THE ROLE OF E.COLI & MICROCOCCUS GRANULATUS CITREUS IN MYCOPLASMA SYNOVIAE INFECTION *

BY

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Introduction

Inflamation of the joints in chickens was reported to be due to many pathogens. Bain & Tempest (1978) reported Staphylococcus aureus as the cause of tendovaginitis in broilers. While Van der Heide & co-workers (1975. 1978) isolated reovirus from cases of tendosynovitis. Mycoplasmal synovitis was first reported in USA by Olson et al (1954) & afterwards by Wills (1955). It was also reported in Canada, France & other countries with intensive poultry industry. In Africa the disease was reported only in South Africa by Buys (1976).

Some reports on the role of viruses in mixed infection with M.synoviae are found in the literature. Springer, Luskus & Pourciau (1974) studied the association of M.synoviae, E.coli & infectious bronchitis virus, in experimental reproduction of airsaculitis in Gnotobiotic chickens. They reported variable severity from various combinations of organisms, but higher mortality as a result of a mixed infection of M.synoviae & infectious bronchitis virus.

The role of bacteria in the mechanism of development of infectious synovitis is not clear. The purpose of this study is to explain the synergistic role of some bacteria in the development of mycoplasmal synovitis.

Materials & Methods

Cultures of M.synoviae, E. coli & Micrococcus granulatus citreus, isolated from chickens, were used. The experiment was conducted in 133 healthy white leg-horn chickens 45 days old, 20 of them were kept as control.

10 chickens were inoculated intratracheally & 10 into the foot pad, with broth culture of M.synoviae in a dose of 0.5 ml; $3X10^7$ cfu/ml concentration. Three to five days later, E. coli or Micrococcus granulatus citreus were injected by the same routes in a dose of 1 ml; $1x10^6$/ml concentration. In another groups 10 chickens each, E.coli or Micrococcus granulatus citreus were inoculated and one day later followed by M. .synoviae. Other groups of 5 or 6 chickens each, were inoculated similarly by M. synoviae, E. coli or Micrococcus granulatus citreus alone.

Body temperature, live weight & pathological changes were studied. In the different groups, erythrocyte & haemoglobin contents, erythrocyte sedimentation rate & leucocyte count were studied. The total protein level was measured by the refractometer (ERF-22). Serum fractions were studied using paper electrophoresis according to Malakhov (1967). The haematological data were subjected to statistical analysis using student T-test method.

Results

Clinical signs 3-5 days after inoculation of M.synoviae & E.coli; 7-10 days after infection with M.synoviae and Micrococcus granulatus citreus, included emaciation; loss of appetite, increased temprature (42,6-42,8°C), lamness and drop in body weight. The average difference in body weight of chickens infected with M.synoviae, E.coli and the control group was 127,5-142,5 gm; between those infected with M.synoviae & Micrococcus granulatus citreus, & the control was 65-95 gm.

The first clinical signs due to M.synoviae & E.coli infection observed in the second day after inoculation, were depression, respiratory symptoms & high body temperature. Clinical signs & mortality among chickens infected with M.synoviae and Micrococcus granulatus citreus were seen after the 20th day after inoculation. Postmortem examination of chickens with clinical symptoms due to mono-i nfection or mixed infection, showed emaciation, enlarged joints with creamy or yellow exudate on the synovial membrane. Hyperaermia of the trachea was observed after intratracheal infection. After inoculation of cultures into the foot pad, changes were observed in the joints chatacterized by catarrhal synovitis, enlarged spleen, congested liver & kidney, together with epicarditis. Postmortem findings were more severe in chickens infected with M.synoviae & E.coli. Data suggested synergistic role of Micrococcus granulatus citreus & E.coli in Mycoplasmal synovitis are shown in table 1.

Results show high infection & mortality ra' in mixed infection. Mortality among chickens infected with M.synoviae was 16,7 % while in mixed infection with E.coli or Micrococcus granulatus citreus reach

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20-40 %. When M.synoviae occured as a secondary pathogen, after a primary infection caused by E.coli or Micrococcus granulatus citreus, the number of clinically diseased chickens reached 80 % after intratracheal inoculation, while mortality ranged between 40-50 %. The above data indicated that, the clinical and the pathological changes of infectious synovitis were more aggravated in mixed infection.

