BACTERIAL AGENTS AFFECTING THE HATCHABILITY RATE OF TURKEY EMBRYOS
(With 4 Tables)

By

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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 embryos through bacteriologically contaminated egg-shell was responsible for a high incidence of unhatched eggs.

The present investigation was carried out to point the bacterial 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 entry 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; JUNGER (1995) 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 meleagris 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. meleagris from dead-in-shell embryos. RHOADES (1981) reported that M. jwowac was a common parasite of turkey known to reduce hatchability; SOLIMAN (1992) isolated both M. gallisepticum and M. meleagris from dead-in-shell, pipped embryos and infertile eggs. NASHED (1981) detected the following organisms: Enterobacteriaceae (38.2%), micrococci (33.3%), Streptococci (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 hatchery 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 bags using sterile wooden tone 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, phenol-red, L-arginine monohydrorchloride, 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- Mycoplasma 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 by sterile 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 cerimid 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 E. coli and Salmonella isolates according to Edwards & Ewing (1972) and Cruickshank et al. (1975);
   c) For the isolation of Mycoplasma 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 (Sarky, 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 hatchery fluffs, dust and litter:

- One table spoonful of hatchery fluffs and dust as well as 2-3 gm, fine-dry floor litter, each was inoculated separately into nutrient broth, selenite-F broth and brain-heart 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 E. coli isolates serotyping are given in Table (2) and Table (3) respectively.
Salmonella isolates were identified as *S. gallinarum* pullorum, *S. typhimurium*, *S. enteritidis* and *S. thompson* which were serologically typed in Table (4).

<table>
<thead>
<tr>
<th>Litter</th>
<th>FluFF²</th>
<th>Egg²</th>
<th>Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>5*</td>
<td>4</td>
<td>3</td>
<td>No.</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>E. coli</td>
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<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>Pseudomonas</td>
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<td>3</td>
<td>4</td>
<td>4</td>
<td>Proteus</td>
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<td>2</td>
<td>2</td>
<td>4</td>
<td>Klebsiella</td>
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<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>Mycoplasma</td>
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<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>Salmonella</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>Staphylococci</td>
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<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Streptococci</td>
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</table>

*Pooled samples from five hatcheries.*

<table>
<thead>
<tr>
<th>Litter</th>
<th>No.</th>
<th>E. coli</th>
<th>Pseudomonas</th>
<th>Proteus</th>
<th>Klebsiella</th>
<th>Mycoplasma</th>
<th>Salmonella</th>
<th>Staphylococci</th>
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<td></td>
<td></td>
<td>124</td>
<td>34.4</td>
<td>126.9</td>
<td>14.5</td>
<td>20.3</td>
<td>9.3</td>
<td>12.9</td>
<td>9.7</td>
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Table (1): Isolation of different bacterial agents from poultry samples.

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Table (2): Typing of 25 Mycoplasma isolates.

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>M. meleagr</th>
<th>M. gallisept</th>
<th>M. iowae</th>
<th>Untyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>18</td>
<td>8</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Fluffs &amp; dust</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Litter</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25</strong></td>
<td><strong>12</strong></td>
<td><strong>5</strong></td>
<td><strong>6</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Eggs (22)</th>
<th>Fluffs,Dust (3)</th>
<th>Litter (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0128</td>
<td>K67 (B12)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>0126</td>
<td>K71 (B16)</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>0119</td>
<td>K69 (B14)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>078</td>
<td>K80 (B-)</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>055</td>
<td>K59 (B5)</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>0125</td>
<td>k70 (B15)</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (4): Serotyping of 14 salmonella isolates.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Antigenic structure</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Somatic &quot;O&quot; antigen</td>
<td>Phase I</td>
<td>Phase II</td>
<td></td>
</tr>
</tbody>
</table>

- S. gallinarum pullorum: 1, 9, 12, 2, 3
- S. typhimurium: 1, 4 (5), 12
- S. enteritidis: 1, 2, 12
- S. Thompson: 6, 7

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 tremendous effect on the survival of embryos and final hatchability rate.

The bacteriological examination of 360 dead-in-shell 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), Klebsiella (21), Mycoplasma (18), Salmonella (14), Streptococci (12) and Staphylococcus (9). Most of these organisms were previously isolated by DHAWEKAR and DHANESAR (1960), EL-EBEEDY (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 Mycoplasma were recovered in this study and identified serologically 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 S. gallinarum - pullorum, S. typhimurium, S. enteritidis and S. thompson; these results are parallel with those recorded by CHOWDHURY et al. (1976), EL-SAWY (1976), EL-TAHER (1977); SHAHATA (1979) and ROKIA (1980).

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

Salmonella, E. coli, Klebsiella, Pseudomonas, Proteus, Staph. aureus, Strept. faecalis and Mycoplasma 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, Micrococcii, Streptococci, Staphylococci and Pseudomonas from hatcheries in which the eggs failed to hatch.

REFERENCES


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