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EFFECT OF USING *LACTOBACILLUS ACIDOPHILUS* ON *E.COLI* CAUSING EMBRYONIC DEATH AND LOW HATCHABILITY IN BALADY HATCHERIES AT DAKAHLIA GOVERNORATE

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ABSTRACT

A total 550 swabs were collected from balady hatcheries egg shell surface (100), infertile eggs (100), dead in shell embryos (200) and newly hatched chicks (150) for bacteriological examination. The following bacteria were isolated and identified: *E.coli, Proteus spp., Pseudomonas aeruginosa, Citrobacter spp., Enterobacter spp., Staphylococcus spp. and Streptococcus spp.* The most predominant isolated bacteria was *E.coli* (25.45%). Experimental infection for eggs were done by dipping in broth culture containing 1×10^6 CFU/ml of *E.coli* and in 3×10^8 CFU of *Lactobacillus acidophilus* for studying the effect of it in controlling *E.coli* infection and improvement of hatchability rate. The criteria used for judgment of therapeutic effect were mortality rate and rate of reisolation of *E.coli* from dead in shell embryos and newly hatched chicks. Dipping eggs in broth culture of *Lactobacillus acidophilus* then after 6 hours in broth culture of *E.coli* gave best results with low mortality rates and rate of reisolation than using them concurrently.

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INTRODUCTION

Bacterial infection of poultry is representing a worldwide important factor in term of their economic losses and public health. Hatchability and the rate of chick survival are one of the major determination factor of productivity in poultry. The hatchery is the greatest source for spread of diseases within the poultry industry. The problem usually starts with contaminated eggs which are incubated under ideal condition for microbiological reproduction. Numerous bacterial pathogens that contaminate hatcheries have been isolated from egg shell, egg content as well as from dead in shell embryos. They included: *Salmonella spp., E.coli, Klebsiella spp., Proteus spp. Staphylococcus aureus* and *Streptococcus* (Ibraheem and Abd El-Latif 1997; Walker *et al.*, 2002; Northcutt *et al.*, 2004; Kim *et al.*, 2007; Al-Khalaf *et al.*, 2010 and Kirunda *et al.*, 2010). Poultry bacterial pathogens are mainly controlled by using chemotherapeutic drugs. Unfortunately, the long term and extensive use of antibiotic for veterinary purpose may eventually result in selection for the survival of resistant microbial species (Aarestrup, 1999). Genes encoding for this resistance also can be transferred to other formerly susceptible bacteria, thereby causing a threat to both animal and human health (Montagne *et al.*, 2003). An alternative approach to therapeutic antibiotic is the using of probiotic which means "for life".

It has been defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). Two genera of bacteria are most reported as probiotic including lactic acid bacteria of genus *Lactobacillus* (Higgins *et al.*, 2008; Yegani and Krover, 2008; Sato *et al.*, 2009; Taheri *et al.*, 2009 and Lee *et al.*, 2010) and *bifodobacteria* (Willis *et al.*, 2010). The effects of some probiotic bacterial were reported, they include modification of the microbial composition and metabolic activity of the intestinal flora, inhibition of infective pathogens like *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* by competitive exclusion and enhancing the growth and development indexes in chickens (Reque *et al.*, 2000; Awad *et al.*, 2009; Higgin *et al.*, 2010). (Fuller, 1977) found that host-specific *Lactobacillus* strains were able to decrease *E.coli* in the crop and small intestine. (Watkin *et al.*, 1982) Similarly observed the competitive exclusion of pathogenic *E.coli* occurred in the gastrointestinal tract of gnotobiotic chicks dosed with *Lactobacillus acidophilus.*

Recent finding indicate that bacteria can be established in the intestine as the embryo develops to stage where the gastrointestinal tract differentiates and close, and the embryo starts ingestion of amniotic fluid (Klasing, 1998). Therefore, probiotic could be introduced into embryonating eggs and established itself as the chick's intestinal microbiota.

This study was designed to isolate and identify the bacterial pathogens that cause low hatchability rate in balady hatcheries at Dakahlia Governorate and Studying the effect of *Lactobacillus acidophilus* on controlling these pathogens and improving the hatchability rate.

