of (GI),(GIII) and about 30% of chickens of (GII).

INTRODUCTION

Mycoplasma gallisepticum (MG) causes a chronic respiratory disease (CRD) in chickens and turkeys resulting in severe economic losses,particularly when present in other respiratory pathogens (Yooder,1984).In most countries control programs for MG are based on maintaining,poultry flocks and commercial breeding stock free from infection (Jordan 1979) and(LEVISOHN and Kleven 2000).

Antibiotic therapy and vaccination with live attenuated MG vaccine are means for controlling losses associated with mycoplasmosis (Kleven et al.,1984 and Evans etal.,2000). None of the vaccine afforded complete protection against infection,but more were effective in suppressing the multiplication of the organism, resulting in less tissue damage followed by faster recovery (Hildebrand et al.,1983,Rodriguez and Kleven et al.,1980).Attempts to prevent mycoplasmosis have included

vaccination with both killed and live attenuated MG strains. Live vaccine provide reduction in clinical signs and have been shown to replace endemic strains when used for several times (Evans et al., 2000). The purpose of this study was to compare and study the pathological lesions of MG field strain and MG live attenuated vaccine.

MATERIALS AND METHODS

1- Materials:

Baby chicks:

Forty one day old chicks were purchased from middle east company. These chicks were proved to be free from avian *Mycoplasma* by Serum Plate Agglutination test (SPA) (Yoder.,1980) and Enzyme Linked Immunosorbant Assay (ELISA) (Talkington et al.,1985)and by culture method (Kleven.,1985).

2- Mycoplasma:

A Mycoplasma Gallisepticum (MG) field isolated and identified strain by growth inhibition test (Clyde.,1964).

Media used:

Liquid and solid Mycoplasma culture media were prepared as described by Frey et al., 1968.

Vaccine:

live attenuated Mycoplasma Gallisepticum F-strain (F Vax MG) of Schering Plough Animal Health Company was used.

2-Methods:

The chicks were divided into four groups, 10 birds for each as follows:

a- Group I (GI) infected only.
b- Group II (GII) vaccinated and infected.
c- Group III (GIII) vaccinated.
d-Group IV (GIV) not infected (control).

Vaccination:

Two –weeks old chicks of GII and GIII received 0.5 ml vaccine (F Vax MG) subcutaneous in neck region.

Challenge:

Four weeks old chicks of GI and GII were challenged by 0.1ml suspension of Mg culture containing 107 colony forming unit (CFU),via intratracheal route as described by Talkington and Kleven.,1985.

Along the experiment time clinical observation were recorded.

At one, two and three-week post challenge (PC), three chickens from each group were scrificed for :

1-Blood samples were used for detection of antibodies by SPA and ELISA.

2-Tissues were used for reisolation of the organism.

3-Samples of air sacs, trachea, lung,larynx and nasal sinus were fixed in 10% neutral buffered formaline.The specimens were treated chemically then sectioned at 5 um and routinly stained with Harris's haematoxylin and eosin, for histopathological evaluation(Culling 1963).

RESULTS

Table (1) shows the reisolation results of MG from respiratory organs (trachea,lung and air sac).MG could not be reisolated from chickens of GV1 but isolated from all chickens of GI,GIII and about 30% of chickensof GII. Serum samples of GV1,were negative for SPA and ELISA along the duration of the experiment.While serum of GIII,were weak positive and suspecious for SPA and ELISA respectively in the 1st week PC,then became positive for both from the 2nd week PC till the end of the experiment. Concerning chickens of GII they were positive for SPA and ELISA during the all period of the experiment. Chickens of GI gave weak positive for SPA and suspect by ELISA in the 1st week PC, then became positive during the next weeks. The vaccine given to chickens resulted into clear antibody reponse as shown in table (2).

Symptoms:

Mild respiratory signs were observed as cough, rales and nasal discharges especially at 1st week PC in GI.No obvious signs were observed in GII and GIII.

Macroscopic lesions:

The air sacs appeared cloudy, caseated Scanty exudate was found in trachea and nasal sinus in GI at 1st week PI.

GII showed only mild cloudness in air sac at 1st week PC where as GIII showed no apparent lesions.

