PATHOGENESIS OF *ESCHERICHIA COLI* IN PREVIOUSLY *MYCOPLASMA GALLISEPTICUM* INFECTED LAYER CHICKS

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SUMMARY: Two weeks-old seventy five layer chicks were divided into 2 groups of 60 (A) and 15 (B) birds. Chicks of group A were inoculated subcutaneously and intratracheally with Mycoplasma gallisepticum while the chicks of group B were left as uninoculated controls. At the age of 3, 4 and 5 weeks, 15 chicks each from group A were inoculated with pathogenic strain of E. coli. Variable clinical signs were recorded in different groups. In Mycoplasma infected birds, coughing, sneezing and mucoid nasal discharge were observed during initial stages and moist rales by the end of 2nd week of inoculation. In mixed infections, onset was rapid with more severe clinical manifestations. In Mycoplasma infected birds, tracheitis and cloudiness of airsacs were the main gross pathological lesions observed. In mixed infections, there were more advanced lesions, including airsacculitis, fibrinous pericarditis and perihepatitis. Airsacs were filled with caseous material in few birds. Serious involvement of the liver, heart, lungs and trachea were also noted. Histopathological studies in Mycoplasma infected birds revealed mononuclear cellular infiltration and epithelioid cells in tracheal mucosa. Mucosal glands of the trachea were also enlarged. In birds inoculated with E. coli after Mycoplasma, there was severe cellular infiltration and sloughing of tracheal mucosa. In lungs, congestion, focal areas of necrosis and emphysema were seen, almost in every case. Congestion, hemorrhages and leukocytic infiltration were recorded in sections of liver from many birds.

Key Words: Escherichia coli, mycoplasma gallisepticum.

INTRODUCTION

Among other factors, infectious diseases are the real threat to the poultry development in Pakistan. Of the various infectious diseases, colibacillosis and mycoplasmosis are of prime importance. These microorganisms persist for reasonably long periods in the flocks and there by lead to high economic losses through mortality, loss of weight and production (2).

Escherichia coli in chicken can cause a variety of disease conditions such as enteritis, septicemia, perihepatitis, pericarditis, peritonitis, synovitis, omphalitis and occasionally salpingitis and coligranuloma (2). Mycoplasma species are mainly encountered in respiratory tract infections but localization in other organs can also occur. *E. coli* cannot readily infect the airsacs unless they are previously invaded by *Mycoplasma gallisepticum* alone or in combination of either infectious bronchitis or Newcastle disease virus (12).

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The present project was therefore designed to study the susceptibility of Mycoplasma infected birds towards *E. coli*, and to investigate clinical signs, gross and histopathological lesions in Mycoplasma infected birds and in chicks superimposed by *E. coli* infection.

MATERIALS AND METHODS

Seventy five day old chicks were randomly divided into two groups (group A having 60 and B,15 chicks). The birds were reared under similar conditions until two weeks of age. Pathogenic strain of *M. gallisepticum* was obtained from Veterinary Research Institute, Lahore and fresh PPLO broth culture was prepared.

Nutrient broth culture of pathogenic strain of *E. coli* was also prepared. The chicks of group A were injected 0.25 ml of 4-5 day old culture of *M. gallisepticum*, subcutaneously and intratracheally, while the group B was kept as uninfected control. Fifteen chicks each, at the age of 3, 4 and 5 weeks from group A (A_1, A_2 and A_3 respectively) were then inoculated subcutaneously with broth culture of a pathogenic strain of *E. coli.* The group A_4 (15 chicks) was kept as Mycoplasma infected control.

All the groups were examined regularly for clinical signs, mortality rate and gross pathological lesions. The morbid tissues from dead and slaughtered birds were collected and were processed for histopathological studies.

RESULTS AND DISCUSSION

The present study was undertaken to investigate the susceptibility of *M. gallisepticum* infected birds to *E. coli.* The clinical signs, mortality, gross and histopathological lesions were recorded in various groups.