MATERIALS AND METHODS

Specimens:
A total of 550 swabs were collected from balady hatcheries in Dakahlia Province. They include 100 egg shell surface, 100 infertile eggs (yolk), 200 from dead in shell embryos (liver, heart, yolk) and 150 from newly hatched chicks (liver, heart, yolk).

Media required:
Selective enrichment media: Nutrient broth, Selenite F broth and Heart infusion broth.

Plating solid media: Nutrient agar and blood agar medium.

Characterization media: McConkey' s agar, S.S agar, Baird Parker agar and Eosin Methylene Blue agar.

Biochemical media: Triple sugar iron medium, Christen's urea agar medium, Simmon's Citrate agar medium, Indol test medium, Methy red and Voges Proskauer test medium and Sugar fermentation medium containing 1 % of the following sugars. (Dlucitol, glucose, lactose, manitol and sucrose)

Experimental bacterial strains:-
E.coli: The isolated *E.coli* streptomycin resistant strain was prepared as described by (Glunder and Siegmann, 1989 and Bisgaard, 1995).

***Lactobacillus Acidophilus*:** It was obtained from Animal Health Research Institute, Dokki, Egypt.

Eggs: A Total of 520 eggs were obtained from Mansoura Poultry Company used for experimental infection.

Methods:-
Collection and treatment of samples:-
Under aseptic conditions swabs were taken from egg shell surface, yolk of infertile eggs. The surface of unhatched eggs was disinfected using 70% ethyl alcohol and flamed. The egg shell was broken and the unhatched embryo was removed with sterile forceps and put in sterile petri dish and opened to expose the internal organs. Swabs from yolk, liver and heart put in sterile test tubes containing nutrient broth.

Also Swabs were taken from liver, yolk and heart of newly hatched chicks by inserting bacteriological loops inside internal organs for cultivation, isolation and identification of microorganisms.

Bacterial isolation:-
Under strict aseptic precautions, the bacteriological samples were inoculated into nutrient and selenite F-broth and incubated aerobically at 37 °C for 24 – 48 hours and then streaked on nutrient agar, blood agar, MacConkey' s agar, S.S. agar, Baird Parker agar, EMB agar and the plates of these media were incubated at 37 °C for 24 hours, different colonies were picked up from selective media and subcultured on slope agar medium.

Identification of bacterial isolates: Morphological characterization and Gram's stain. The bacterial isolates were biochemically identified according to Finegold and Martin (1982).

Experimental infection:
This method was done according Zeinab *et al.* (2011). A total of 520 eggs were used. Twenty of them were examined to establish their freedom from *E.coli.* The remain 500 egg were divided into 5 groups. Each group contain 100 eggs (1, 2, 3, 4, 5) table (1).

Group (1): Eggs were infected by dipping in broth culture containing 1×10^6 CFU/ml of *E.coli* for 15 minutes.

Group (2): Eggs were infected by dipping in broth culture containing 1×10^6 CFU/ml of *E.coli* and 3×10^8 of *Lactobacillus acidophilus* for 15 minutes.

Group (3): Eggs were infected by dipping in broth culture containing 3×10^8 CFU/ml of *Lactobacillus acidophilus* for 15 minutes then after 6 hours dipped in broth culture containing 1×10^6 CFU/ ml of *E.coli* for 15 minutes.

Group (4): Eggs were infected by dipping in broth culture containing 3×10^8 CFU/ml of *Lactobacillus acidophilus* for 15 minute.

Group (5): Eggs were dipped in sterile nutrient broth as a control.

Eggs of all groups were incubated for 21 days at 37°C and humidity 60 – 70% with daily observation for embryo livability or mortality. Specimens including yolk sac, liver, heart of dead embryos were collected and cultured for bacteriological examination for *E.coli* re-isolation.

The newly hatched chicks from each group were killed and specimens from heart, liver and yolk were collected and cultured for bacteriological examination for *E.coli* reisolation.

RESULTS

Bacterial isolation:
Out of 550 samples (100 eggshell, 100 infertile eggs, 200 dead in shell embryos and 150 newly hatched chicks) 220 bacterial isolates were obtained (Table 2). The isolated bacteria were *E.coli* (56), *Proteus spp.* (43), *Pseudomonas Aeruginosa* (33), *Citrobacter spp.* (22), *Enterobacter spp.* (26), *Staphylococcus spp.* (20) and *Streptococcus spp.* (20). The most predominant isolated bacteria was *E.coli* (25.45%) of isolated bacteria.