Microscopic lesions:

Air sac showed severe lesions at 1st and 2nd week PC in GI as marked thickening in the mucosa due to hyperplasia of the

epithelium which form polyps like appearance (Fig1). Submucosal congested blood vessels was seen. At the 2nd week PC perivascular lymphocytic aggregation in addition to heterophils,plasma cells with severe oedema was shown (Fig 2). Three weeks PC the lesion were degradated as hyperplasia,oedema and mild congested blood vessels. The air sac of GII after 1st week PC revealed mild oedema and hyperplastic epithelium. Few aggregation of lymphocytes and granulocytes in the lamina propria at 2nd week PC. These lesions were minimize by the 3rd PC. GIII exhibited mild focal hyperplasia at 1st week PC with mild oedema in lamina propria. After 2nd and 3rd week PC mild oedema only was recorded.

Tracheal lesions were severe after 1st and 2nd week PC and decrease after 3rd week PC in GI. There was loss in the epithelial layer and deciliation.Severe thickening in the mucosa (Fig3) due to multiple lymphocytic nodules (Fig 4) with lymphocytic infiltration in the lamina propria were shown inaddition to severe congested blood vessels. Activation of mucous glands was observed.The lumen cotain sloughed epithelium with inflammatory cells.

At the 2nd week PC the tracheal epithelium revealed hyperplasia and metaplasia. Lamina propria had lymphocytic nodules sometimes tangled with RBcs. Tracheal lesions in GII and GIII decreased after 2nd and 3rd week PC. GIII showed mild thickening in the mucosa at 1st week PC due to few lymphocytic infiltration.GII showed activation of mucous glands with marked focal aggregation of lymphocytes.

Lesions of lung in GI at 1st week PC exhibited endotheliosis with proliferation of the endothlial lining of the blood vessels. Severe congested blood vessels inaddition to perivascular and interstitial haemorrhage(Fig 5) were observed. Global perivascular and interstitial lymphocytic infiltration. Presence of granulomas surrounded by inflammatory cells and giant cells (Fig 6). Fibrinous pneumonia (Fig7), pleuricy and emphysema (Fig8) can also be seen.The lesions progressed after the 2nd week PC as the 2ry bronchial epithelium suffered from severe folded hyperplastic epithelium (Fig 9) with activation of mucous glands. Several lymphocytic nodules tangled with RBcs were found
(Fig 10). Multiple granulomas also were seen.

Thickening in the atrial wall (Fig 11) with oedema can also be observed. The severity of lesions will reduce at 3rd week PC. At GII after 1st and 2nd week PC the lung revealed mild fibrinous pneumonia and oedema.
At 3rd week PC mild hyperplasia and metaplasia of bronchial epithelium with hypertrophy in mucous glands were recorded. 1st and 2nd week PC of G111 revealed mild fibrinous pneumonia with heterophilic infiltration (Fig 12) and thickening in the atrial wall. At 3rd week PC mild hyperplasia and ballooning bronchial epithelium were seen.

The larynx in case of GI in 1st week PC showed thickening in the mucosa due to hyperplasia of the epithelium and activation of mucous glands with the presence of prominent lymphocytic nodules in the lamina propria tangled with RBcs (Fig 13) in addition to submucosal congestion and haemorrhage.The lesions became more severe at 2nd week PC and characterised by presence of several nodules of lymphocytes in the lamina propria tangled with RBCs in addition to inflammatory cells infiltration.The lesions declined at 3rd week PC. GII showed moderate submucosal oedema and congestion at 1st and 2nd week PC.

Hyperplasia of laryngeal epithelium inaddition to congested blood vessels were observed in GIII at 1st week PC. The lesions reduced at the 2nd and 3rd week PC.

Nasal sinuses in case of GI at 1st week PC exhibited hyperplasia of lining epithelium, submucosal haemorrhage and presence of lymphocytic nodules (Fig 14) tangled with RBCs at 2nd week PC with activation of mucous glands at 3rd week PC. GII and GIII showed mild to moderate lesions as hyperplasia in the lining epithelium and submucosal oedema with few aggregation of lymphocytes in the lamina propria at 1st week PC.Milder lesions were observed at 2nd and 3rd week PC.

DISCUSSION

The protection effect of MG vaccine was examined serologically and culturally. The vaccine stimulated the humeral response in chickens as detected by SPA and ELISA. Laboratory studies have shown that F strain vaccine provides significant protection against MG infection Cummings and Kleven (1986). Levisohn and Kleven (1981) concluded that,the use of F strain

vaccine ineach replacement flock over a period of time might result in eventual displacement of the original field strain.
In the present study, the chickens of GII and GIII received 0.5 ml vaccine (F Vax MG) at the age of 2 weeks subcutaneously in neck region for protection of chicken trachea against colonization by MG.