Haematological studies (table 11) showed significant difference in erythrocyte count due to M.synoviae & E.coli infection. Erythrocyte count 15-30 days after experimental infection was $2,9 \pm 0,09$-$3,42 \pm 0,12 \times 10^6$/mm compared to $3,31 \pm 0,09$-$4,05 \pm 0,15 \times 10^6$/mm in control groups. The haemoglobin content decreased; $8,2 \pm 0,28$-$8,83 \pm 0,29$, and was $9,95 \pm 0,16$-$10,10 \pm 0,3$ gm % in the controls.

Mixed infection resulted in changes in the physio-chemical blood composition, as a result of which slow erythrocyte sedimentation rate was observed which was more pronounced in 45-60 minute time. In chickens infected with M.synoviae & E.coli, the leucocyte count in 10-15 days after infection was $33,25 \pm 0,54 \times 10^3$/mm, while in the controls it was $31,9 \pm 0,048 \times 10^3$/mm. White blood cells dropped to the normal level 15-30 days after infection.

The decrease in erythrocyte count & haemoglobin content were observed in chickens infected with M.synoviae & Micrococcus granulatus citreus. Erythrocyte count of infected birds was $2,966 \pm 0,09$-$3,8 \pm 0.21 \times 10^6$/mm& that of the control group was $3,31 \pm 0,09$-$4,04 \pm 0,15 \times 10^6$/mm.The haemoglobin level was $9,5 \pm 0,38$ — $8,52 \pm 0,7$: $10,0 \pm 0,2$-$10,1 \pm 0,3$gm % in control groups. Slow erythrocyte sedimentation rate was also observed as a result of changes in red cells parameters. Leucocyte count increased 15 days after infection in comparison with the controls ($31,87 \pm 0,45$ & $30,9 \pm 0,48 \times 10^3$/mm); while in later observations it reached $34,1 \pm 0,92$ & $30,05 \pm 0,15 \times 10^3$/mm in the control groups.

E.coli & Micrococcus granulatus citreus in mixed infection with M.synoviae resulted in pronounced changes in the haematological picture. Result of biochemical blood composition 15 days after infection with M.synoviae, E.coli or Micrococcus granulatus citreus showed clear changes in total blood protein which in comparison with the control groups was found to be $3,9 \pm 0,1$-$3,8 \pm 0,2$ compared to $4 \pm 0,1$ gm % in the control group. Albumin content dropped after 30 days to 5.3 %.

Mathmetical analysis of the results showed decrease of albumin/globulin coefficient (A/G): from $0,6 \pm 0,1$ in control group to $0,5 \pm 0,1$ in infected due to disorder in albumin- globulin relationship.

Increase in & decrease in -globulins was also observed. In chickens infected with M.synoviae after E.coli or Micrococcus granulatus citreus inoculation, changes in total protein & albumin content have nearly similar character, nevertheless drop in globulin level was more pronounced. In chickens infected with Micrococcus & M. synoviae drop in globulin level was $2,2 \pm 0,2$ gm %, while it was $2,6 \pm 0,4$ gm % in the controls. γ-globulin decreased to 1,8 % (2,0gm %) in comparison with the control.

Infection of chickens with M.synoviae & then Micrococcus granulatus citreus was characterized by increase of the total protein ($3,9 \pm 0,2$-$4,1 \pm 0,04$ gm %) 30 days after inoculation. In groups infected with M.synoviae & E.coli it reached $3,8 \pm 0,3$-$3,5 \pm 0,3$ & $3,6 \pm 0,5$ gm % in the control groups. Studies of protein fractions in mixed infection showed irregularity in α, β & γ -globulin relationship. Increase in β -globulin level specially observed in chickens infected with M.synoviae & E.coli ($7,6 \pm$,6-$10,6 \pm 0,2$ %) & $4,8 \pm 0,4$ % in controls. In statistical analysis of the data $P < 0.01$.

Discussion

The synergistic effect of bacteria is quite important in the pathogenicity Pathogenesis of mycoplasmal synovitis. Mycoplasmal infection is more acute when chickens are inoculated with M.synoviae together with E.coli or Micrococcus granulatus citreus. There are few reports about the role of viruses or their vaccines in the development of mycoplasmal synovitis; (Kleven et al 1972, 1973,1975); Vardaman, 1975, but the role of bacteria recieved no attention.