Clinical manifestations

Mycoplasma infected birds revealed clinical signs 7th day post-infection, firstly depression and low feed intake, later on coughing, sneezing and mucoid nasal discharge in some cases. Moist rales and gargling voices were observed in few birds. Cyanosis of the comb and nervous involvement was also seen in some chicks, similar clinical signs have been reported by Yorder (13). The birds inoculated with *E. coli* after 1st, 2nd and 3rd weeks post Mycoplasma infection resulted almost similar clinical manifestations, but more aggravated and there were relatively high morbidity and mortality. The course of disease was short with rapid development of clinical manifestations i.e. 2-3 days post-infection. Greenish diarrhea which is also reported by Sponenberg *et al.* (7) was seen in some birds which might be due to low feed intake. The respiratory signs and loss of condition were the main clinical manifestations.

In Mycoplasma infection alone (group A_4), the mortality recorded in 6 weeks period, appeared late and continued thereafter (Table 1). Yorder (13) reported, Mycoplasma as chronic infection which leads to mortality in later course of disease. It causes firstly air sacculitis which leads to anoxia resulting in poor oxygenation of blood and ultimately damage the other visceral organs. This tends to decrease the birds viability, low feed intake and conversion, and death during later course of the disease. The mortality in groups A_1, A_2 and A_3 was relatively high and started very early in the same week of super-infection with *E. coli* (Table 1).

Table 1: Week-wise mortality in various groups.

Age in weeks	Weeks post-initial infection	No. of chicks died in various groups				
		A_1	A_2	A_3	A_4	B
3	1st week	-	-	-	1	-
4	2nd week	3	-	-	2	-
5	3rd week	2	4	-	1	-
6	4th week	2	3	-3	1	-
7	5th week	1	2	3	-	-
Total mortality		8	9	6	5	-

Gross pathology

The chicks of group A_4 revealed mucoid/catarrhal exudate in nasal passages, trachea, bronchi and airsacs. Lungs showed congestion and light colored foci. Mycoplasmosis is primarily a disease of respiratory system, initially of airsacs and later intends to trachea and upper respiratory passages. The accumulation of the exudate in air passages is responsible for typical sneezing and tracheal rales (14). There was more serious involvement of the lung, heart, liver and trachea in groups A_1, A_2 and A_3. The liver was enlarged with mild hemorrhages and covered by white cloudy covering i. e., perihepatitis. Lungs revealed dark red appearance and hemorrhages in few cases. In few cases, the heart was enlarged but covered with white cloudy covering in most of the cases (Figure 1). Congestion, hemorrhages and urate deposition was recorded in kidneys in most of the cases. Trachea revealed tenacious exudate and linear hemorrhages, cloudy airsacs in some cases containing caseous material were also observed. Splenomegaly was recorded in few birds. Predisposition of the flocks to colibacillosis previously infected with *M. gallisepticum* and similar gross lesions have been reported by Thornton (9), Hofstad *et al.* (2), Ibragimov *et al.* (3).

Figure 1: Photograph of liver showing while thick covering of fibrin i.e.; Pericarditin.

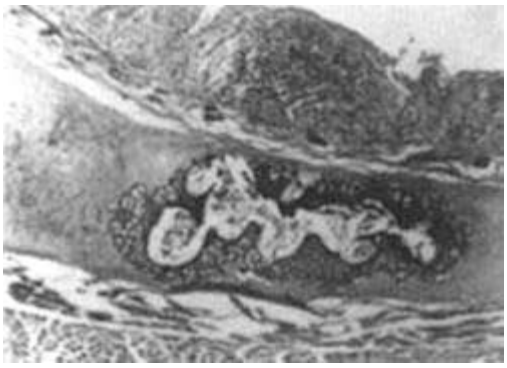
Histopathology

In chicks of group A_4, i.e. affected with Mycoplasma infection, showed most consistent histopathological alterations in trachea and lungs. In trachea, sloughing of mucosa of varying degree and mononuclear cells

infiltration was seen in mucosa and sub-mucosa. The exudate was also seen in the lumen of the trachea. The lungs showed congestion, focal hemorrhages, focal necrosis and leukocytic infiltration (lymphocytes and polymorphs). In few cases, emphysema and exudate in the alveoli was also recorded. Similar histopathological findings in Trachea and lungs have been reported by Johnson (5), Surdom and Stoenescu (8).