Mycoplasma could not be reisolated from about 60-70% from chickens of GII during the all period of the experiment, there for the vaccine provided a pronounced protection of birds. These results agreed with those mentioned by Yagihashi et al.,(1981) and Frosyth et al.,(1992) who concluded that MG vaccine inhibited the attachment of radiolabelled MG to the trachea of birds by more than 60%. This degree of inhibition suggests the role of vaccine in the process of cytoadherence.

Cummings and Kleven (1986) and Barbour et al.,(2000) mentioned that, vaccination of broiler chicken breeders with live MG vaccine (Ts-11) prevented reisolation of field MG from trachea and infraorbital sinus of broilers. Papazisi et al.,(2002) stated that,the antibodies elicited by vaccination of birds with live vaccine of MG are responsible for blocking the initial colonization of MG thereby resulting in protection. Regarding antibodies in serum of chickens detected by SPA and ELISA, chickens of GIII gave weak positive for SPA in the 1st week PC and suspecious for ELISA, meanwhile GII gave positive SPA and ELISA (970). These results were agreed with those mentioned by Talkington et al., (1984), who stated that SPA showed no activity at 0-10 daysPC, then by time the percentage of SPA and ELISA procedures increased to 90% and 100% in the 3rd week PC. In the present work, the highest titer of antibodies detected by ELISA was that of GII (2260) in the 3rd week PC,while it was (1211) for GIII in the same week. These results were agreed with the mentioned by Elshater et al (2001) and Noormohammadi et al., (2002) who said that,the antibodies in sera of birds vaccinated with TS-11, challenged with MG begins low and increase gradually in the 3rd and 4th week PC. This study revealed mild respiratory signs begin after 1st week PC in GI and reduced gradually till the 3rd week PC. This may be attributed to the irritation of the mycoplasmas that attached and colonized on the affected cells.

These finding were similar to those mentioned by Calnek et al.,(1997).The gross lesions of the experiment were more or less

simulates that recorded by Pruthi and Kharole (1980) and Ritchie et al., (1994). Those findings were most probably due to the adsorption of the organisms to the surface of host cells at which the multiplication of mycoplasmas takes place that altered the function of host cells and the membrane integrity. Concerning the microscopic lesions of the examined organs revealed hyperplasia and degeneration in the lining epithelium with deciliation. This is might be due to the irritation of mycoplasma which colonized on the host cells by special organelles and may be hidden in the recesses of the host cells by cytoadsorption and protected itself from the elimination of clearing function of cilia. Could be also possible take up some nutrients from the host cells resulting in degenerative changes. Those finding agreed with Tajima et al.,(1979) and Ritchie (1994). Presence of several nodules of lymphocytes in the lamina propria of the affected tissues may be owed to the toxic substances and the hydrogen peroxide that produced by mycoplasma which causing cell damage and evoke the hyper sensitivity reaction of the cells. Aforementioned results more or less agree with Tajima et al., (1971) and Cherry and Robinson (1971). On comparing the pathological lesions in all groups we found that lesions of GI more severe than GII and GIII. By the advance of time we found that the severity of lesions decrease in all examined organs in GI. Mild lesions appeared at 3rd week PC this is due to the increase in the antibody titer, which reflect the improvement of the immunity of the chickens. Lesions in GIII milder than in GII and GI at 1st week PC then it would declined during the 2nd and 3rd week PC.

Although GII represent the vaccinated challenged group, we found moderate lesions in all examined organs especially at 1st week PC and this is may be the F strain colonization on tracheal epithelium did not block infection by the pathogenic mycoplasma Gallisepticum as those mentioned by Calnek et al., (1997). These results also in agreement with Forsyth et al., (1992) who stated that the MG vaccine inhibited the attachment of radiolabelled MG to the trachea of birds by more than 60%.

In conclusion; live attenuated MG vaccine (F strain) can produce mild lesions in all examined tissues with no symptoms. So further work was needed to reach complete protection against infection, whereas the presence of diseased tissue considered as predisposing factor for the infection by other pathogens.

