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# BACTERIAL AGENTS AFFECTING THE HATCHABILITY RATE OF TURKEY EMBRYOS

(With 4 Tables)

By

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العوامل البكتريولوجية المؤثرة على نسبة نقس أُجنة الديوك الرومي إحمد صادق ، خالد حسانين ، أحمد عبدالفتاح ، عادل سسليمان

يعتبر الانخفاض في معدل الفتص من أهم المشاكل الاقتصادية في مزارع انتاج الديوك الرومي والتي يمكن أن تعزى بصفة أساسية الى مصادر بكتريولوجية وأن عدوى الأجنة مسن خلال التلوث البكتريولوجي لقشرة البيض من أهم المؤثرات في احداث نسب عالية من عسدم فقص البيض كما أن ظاهرة الفاقد في فقص أجنة البيض قد بحثت في أبحاث كثيرروكان السبب الرئيسي لها هر التلوث البكتريولوجي للأجنة وأجريت هذه الدراسة لتحديد العوامل البكتريولوجية التي تمنع عملية فقص أجنة بيض الديوك الرومي مع معرفة المصادر التي يمكن أن يتأتى منها مثل هذا التلوث للبيض وقد أمكن عزل عديد من أصناف البكتريا من بين العينات التي تم أخذها من عينات بيض لم تفقص أجنته وكذلك من عينات الأثربة والزغب تلموجود في المفرخات وتوصى الدراسة بالعمل على تنظيف البيض المعدد للتفريخ بالغمل ثم بالتطهير وكذلك غرف التفريخ لما لهذه العملية من أهبية في تقليل نسبة عسدم الفقص لأجنة الديوك الرومي.

### SUMMARY

Decrease of hatchability rate represents till now an economic problem in turkey breeding farms, one of the most important factors is that of bacteriological origin.

Infected embryoes through bactericlogically contaminated egg- sheel was responsible for a high incidence of unhatched eggs.

The present investigation was carried out to point the beterial agents which would interfere with hatchability, as well as the sources and reservoir of egg-shell contamination.

Non-hatched eggs samples of fluffs and swabs from litters of the hatcheries were collected and examined bacteriologically.

E.coli, Proteus, Pseudomonas, Klebsiella, Mycoplasma, Salmonella, Staphylococci and Streptococci were isolated and identified, among which E.coli represents the main isolate.

Cleaning and disinfection of eggs for hatching as well as the hatcheries are recommended to decrease hatching losses.

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#### INTRODUCTION

Bacterial entery into the egg-shell has been a subject of considerable interest for a number of workers. Diseases control measures should start in the farm to minimize the risk of contamination of farm eggs.

Drastic drop in hatchability rate (40%) was recorded among the breeding turkey flocks at El-Wadi El-Gadid Turkey Farms; JUNGHER (1935) concluded that hatchability losses was attributed to non-specific bacterial infections. DHAWEDKAR and DHANESAR (1960) recovered Streptococci, Staphylococci, Micrococci, Aerobacter, Proteus, Salmonella, Pasteurella, Corynebacterium and Aspergillus niger from dead in shell embryos. REID et al. (1961) demonstrated that shell penetration by a field isolated strain of E.coli could markedly reduce hatchability; YAMAMOTO and ORTMAYER (1967) recorded that Mycoplasma meleagridis was prepetuated primarily.

SEVIOUR et al. (1972) reported that coliform organism, micrococci, Pseudomonas, Actinobacter, Bacillus species were the principle egg-shell contaminants penetrating the egg-shell during during incubation; EL-EBEEDY (1973) isolated M.meleagridis from dead-in-shell embryos. RHOADES (1981) reported that M.iowac was a common parasite of turkey known to reduce hatchability; SOLIMAN (1982) isolated both M.gallisepticum and M.meleagridis from dead-in-shell, pipped embryos and infertile eggs. NASHED (1981) detected the following organisms: Enterobacteriaceae (38.2%), micrococci (33.3%), Strepto-cocci (12.2%). Pseudomonas (11.1%) and Anthracoid (5.2%) out of 500 unhatched chicken eggs at Assiut province.

The present study was planned to cover the following points:

- 1- Detection of the microbial agents associated with hatchary losses from unhatched egg, hatcheries and litters (Turkey farm).
  - 2- Recommendations for the improvement of hatchability rate.