Our results showed, that the clinical & pathological changes of mycoplasmal synovitis are more severe in combination of E.coli & Micrococcus granulatus citreus infection, which reflect the synergistic action of these microorganisms. In chickens infected with M.synoviae alone mortality rate was not higher than 16,7 %, while in combination with E.coli or Micrococcus granulatus citreus it reached 20-40 % & 50 % respectively.

According to many authors, changes in the physi-

cal & biochemical blood composition characterize the mechanism of the disease onset (Evanov et al 1969). Haematological changes were observed in mixed infection of M.synoviae & E.coli. There was a decrease in red blood cells & haemoglobin which resulted in anaemia; also there was slow erythrocyte sedimentation rate & high level of leucocyte count. According to Ceel (1960) changes in leucocyte parameters, together with other haematological changes are considered as a sign of stress condition.

In mixed infection of E.coli or Micrococcus granulatus citreus with M.synoviae there is a change in the total protein level in infected chickens, and decreased albumin content. The above changes highly affect the albumin globulin coefficient and blood protein fractions. It is important to state, that in mixed mycoplasmal synovitis with E.coli infection,there is an increase in β -globulin, which may indicates the toxogenic factor of E.coli in the pathogenesis of mixed infection. Also there was a decrease in γ -globulin which support Springer et al (1974), and Vardaman et al (1974). Kleven et al (1972) findings about the disorder in protein fraction parameters in mycoplasmal synovitis & decrease of antibody response to M.synoviae in mixed infection or M.synoviae with other microorganisms.

Disorder in protein metabolism characterized the pathological process in mixed infection, which leads o disturbances in active body resistance & aggravated clinical signs.

Summary

Infection with E.coli or Micrococcus granulatus citreus in combination with M.synoviae resulted in high infection and mortality rate of chickens. In-mono-infection mortality rate reached 16,7 % while in mixed infection it reached 20,40 %. In primary infection with E.coli or Micrococcus granulatus citreus with M.synoviae morbidity reached 60-80 % and mortality 30-50 %.

Drop in erythrocyte and increase in leucocyte count were observed together with low haemoglobin level and slow erythrocyte sedimentation rate. Changes in the total protein, albumin and globulin levels were clearly demonstrated. These changes correlated well with the disorder in α , β & γ -globulin prameters.

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Table I

Results of experimental inoculation of chickens with **M.synoviae** in combination with **E.coli** or **Micrococcus granulatus citreus.**

Inoculum	No. at risk		No. with symptoms		Mortality	
	Intratracheal	Foot pad	Intratracheal	Foot pad	Intratracheal	Foot pad
M.synoviae	6	6	2(33.3 %)	3(50 %)	16.7	0
E.coli	6	5	3(60 %)	2(40 %)	16.7	40
M.granulatus citreus	5	5	1(20 %)	2(40 %)	0	0
M.synoviae and E.coli	10	10	6(60 %)	7(70 %)	40	20
M.synoviae and M.granulatus citreus	10	10	5(50 %)	3(30 %)	40	0
E.coli and M.synoviae	10	10	8(80 %)	6(60 %)	50	30
M.granulatus citreus and M.synoviae	10	10	4(40 %)	2(20 %)	40	0
Control 20		—	—	—		

Table II

Biochemical results of chickens infected with **M.synoviae** **E.coli** and **Micrococcus granulatus citreus.**

Inoculum	Erythrocyte count (10^6 %mm)	Haemoglobin content (g %)	Leucocyte count (10^3/mm)	Total Protein g%	Protein fractions albumin (g %)	Protein fractions globulin (g %)
M.synoviae & E.coli	2.9 ± 0.1	8.2 ± 0.3	33.3 ± 0.5	3.5	1.0	2.5
M.synoviae & M.granulatus citreus	3.0 ± 0.1	8.5 ± 0.7	34.1 ± 1.0	3.9	1.5	2.4
E.coli & M. synoviae	2.8 ± 0.2	8.3 ± 0.9	32.3 ± 0.5	3.8	1.3	2.5
M.granulatus citreus & M.synoviae	3.3 ± 0.2	8.6 ± 0.4	34.1 ± 0.9	3.3	1.1	2.2
*Control	3.3 ± 0.1	10.1 ± 0.3	31.9 ± 0.1	3.6	1.6	2.0