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Table (1) showing the reisolation of MG from different groups

GROUP	WEEK PC	RESPIRATORY ORGANS		
		TRACHEA	LUNG	AIR SAC
1	1ST	+	+	+
2		WEAK+	WEAK+	WEAK+
3		+	+	+
4		-	-	-
1	2nd	+	+	+
2		WEAK+	WEAK+	WEAK+
3		+	+	+
4		-	-	-
1	3rd	+	+	+
2		WEAK+	WEAK+	WEAK+
3		+	+	+
4		-	-	-

Table (2) Results of SPA and ELISA titer in different groups

GROUP	WEEK PC	Serological tests		
			ELISA	
		SPA	Titer	AIR SAC
1	1ST	WEAK+	200	Suspicious
2		WEAK+	180	Suspicious
3		+	970	+
4		-	40	-
1	2nd	+	892	+
2		+	1020	+
3		+	1180	+
4		-	60	-
1	3rd	+	1530	+
2		+	1211	+
3		+	2260	+
4		-	72	-

Negative (-): 0 – less than 149. Suspicious: 149 – 744.
Positive: more than 744.

Fig (1): Section through the air sac of chickens G1 1st w PC:
Showing hyperplasia of the lining epithelium (H&E, x250).

Fig (2): Section through the air sac of chickens G1 2nd w PC:
Showing oedema, congested blood vessels and lymphocytic aggregation (H&E, x40).

Fig (3): Section through the trachea of chickens G1 1st w PC:
Showing severe thickening of the mucosa (H&E, x100).

Fig (4): Section through the trachea of chickens G1 1st w PC:
Showing lymphocytic nodules and submucosal oedema (H&E, x100).

Fig (5): Section through the lung of chickens G1 1st w PC:
Showing perivascular and interstitial haemorrhage (H&E, x250).

Fig (6): Section through the lung of chickens G1 1st w PC:
Showing granuloma with giant cells (H&E, x400).

Fig (7): Section through the lung of chickens G1 1st w PC:
Showing fibrinous pneumonia (H&E, x250).

Fig (8): Section through the lung of chickens G1 1st w PC:
Showing emphysema (H&E, x100).

Fig (9): Section through the lung of chickens G1 2nd w PC:
Showing hyperplasia of 2ry broncheol, activation of mucous glands and submucosal lymphocytic nodules (H&E, x100).

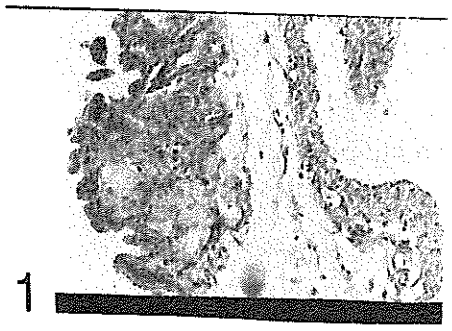
Fig (10): Section through the lung of chickens G1 2nd w PC:
Showing several lymphocytic nodules tangled with RBcs (H&E, x40).

Fig (11): Section through the lung of chickens G1 2nd w PC:
Showing thickening in the atrial wall with aggregation of lymphocytes (H&E, x250).

Fig (12): Section through the lung of chickens G111 1st w PC:
Showing aggregation of granulocytes (H&E, x400).

Fig (13): Section through the larynx of chickens G1 1st w PC:
Showing lymphocytic nodules tangled with RBcs (H&E, x100).