### MATERIAL and METHODS

- I- Samples of 360 dead turkey embryos of different ages were collected from Elwayd El-Gaded Turkey Production Farm, cleaned and sterilized externally to get rid of surface contamination.
- II- Hatcher/fluffs and dust were collected into sterile plastic begs using sterile wooden tonque blades.
- III- Samples of 2-3 gm. fine, dry floor litter from the upper 1-2 inches of litter were collected into sterile plastic bags.
- IV- Media: Nutrient broth, Selenite F-broth, Brain-heart infusion broth and agar, S-S agar, MacConkey agar, Blood-azide agar, Manitol salt agar and cetrimid-agar.
  - V- Characterization media and Reagents:

Triple sugar iron agar, urea agar base, Gelatine media, Kosor's citrate medium, glucose-phosphate medium. Kovac's, methyl-red, urea, rabbit plasma, phencl-red, L-arginine monohydrochloride, yeast-extract, Thallum acetate (ERNO & STIPKOVITIS, 1973 and CRUICKSHANK et al., 1975).

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#### VI- Antisera:

- 1- E.coli antisera were obtained from Behringwerk, Marbarg, W. Germany.
- 2- Polyvalent o, H and monovalent salmonella agglutination sera were obtained from Wellcome Research Laboratories, Beckenham, England.
- 3- Myocplasma antisera were obtained from intervet, Bexneer-Holland.

VII- Embryonating-Eggs: 300 embryonated turkey aggs (5-7 dys old) were supplied from general poultry Company (Bahtem).

## (1) Isolation and Identification of Microbial agents:

- 1- Bacterial agents of dead-in-shell embryos:
- a) About one-ml of yolk sac was taken bysterile pipette, inoculated in 10 ml Selenite F-broth incubated for 16 hours, followed by Subculturing on S-s agar, blood-azide media then incubated at 37°C for 24-48 hours;
- b) A loopful from yolk-sac was directly streaked on each of: MacConkey agar, blood-azide media, manitol-salt-agar and certimid agar. The plates were incubated at 37°C for 24-48 hours.

Suspected colonies were picked up for purification. For further identification the colonies were studied for its staining affinity, and biochemical reactions as well as serological typing in case of <u>E-coli</u> and Salmonella isolates according to EDWARDS & EWING (1972) and CRUICKSHANK et al. (1975);

c) For the isolation of Myocplasma sterile cotton swab was used to swab the yolk-membrane and to take a sample from yolk into 5 ml. of brain-heart infusion broth, the broth was incubated at 37°C for 3 days then subcultured on Brain-heart infusion agar plate. The plates incubated in moist candel jar under low oxygen tension at 37°C (SABRY, 1968). After 3 days incubation the plates were examined microscopically for appearance of characteristic fried egg colonies. The suspected colonies were subjected directly to the immunofluorescence test (IFT) after AL-AUBAIDI and FABRICANT (1971).

# (2) Bacterial agents of hatchary fluffs, dust and litter:

- One table spoonful of hatchary fluffs and dust as well as 2-3 gm, fine-dry floor litter, each was inoculated separtely into nutrient broth, selenite-F broth and brainheart infusion broth, 50 ml of each, the same procedure as previously mentioned was carried out for isolation and identification of bacterial agents.
- The Salmonella isolates were found to be serologically typed according to Modified Kauffman-white scheme for Salmonella and Arizona (1977).

### RESULTS

Results of isolation and identification of bacterial agents from dead-in-shell, litter, fluffs and dust are illustrated in table (1).

Results of serological typing of Mycoplasma (Table 2), and  $\underline{\text{E.}}$  coli isolates serotyping are given in Table (2) and Table (3) respectively.

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Salmonella isolates were identified as  $\underline{S}$ .  $\underline{gallinarum}$  pullorum,  $\underline{S}$ .  $\underline{typhimurium}$ ,  $\underline{S}$ .  $\underline{enteritidis}$  and  $\underline{S}$ .  $\underline{thompson}$  which were serologically typed in Tabe (4).