In chicks of groups A_1, A_2, and A_3 the most consistent histopathological findings were seen in trachea, lungs, liver, intestine, kidney and heart. The trachea revealed hyperplasia of glands with degenerated cells, infiltration of lymphocytic cells in mucosa (Figure 2) along with exudate in the lumen, degeneration and necrosis of mucosal epithelium at some places and at other hyperplasia of the mucosal epithelium. The lungs showed congestion and focal hemorrhages in some cases. Caseous exudate in the lumen of bronchiole, alveoli was also seen with oedema of the parenchyma. Emphysema, focal necrosis and lymphocytic and polymorphs infiltration was also observed in most of the cases. Almost similar findings have been reported by Buxton and Fraser (1) and Johnson (5). The liver showed congestion, leukocytic infiltration (lymphocytic and polymorphonuclear), degeneration and necrosis of the hepatocytes (Figure 3). Hemorrhages were also seen in some of the sections with golden colored pigmentation. Trauscott *et al.* (10) and Waheed and Siddique (11) have reported almost similar histopathological findings in *E. coli* infections.

Figure 2: Photomicrograph of Trachen moving lymphocytic infiltration in mucosa and degeneration.



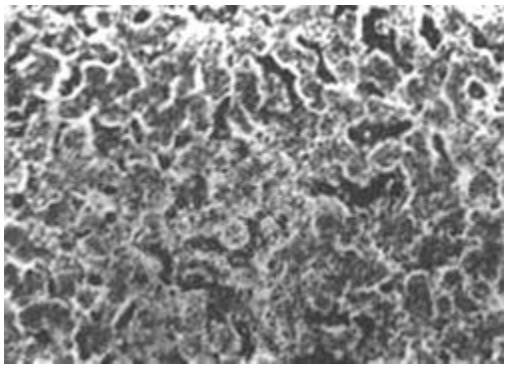


Figure 3: Photomicrograph of liver moving, congestion and necrosis of the hepatocylis.

The heart showed, congestion, degeneration of cardiac muscles with variable degree of leukocytic infiltration and pericarditis. These findings were in agreement with those of Trauscott *et al.* (10) and Waheed and Siddique (11). Histopathological findings in intestine were congestion sloughing and degeneration of mucosal epithelium, lymphocytic and polymorphonuclear infiltration, hemorrhages, degeneration and necrosis of intestinal glands. Sporenberg *et al.* (7) and Inove *et al.* (4) had reported similar findings in *E. coli* infected birds. Histopathological findings in kidneys were similar as reported by Nagi and Khanna (6). There was congestion, degeneration and necrosis of the tubules.

All the chicks of uninfected control group were in good health and none died in that group during the course of study.

It is evident from the present study that the morbidity and mortality is high in mixed infection of *E. coli* and Mycoplasma and both the infections are real threat to the farmers. Control to one can reduce the mortality to an extent as any one predispose the birds to its counterpart and hence leads to high mortality. Probably Mycoplasma weakens the body defenses by producing chronic and debilitating disease, simultaneously injuring the body tissues exposing for opportunist pathogens. *E. coli* is very widely distributed in the whole environment, gets an easy chance and aggravates the condition, this way leads to great economic losses through high mortality. Hence the control of Mycoplasma is helpful in reducing the losses through avoiding the opportunity for *E. coli*.

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