COMPETITIVE EXCLUSION OF PROTEUS MIRABILIS CAUSING CLOACAL PROLAPSE AND EMBRYONIC CHICKEN DEATHS

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ABSTRACT

Out of 50 bacteriologically examined Ross-laying hens suffered from cloacal prolapse, *Proteus mirabilis* was isolated in pure culture with a percentage of 44.0% and in mixed form with other enteric organisms, it was in a percent of 34%. It produced cloacal prolapse in 60.0% of experimentally infected layers, decreased to 40.0% when inoculated simultaneously with *Lactobacillus acidophilus* and 20.0% when *Lactobacillus acidophilus* precedes *Proteus mirabilis* with 6 hours. The penetrating power of *Proteus mirabilis* through eggs shells in fertile eggs as well as the mortality rates in embryos were studied. *Proteus mirabilis* caused 68.0% dead-in-shell-embryos when bathed alone, decreased to 44.0% when bathed simultaneously with *Lactobacillus acidophilus* and decreased again to be 28.0% when *Lactobacillus acidophilus* precedes *Proteus mirabilis* with 6 hours.

INTRODUCTION

Genus Proteus is considered as one of the family Enterobacteriaceae, which is characterized by swarming appearance on solid media. Proteus was isolated from dead-in-shell chicken embryos (**Orajaka and Mohan, 1985**) and sick ducklings (**Safwat *et al.*, 1984**).

Penetration of eggs and survival within the egg are affected by temperature (**Al Aboudi *et al.*, 1988**).

Septicaemia has occurred in quails (**Sah *et al.*, 1983**) and broilers suspected of having immunological deficiency (**Radall *et al.*, 1987**). *Proteus mirabilis* has been recovered from salpingitis lesions in layers with low percentage (**Bisgaard and Dam, 1981**) and caused up to 65% mortality in quail chicks aged up to 2 weeks (**Myint, 1987**). On the other hand, **Venkanagouda *et al.*, (1996)** reported that *Proteus mirabilis* infection mainly causing mortality in young chicks up to 4 weeks of age with suppurative osteomylitis.

This work was planned in order to study:

1- The epidemiological status of *Proteus mirabilis* in layers suffered from cloacal prolapse.
2- The penetrating power of *Proteus mirabilis* through egg shell.
3- Experimental infection in layers through cloaca.
4- The role of *lactobacillus acidophilus* of chicken origin as a competitive inhibitor to *Proteus mirabilis* infection.

MATERIAL AND METHODS

Laying hens:
A total of 50 Ross 37 weeks old laying hens suffering from cloacal prolapse were studied bacteriologically for isolation of potentially pathogenic bacteria related to the case.

Media used:
Tryptone-soy-agar, brain-heart-infusion agar, sheep-blood agar, MacConkey-bile salt-lactose agar, Hektoen enteric agar, Salmonella-Shigella agar and thiosulphate-citrate-bile salt-sucrose agar media (Oxoid) were used for bacteriological examination of cloacal swabs from diseased hens.

Diagnostic strips:
The API 20 E strips (Bio Merieux):
The API 20 E strips containing 18 enzymatic reactions as well as motility, growth on MacConkey agar medium, oxidation-fermentation tests, cytochrome oxidase on filter paper, nitrate production and reduction to nitrogen gas were used for biochemical identification of the bacterial isolates.
Lactobacillus acidophilus (B.N.L TB 002, Microbiotich. USA) was used as competitive exclusion product against challenge with *Proteus mirabilis* experimentally inoculated through cloaca in layers and through shell in embryonated chicken eggs.

Experimental chickens and Embryonated chicken eggs:
A total of 40 healthy 37-weeks old Ross laying hens were bacteriologically examined by direct swabbing from the cloaca and proved to be free from potential pathogens were used for experimental infection through the cloaca. On the other hand, a total of one-hundred 11-days-old embryonated chicken eggs collected from the same flock were used for experimental infection through the egg-shell.

Experimental design:

1- Laying hens: were divided into four equal groups each of 10 hens.