Eggs 360 No.	- 100
5* 5* No.	1
	2 310
4 3 2 NO	
	530
E. coli	
	100
Pseudomonas	
D %	3
4	
3 4 15 No	
A 15 No Proteus	
-	-
N N N N N N N N N N N N N N N N N N N	0.07
Klebsiella	1
No Klebsiells	
1 2 1 1 1	2
Mycoplasma	
4 W Mycoplasma	Z
The second secon	
Salmonella	No. of recovered isolates
7 %	cec
ù	0 7 6
2	erec
Staphylococ	ci   -
W at	901
3 2 No	ate
	0
o se streptococci	
and the second second	C7- 15.
124 Total 23	-
34.4	

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Table (2): Typing of 25 Mycoplasma isolates.

	No. of isolates	M. meleagr;	M.gallisep.	M. iowae	Untyped
Eggs	18	8	4	5	1
Fluffs &					
dust.	3	2	-	1	-
Litter	4	2	1	-	1
Total	25	12	5	6	. 2

Table(3): Serotyping of E.coli isolates:

			-			
				Eggs (22)	Fluffs,Dust	Litter (4)
0128:	K67	(812)		4	1	1
0126:	K71	(B16)		3	-	-
0119:	K69	(814)		5	2	1
<b>9</b> 78 :	K80	(B-)		3	-	1
055 :	K59	(B5)		3		-
0125:	k70	(B15)		4	a 14 -	1

Table (4): serotyping of 14 salmonella isolates.

Serotypes	Antigenic structure					
	Somatic"o"antigen	Flagellar "H"antiger				
		Phase I	Phase II			
S.gallinarum pullorum	1,9,12,2,3	-	- 1			
S.typhimurium	1,4 (5), 12	1	1,2			
S.enteritidis	1,2, 12	GM	1,7			
S. Thompson	6,7	κ .	1,5			

## DISCUSSION

The hatchability rate is the most essential measure for the reproductive efficiency of birds. It depends on many factors; bacterial contamination is considered to be the one having termendous effect on the survival of embryos and final hatchability rate.

The bacteriological examination of 360 dead-in-sheel embryos collected from turkey farms showing drastic drop of hatchability rate (40%) revealed the recovery of the following organisms: E. coli (22), Pseudomonas (13), Proteus (15), Kelbsiella (21), Myocplasma (18), Samonella (14), Streptococci (12) and Staphylococcus (9). Most of these organisms were previously isolated by DHAWEDKAR and DHANESAR (1960). ELEBEEDY (1973), VENUGOPOLAN et al. (1974), KARIM and ALI (1976). CHOWDHURY et al. (1976), FALADE (1977), ROKIA (1980), NASHED (1981), RHOADES (1981), KAMEL (1982), SOLIMAN (1982) and ASHGAN (1988).

18 isolates of Myocplasma were recovered in this study and identified seroligically by (IFA) test into M. meleagridis M. gallisepticum and for the first record in Egypt M. iowae. The same findings were previously recorded by YAMAMOTO and ORTMAYER (1967), EL-EBEEDY (1973), RHOADES (1981), LIN and KLEVEN (1982) and SOLIMAN (1982).

Serological typing of 14 strains of Salmonella revealed the isolation of <u>S. gallina-rum</u> - pullorum, <u>S. typhimurum</u>, <u>S. entritidis</u> and <u>S. thompson</u>; these results are parallel with those recorded by CHOWDHURY et al. (1976), EL-SAWY (1976), EL-TAHER (1977); SHAHATA (1979) and ROKIA (1980).

 $\underline{\text{E. coli}}$  isolates were serotyped (Table 3), REID  $\underline{\text{et}}$  al. (1961) and SAVOV (1966) recorded that between 0.5 to 60% of examined egg samples laid by normal nens were contaminated by  $\underline{\text{E. coli.}}$  Most of the isolated serotypes in this study was previously recovered by KAMEL (1982).

Salmoneila, E. coli, Klebsiella, Pseudomonas, Proteous, Staph, aureus, Strept, faecalis and Myocoplasma were recovered from hatcheries and litter samples. These results are in agreement with those reported by PLESSER et al. (1975) who recovered Klebsiella from hatcheries containing unhatched eggs. BESIDES, BRUCE and johnson (1978) recovered members of Enterobacteriaceae, Micrococci, Streptococci, Staphylococci and Pseudomonas from hatcheries in which the eggs failed to hatch.

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