Experimental infection:
The mortality rate of embryonating chicken eggs that infected with *E.coli* and *Lactobacillus acidophilus* is shown in table (3).The mortality rate was (52%) in group (1) that infected by dipping in broth culture of *E.coli* while it was (45%) and (35%) in group (2) and group (3) that infected by dipping in broth culture of *E.coli* and *Lactobacillus acidophilus.*

Table (4) reveals that the rate of reisolation of *E.coli* from dead in shell embryos was high in group (1) (86.53%) as compared with group (2) (75%) and group (3) (63.3%). Also the rate of reisolation of *E.coli* from newly hatched chicks in group (1) was high (83.3%) as compared with group (2) (74.5%) and group (3) (57.1%).

Table 1: Represents experimental infection design.

Group No.	*M.O	No. of dipping eggs	Time of dipping
1	*E. coli*	100	15 minutes
2	*E. coli* and *Lactobacillus acidophilus*	100	15 minutes
3	*Lactobacillus acidophilus*	100	15 minutes after 6 hr
	E. coli		15 minutes
4	*Lactobacillus acidophilus*	100	15 minutes
5	Control	100	15 minutes

M.O.: Microorganism

Table 2: Illustrates the results of bacterial isolation from egg shell surface, infertile eggs, dead in shell embryos and newly hatched chicks obtained from balady hatcheries in different areas of Dakahlia Governorate.

Recovery site	No. of samples	Total isolates	*E. coli*	*Proteus spp.*	*Pseudomonas aeruginosa*	*Enterobacter spp.*	*Citrobacter spp.*	*Staph spp.*	*Streptococcus spp.*
Egg shell	100	33	7	6	3	5	3	4	3
Infertile eggs	100	45	10	9	8	5	4	4	5
Dead in shell embryos	200	85	23	15	13	9	9	8	8
Newly hatched chick	150	57	16	13	9	7	6	4	4
Total	550	220	56	43	33	26	22	20	20
Prevalence of bacterial isolates	-	-	25.45%	19.55%	15%	11.82%	10%	9.09%	9.09%

*Percentage compared with total number of isolated bacteria

Table 3: Shows mortalities of embryonating chicken eggs after dipping in1 $\times 10^6$ *E. coli* and 3×10^8*Lactobacillus acidophilus.*

Group No.	No. of dipped eggs	**M.O.	Time of dipping	Embryo mortalities	
				No.	*%
1	100	*E. coli*	15 minutes	52	52%
2	100	*E.coli and Lactobacillus acidophilus*	15 minutes	45	45%
3	100	*Lactobacillus acidophilus 6 hr → E. coli*	15 minutes then after 6 hr, *E. coli* for 15 minutes	30	30%
4	100	*Lactobacillus acidophilus*	15 minutes	9	9%
5	100	Control	15 minutes	10	10%

*Percentage compared with total number of dipped eggs.
** M.O.: Microorganism

Table 4: Shows the results of reisolation of *E. coli* from dead in shell embryos and newly hatched chicks.

Group No.	Reisolation from dead in shell embryos			Reisolation from newly hatched chicks		
	No. of dead embryos	No. of positive cases	*%	No. of chicks	No. of positive cases	*%
1	52	45	86.53	48	40	83.3
2	40	30	75	55	41	74.5
3	30	19	63.3	70	40	57.1
4	9	-	-	91	-	-
5	10	-	-	90	-	-

group (1) = eggs dipped in 1×10^6 CFU/ml of *E. coli*
group (2) = eggs dipped in 1×10^6 CFU/ml of *E. coli* and 3×10^8 CFU of *Lactobacillus acidophilus*
group (3) = eggs dipped in 3×10^8 CFU/ml of *Lactobacillus acidophilus* and after 6 hr dipped in 1×10^6 CFU of *E. coli*
group (4) = eggs dipped in 3×10^6 CFU *Lactobacillus acidophilus*
group (5) = control
*Percentage compared with number of examined dead in shell embryos and newly hatched chicks

DISCUSSION

Hatchery industry considered one of the major steps in poultry production cycles, so must obtained good sanitation and low bacterial contamination that play an important role in lowering hatchability and decreasing the performance of hatched chicks.