The 1st group: was inoculated through the cloaca with 1 x 10^6 CFU/ml of *Proteus mirabilis* (**Youseif, 1985**).

The 2nd group: was inoculated with *Lactobacillus acidophilus* (1 x 10^6 CFU/ml) and *Proteus mirabilis* (1 x 10^6 CFU/ml) simultaneously through the cloaca.

The 3rd group: was inoculated with *Lactobacillus acidophilus* (1 x 10^6 CFU/ml) through the cloaca and kept for 6 hours then reinoculated with *Proteus mirabilis* with the same dose.

The 4th group: was swabbed with sterile saline and kept as a control group.

All groups were floor-reared on clean litter and observed daily for naked eye pathological lesions for a period of one month.

2- Embryonated chicken eggs:

One hundred 11-days-old embryonated chicken eggs were divided into four equal groups each comprising 25 eggs.

The 1st group: Eggs in this group were bathed for 15 minutes in sterile distilled water containing *Proteus mirabilis* with a concentration of 1x 10^6 CFU/ml and the water temperature was adjusted to be 37°C.

The 2nd group: were bathed for 15 minutes in sterile distilled water containing *Proteus mirabilis* as well as *Lactobacillus acidophilus* simultaneously with a concentration of 1 x 10^6 CFU/ml for each microorganism.

The 3rd group: Eggs in this group were firstly bathed in sterile water containing 1 x 10^6 CFU/ml of *Lactobacillus acidophilus* for 15 minutes and after 6 hours rebathed in sterile water containing 1 x 10^6 CFU/ml of *Proteus mirabilis* for 15 minutes.

The 4th group: Eggs in this group were bathed in sterile distilled water for 15 minutes and kept as a control group.

Eggs of all groups were incubated in egg incubators and examined daily for dead-in-shell embryos till hatching.

RESULTS

1-Bacteriological examination of cloacal swabs:

Twenty-two isolates of *Proteus mirabilis* were recovered in pure culture from the bacteriologically examined layers. On the other hand, 17 laying hens harboured *Proteus mirabilis* in association with other enteric microorganisms,

namely, *Enterobacter cloacae* (6 hens), *Citrobacter freundii* (5 hens), *Hafnia alvei* (4 hens) as well as *Morganella morganii* (2 hens), while 11 of the examined layers recover neither *Proteus mirabilis* in pure form nor in a mixed form with other enteric organisms.

2- Results of experimental infection of laying hens with *Proteus mirabilis* through the cloaca:

The 1st group:

This group was inoculated through the cloaca with *Proteus mirabilis* strain isolated in pure culture from laying hens with cloacal prolapse.

The results indicated that, the signs of inflammation were noticed 6 days post inoculation in 6 hens and 9 days post inoculation in two other hens. On the other hand, two layers were resistant without any sign of inflammation. Examination of the experimentally infected hens revealed that cloacal prolapse was obvious in 6 layers after 14 to 17 days post inoculation. Reisolation of *Proteus mirabilis* in pure culture from 7 layers was recorded.

The 2nd group:

This group was inoculated with *Lactobacillus acidophilus* and *Proteus mirabilis* simultaneously. The first sign of inflammation was recorded 8 days post inoculation in 5 layers and 10 days post inoculation in one hen.

Only 4 laying hens in this group were suffered from cloacal prolapse from 15 to 19 days of experimental infection.

Reisolation of *Proteus mirabilis* was recorded in two hens and reisolation of *Lactobacillus acidophilus* was recorded in four other laying hens.

The 3rd group:

This group was inoculated firstly with *Lactobacillus acidophilus* and 6 hours later reinoculated with *Proteus mirabilis*. Signs of inflammation were recorded in four layers 10 days post inoculation. Cloacal prolapse was obvious in two layers only 22 days post inoculation and till the end of the experiment.

Lactobacillus acidophilus was reisolated from 6 laying hens meanwhile, *Proteus mirabilis* was reisolated from one hen only in pure culture.

The 4th group:

Daily examination of this group revealed that there was no signs of inflammation or prolapse during the course of the experiment with regular laying.