Fig (14): Section through the nasal sinus of chickens G1 2nd w PC:
Showing submucosal lymphocytic nodules tangled with RBcs (H&E, x100).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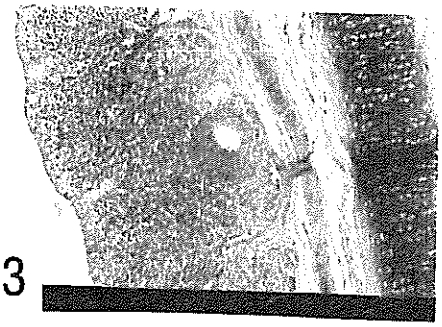
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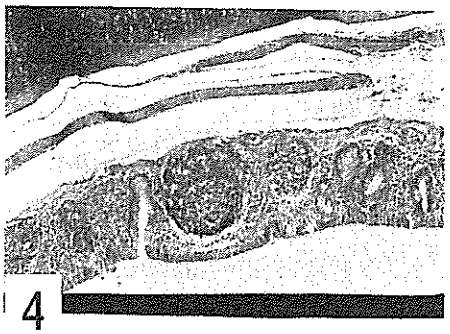
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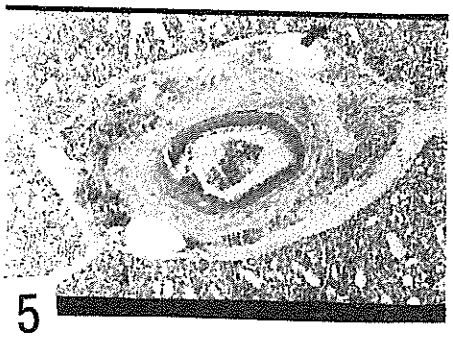
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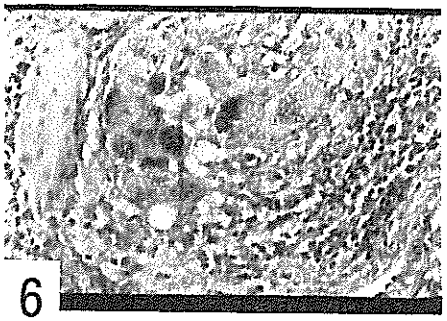
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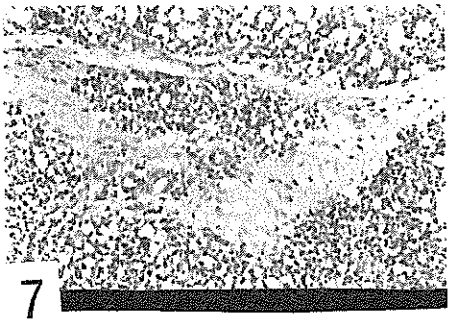
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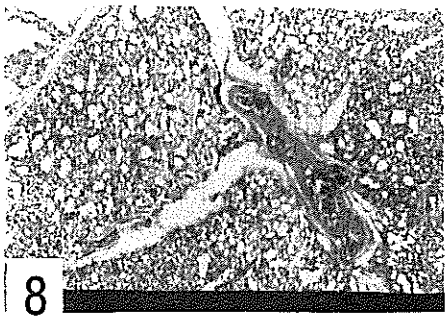

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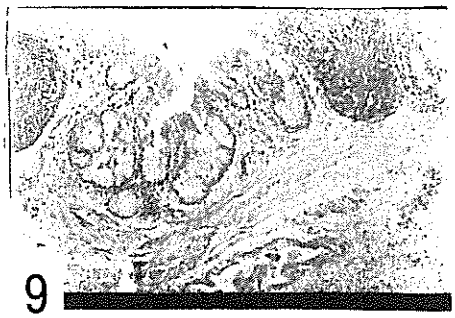

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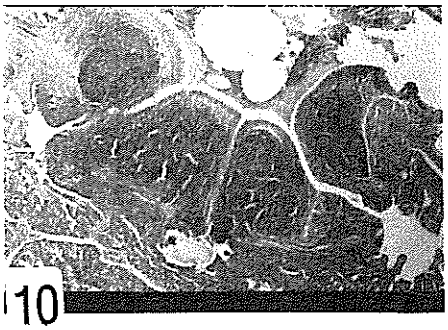

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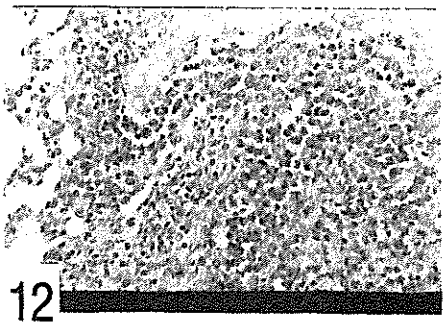

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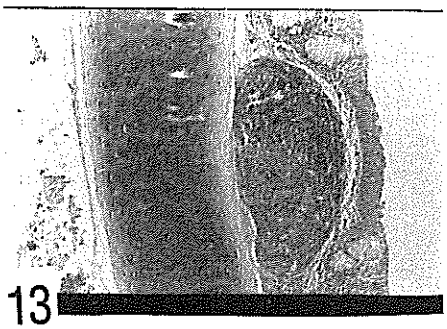

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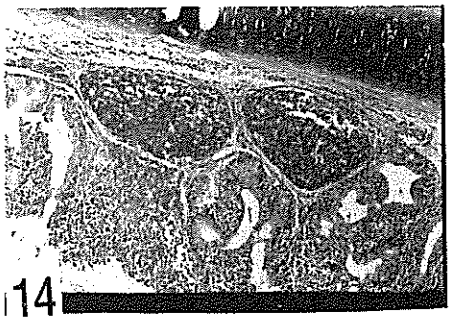

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