In this study we obtained 220 bacterial isolates which were identified as *E.coli, Proteus spp., Pseudomonas aeruginosa, Citrobacter spp., Enterobacter spp., Staphylococcus spp.*, and *Streptococcus spp.* This result is agreement with (Al-Khalaf *et al.*, 2010 and Kirunda *et al.*, 2010) who could isolated the same bacterial strains from egg shell, infertile eggs, dead in shell embryos and newly hatched chicks.

E.coli is the most prevalent isolate in this study and it was isolated with prevalence of 25.45% of isolated bacteria. Transmission of avian pathogenic *E.coli* strains through contamination of incubated eggs or embryo infection has been described (Saif *et al.*, 2003).

(Cortes *et al.*, 2004) reported that the contamination of hatching eggs with *E.coli* is a major cause of yolk sac infection and the presence of germ on the egg shell increase the risk of omphalitis and shell mortalities.

(Al-Khalaf *et al.*, 2010; Azmy, 2010 and Kirunda *et al.*, 2010) isolated *E.coli* at a rate of 25.9%, 24% and 27.9% respectively. While (Shalaby and Abd El-Hamid, 1987 and Raji *et al.*, 2007) isolated *E.coli* from unhatched eggs in prevalence of 44% and 47% respectively. In addition, (Abd El- Galil *et al.*, 1984 and Azmy, 1996) isolated *E.coli* from newly hatched chicks at a rate of 15% and 62% respectively.

Proteus spp. was isolated with mean prevalence of (19.55%). (Fyrouz *et al.*, 2011) reported that *Proteus*

reduced hatchability rate into 58% in embryonated eggs. (Abd El-Latif, 1995 and Al-Khalaf *et al.*, 2010) isolated *Proteus* from dead in shell embryos with various prevalence. (Abd El- Gawad, 1989 and Azmy, 1996) isolated *Proteus* from newly hatched chicks.

Pseudomonas aeruginosa was isolated with mean prevalence of 15%. (Walker *et al.*, 2002) suggested that *Pseudomonas aeruginosa* can invade fertile eggs causing death of embryos and newly hatched chicks. (Karaman, 1980; Al-Khalaf *et al.*, 2010 and Azmy, 2010) isolated *Pseudomonas aeruginosa* from dead in shell embryos and infertile eggs at a rate of 17.2%, 17.11and 14.63% respectively. (Hebat-Allah, 2004) isolated *Pseudomonas aeruginosa* from dead in shell embryos and newly hatched chicks in percentage of 21% and 17.6% respectively.

Moreover, *Enterobacter spp.* and *Citrobacter* spp. were isolated at a rate of 11.82% and 10% respectively (Abd El-Latif, 1995; Husseina *et al.*, 2008 and Al-Khalaf *et al.*, 2010) isolated them with various prevalence.

Staphylococcus spp. and *Streptococcus spp.* were isolated with mean prevalence 9.09% for both of isolated bacteria. (Azmy, 2010) isolated *Staphylococcus spp.* and *Streptococcus spp.* from dead in shell embryos at prevalence of 7% and 3.7% respectively. *Staphylococcus aureus* was isolated from newly hatched chicks by (Abd El- Galil *et al.*, 1984 and Azmy, 1996) at prevalence of 14.7% and 15% respectively.

Following the discovery that chicks can hatch with bacteria already in their intestine the idea of establishing an intestinal community of healthy bacteria during incubation to make it more difficult for pathogens to establish themselves and cause disease. A suspension of probiotics can be administered in the hatchery as spray into eggs inside incubator, In ovo (Navid Hossein *et al.*, 2011) and dipping eggs in it (Fyrouz *et al.*, 2011).

In the experimental infection we dipped the eggs in broth culture containing 1×10^6 CFU/ml of *E.coli* which represent the top of isolated pathogens and in broth culture containing 3×10^8 CFU of *Lactobacillus acidophilus* as competitive exclusion product against challenge with *E.coli.*

The embryonic mortality rate was 52% in group (1) that infected with *E.coli* by dipping for 15 minutes as compared with 10% in control group (5). This indicated the responsibility of *E.coli* for lowering hatchability rate.