3- Results of experimental infection with *Proteus mirabilis* through egg shell:

The 1st group:

Comprising 25 fertile eggs bathed in a suspension of *Proteus mirabilis* with a concentration of 1 x 10^6 CFU/ml. With daily observation of embryonated eggs,

the first embryonic death was recorded 3 days post infection in 13 eggs, and 24 hours later, in other 4 eggs. *Proteus mirabilis* was reisolated form all dead embryos as well as from the yolk sac of 3 hatched chicks.

The 2nd group:

This group was bathed in *Proteus mirabilis* as well as *Lactobacillus acidophilus* simultaneously. Dead embryo in shell was recorded 3 days after bathing in 7 fertile eggs followed by 3 eggs 2 days later and finally one fertile egg at the day of hatching. Isolation of *Proteus mirabilis* was recorded in 9 out of the 11 dead embryos as well as 5 hatched chicks, meanwhile, *Lactobacillus acidophilus* was isolated from two dead embryos as well as two hatched chicks.

The 3rd group:

This group was previously bathed in a suspension of *Lactobacillus acidophilus* and after 6 hours, rebathed in a suspension of *Proteus mirabilis*.

The first embryonic death was recorded four days post infection in 5 eggs. Three days later, there was an embryonic death in two other fertile eggs. At the day of hatching; there was 18 living chicks out of the 25 bathed fertile eggs. Isolation of *Proteus mirabilis* was recorded in a mixed form with *Lactobacillus acidophilus* in all dead embryos. Regarding hatched chicks, *Proteus mirabilis* was isolated from the yolk sac of three chicks and *Lactobacillus acidophilus* was recovered from four other chicks in pure form (Table 2).

The 4th group:

Comprising 25 fertile eggs bathed in warm distilled water (37ºC) for 15 minutes and kept as a control group. At the day of hatching there was 23 living chicks in this group.

Table (1): Results of experimental infection of layers through Cloaca.

Group No.	Experimental infection with	First sign of inflammatory response	cloacal prolapse		Reisolation rate			
					Proteus mirabilis		*Lactobacillus acidophilus*	
			No.	%	No.	%	No.	%
I (10 hens)	*Proteus mirabilis*	6 days post infection	6	60%	7	70%	0	0%
II (10 hens)	*Proteus mirabilis* + *Lactobacillus acidophilus* simultaneously	8 days post infection	4	40%	2	20%	4	40%
III (10 hens)	*Lactobacillus acidophilus* then *Proteus mirabilis* 6 hours later	10 days post infection	2	20%	1	10%	6	60%
IV (10 hens)	Sterile saline	-	-	-	-		-	-

Table (2): Results of experimental infection through egg shells.

Group No.	Experimental infection with	First embryonic death post infection	Embryonic death		Reisolation rate from							
					Dead embryos				Hatched chicks			
					Proteus mirabilis		*Lactobacillus acidophilus*		*Proteus mirabilis*		*Lactobacillus acidophilus*	
			No.	%	No.	%	No.	%	No.	%	No.	%
I (25 fertile eggs)	*Proteus mirabilis*	3 days	17	68.0	17	100	0	0	3	37.5	0	0
II (25 fertile eggs)	*Proteus mirabilis* + *Lactobacillus acidophilus* simultaneously	3 days	11	44.0	9	81.8	2	18.2	5	35.7	2	14.3
III (25 fertile eggs)	*Lactobacillus acidophilus* then *Proteus mirabilis* 6 hours later	4 days	7	28.0	7	100	7	100	3	16.7	4	22.2
IV (25 fertile eggs)	Distilled water	17 days	2	8.0	0		0		0		0	

DISCUSSION

Recognition, treatment or prevention of disease is of crucial importance and the subject of much investigation and research. A healthy bird with a stable microflora level is important as a barrier against colonization by potentially pathogenic microorganisms. The use of antibiotics in this respect has negative influences on the composition of microflora.

Forty-four percent of layers suffered from cloacal prolapse recovered *Proteus mirabilis* in pure culture. On the other hand, 34% of the examined hens with prolapse, revealed *Proteus mirabilis* in a mixed form, with other enteric organisms.