This finding assumed the possibility of transmission of *E.coli* via egg shell penetration. This result supporting the result of (Al-Bahry *et al.*, 2012). The mortality rates were reduced into 45% and 30% in group (2) and group (3) that dipped in broth culture of *E.coli* and *Lactobacillus acidophilus*. This result confirm concept of early administration of probiotic in hatchery may help gut maturation, improve gut health and so aid in the prevention of colonization by pathogens such as *Salmonella, E.coli* and *Campylobacter* (Helen Houghton, 2011).

The mortality rate was (45%) in group (2) that dipped in broth culture of *E.coli* and *Lactobacillus acidophilus* concurrently and the mortality rate was(38%) in group (3) that dipped first in broth culture of *Lactobacillus acidophilus* then after 6 hours dipped in broth culture of *E.coli.* This result agreement with (Fyrouz *et al.*, 2011) who reported that dipping of eggs in broth culture *of Lactobacillus acidophilus* then after 6 hours eggs dipped in broth culture of *Salmonella* or in broth culture of *Proteus* improved hatchability rate than dipping them concurrently.

Comparing mortality rate (9%) in group (4) that dipped in broth culture of Lactobacillus acidophilus to the mortality rate (10%) in group (5) control group we noticed slight improvement in hatchability rate. (Lalev *et al.*, 2011) noticed slight improvement of hatchability rate (91.29%) for eggs from broiler breeder feed on probiotics as compared to the control group (91.%).

The rate of reisolation of *E.coli* from group (1) that dipped in broth culture of *E.coli* was higher as compared to groups (2) and group (3) that dipped in broth culture of *E.coli* and *Lactobacillus acidophilus.* This result confirm the concept of metabolites produced by *Lactobacillus acidophilus* have bacteriostatic effects and antimicrobial agents for pathogenic bacteria such *as E.coli* and *Staphylococcus aureus* (Amin *et al.*, 2009; Gharaei – Fathabad and Eslamifar, 2011and Hassan *et al.*, 2013).

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تأثير استخدام اللاكتوباسيلس اسيدوفيلس على بعض أنواع البكتيريا المسببة لنفوق أجنة البيض وانخفاض معدل الفقس في المفرخات البلدية بمحافظة الدقهلية

غادة علام عبد الدايم ، شيرين سامي مصطفى

تم جمع ٥٥٠ مسحه من المفرخات البلدية (قشرة البيض (١٠٠) والبيض غير المخصب (١٠٠) والأجنة الميتة داخل القشرة (٢٠٠) والكتاكيت الفاقسة حديثًا (١٥٠) بغرض الفحص البكتريولوجى وقد تم عزل ٢٢٠ نوع من البكتيريا بنسب مختلفة وتم تصنيفها كالتالي الميكروب القولونى والبروتيس والسيدوموناس ايروجنوزا والسيتروباكر والأنتيروبكتر والمكور العنقودى والمكور السبحي وكان الميكروب القولونى هو أكثر الميكروبات عزلا (٢٥.٤٥%) بالنسبة للميكروبات المعزولة. وقد تم إجراء العدوى الصناعية عن طريق التغطيس في محلول يحتوى (على ١٠٦×١ CFU/ml) من الميكروب القولوني و(١٠٨×٣ CFU/ml) من بكتيريا اللاكتوباسيلس اسيدوفيلس لدراسة مدى تأثيرها في مقاومة العدوى بالميكروب القولوني وتحسين نسبة الفقس. وقد تم اتخاذ نسبة وفيات الأجنة وإعادة عزل الميكروب القولوني من الاجنه الميتة والكتاكيت الفاقسة كوسيلة للحكم على مدى تأثير اللاكتوباسيلس اسيدوفيلس. وقد أظهرت النتائج تحسن في نسبة الفقس في المجموعات التي تم تغطيسها في محلول اللاكتوباسيلس أسيدوفيلس وبعد٦ ساعات تم تغطيسها في محلول الميكروب القولونى عن تلك المجموعات التي تم تغطيسها في محلول به الميكروب القولونى واللاكتوباسيلس اسيدوفيليس معا أو الميكروب القولوني.