Successful experimental induction of cloacal prolapse in 60% of layers experimentally infected with *Proteus mirabilis* through cloaca lowered to be 40% by using *Proteus mirabilis* simultaneously with *Lactobacillus acidophilus* as a competitor and lowered again to be 20% only with the precedence of *Lactobacillus acidophilus* by 6 hours as well as delaying the inflammatory response, two and four days respectively, investigated the protective effect of *Lactobacillus acidophilus* as a colonization resistance factor.

Lactobacilli, which constitute the largest part of the aerobic microflora in the intestines due to production of lactic acid and lowering of the pH, result in less favourable growth conditions for several microorganisms. Lactobacilli are also able to produce bacteriocins which have a bacteriocidal or bacteriostatic effect towards many other bacteria. Lactocins formed by lactobacilli are acidophilin, lactolin, reuterin and acidolin. These compounds are responsible for the antibacterial effect against potential pathogens (**Mulder, 1991**).

Table (2) shows the results of experimental infection with *Proteus mirabilis* through egg shells. Embryonic death was 68% on using a pure culture of *Proteus mirabilis*, decreased to 44.0% by using a mixture of *Proteus mirabilis* and *Lactobacillus acidophilus* simultaneously and decreased again to be 28.0% when *Lactobacillus acidophilus* precedes *Proteus mirabilis*. **Al-Aboudi *et al.*, (1988)** studied the penetrating rate of *Proteus mirabilis* through egg shell and egg membranes and proved that the organism survived on the shell and in the yolk for 21 days. Also, **Abd-El-Galil *et al.*, (1995)** isolated Proteus species with a percentage of 22.6% of infertile eggs, meanwhile, **Lin *et al.*, (1996)** isolated Proteus species (8.3%) from dead-in-shell embryos. The physiological indigenous microflora has a key role in influencing the appropriate development of the mucosal immune system. It has been suggested that this effect of microflora may be medicated by the adhesion of the bacteria to surface epithelium (**De Simone *et al.*, 1993**).

Link-Amster *et al.*, (1994) when used *Lactobacillus acidophilus* as a competitive inhibitor to Salmonella, there was a significant increase in the titre of specific serum IgA in comparison to the control group.

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الملخص العربى

التنافس التزاحمى ضد ميكروب البروتيس ميرابلس المسبب لانقلاب قناة البيض ونفوق الأجنة فى الدجاج

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تم دراسة الدور الذي تلعبه ميكروبات البروتيس ميرابلس كمسبب لانقلاب قناة البيض في عدد خمسين من أمهات الدجاج المنتجة لكتاكيت التسمين (روس) وتم عزل الميكروب في صورة نقية من فتحة المجمع من ٢٢ دجاجة وعزل أيضا مع ميكروبات أخرى من ١٧ دجاجة بنسبة عزل ٤٤% ، ٣٤% على التوالي.

عند إجراء العدوى التجريبية باستخدام الميكروب المعزول فإنه قد حدث انقلاب قناة البيض بنسبة ٦٠% ، انخفضت هذه النسبة لتصبح ٤٠% عند استخدام ميكروب اللاكتوباسيلس أسيدفيلس كمنافس تزاحمي في ذات الوقت وانخفضت النسبة أيضا لتصبح ٢٠% عندما استخدمت اللاكتوباسيلس أسيدفيلس كمنافس تزاحمي قبل ميكروب البروتيس ميرابلس بست ساعات.

تم دراسة قدرة ميكروب البروتيس ميرابلس على اختراق قشرة البيض وإحداث نفوق في الأجنة من خلال إجراء عدوى تجريبية بالتغطيس. وقد أحدث الميكروب نسبة نفوق ٦٨% عند استخدامه بصورة نقية. انخفضت هذه النسبة إلى ٤٤% عند استخدام ميكروب اللاكتوباسيلس أسيدفيلس كمنافس تزاحمي في ذات الوقت ووصلت إلى ٢٨% عند إجراء التغطيس بميكروب اللاكتوباسيلس أسيدفيلس قبل استخدام ميكروب البروتيس ميرابلس بست ساعات.