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Bacteriological studies on dead in shell chicken embryos

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ABSTRACT

A total of 267 samples of fertile eggs containing dead-in-shell chicken embryos were collected from different hatcheries located in Ismailia Governorate, Egypt. The percentage of positive samples was (58.8%). *E.coli* isolates were isolated with an incidence of (10.8%), *Salmonella* spp. (4.4%). They were serologically identified. *Pseudomonas* spp. also were isolated (15.9%), *Klebsiella spp*. (10.8%), *Proteus* spp. (18.4%), *Staphylococci* spp.(20.3%), *Streptococci* spp. (12.7%) and *B. anthracoid* (6.3%). These isolates were biochemically identified. The penetration ability of *Salmonella enterica* serotype *enteritidis* to the intact and cracked egg shells was studied. The results revealed that it could penetrate the intact egg shells after 72 hours but it could penetrate the cracked ones after only 24 hours from artificial contamination.

KEYWORDS

Bacteriological studies;
Dead in shell;
Chickens.

INTRODUCTION

It is known that the healthy day-old chick is considered the nucleus of poultry industry in Egypt. Hatching in Egypt is done either by old (balady) hatcheries or modern ones, the balady ones still represent an important and continuous source of day old chick. Many problems involved the balady hatching process. Such construction of buildings, the lack or even complete absence of hygienic measures, unsanitary conditions of egg collection, unsuitable storage and hatching process are the main causes of bacterial infections such infections lead to early embryonic death, lowering hatchability and reduction of the fertility of hens egg[1].

Several microorganisms were incriminated as a cause of embryonic deaths and lowering hatchability. Bacterial contamination appeared to be one of the infectious agents having tremendous effect on the survival of embryos and final hatchability rate[45].

Microorganisms may enter eggs by two routes. The first route involves invasion from the exterior via the shell[47], while the second route is by trans-ovarian infection during the development of the egg[26].

The purpose of this study is to evaluate, in the first step, the prevalence of microbial agents in both native and foreign breeds causing early embryonic deaths of fertile hen eggs in Ismailia Governorate. In the second, the penetration ability of *Salmonella enterica* serotype *enteritidis* to egg shell into egg contents.

MATERIAL AND METHODS

Collected samples

A. A total of 267 fertile chicken eggs were collected

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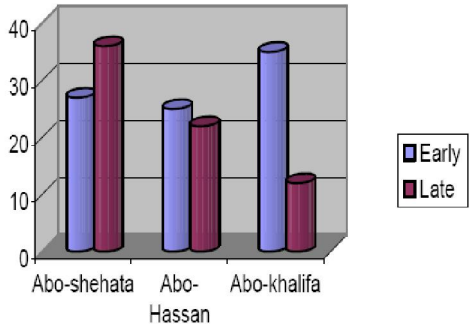

Figure 1 : Results of bacterial isolates from dead-in-shell chicken embryos at different localities

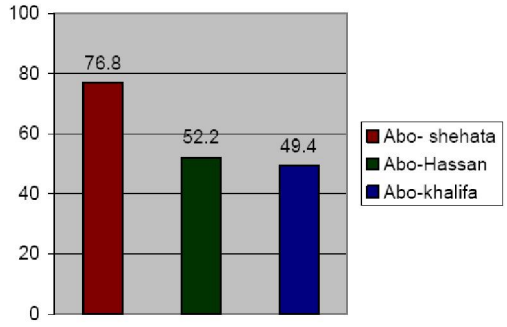

Figure 2 : Percentage of positive bacterial isolates at different localities

from native and foreign breeds under complete aseptic conditions and transported to the Animal Health Research Institute, Ismailia branch with minimum of delay (TABLE 1).

B. Eggs for experiment: Fifty fresh fertile eggs were purchased from native breeder flocks. These eggs were freshly laid and free from cracks or deformities. Random samples from these eggs were bacteriologically examined and proved to be free from *S. enteritidis* contamination.

C. *Salmonella enterica* serotype enteritidis: Strain of *S. enteritidis* (previously isolated from poultry by Animal Health Research Institute, Dokki) was used in the experiment.

Bacteriological examinations

(1) Bacterial isolation

A total of 267 samples of dead in shell chicken embryos were randomly collected from native and foreign breeds from farms of Ismailia Governorate. These samples were subjected to bacteriological examination to distinguish the possible bacterial causative agent.

All samples were macroscopically examined. Eggs with cracks and those embryos that pipped the shell but failed to hatch were discarded to minimize the incidence of external contamination. The surface of each egg was disinfected with tincture iodine then with an aid of sterile scissor a sufficient area around the air sac was removed and the egg content was drained into sterile Petri dish. Under strict aseptic precautions a loopful from yolk only in early embryonic deaths and yolk, liver, heart blood in late embryonic deaths were inoculated into nutrient broth and selenite F. broth as enrichment media, aerobically incubated at 37°C for 18-20 h. Sub culturing was carried out onto the following, media, Nutrient agar, blood agar, Baird parker media; MaConkey's but S.S. agar or XLD were cultured from selenite F broth and Enterococci selective differential media (ESD). Subculturing onto Pseudomonas selective agar medium from nutrient agar but subculturing onto EMB medium from MaConkey's.

Growth at 42°C or 4° C for differentiation between *Ps.aeruginosa* (which grows at 42°C) and *Ps.fluorescens* (which grows at 4°C). Also solubility in chloroform and water is examined as *Ps.aeruginosa* soluble in both chloroform and water while *Ps.fluorescens* soluble in water only.

(2) Bacterial identification

a. Morphological identification: Direct bacteriological smears were made from separate colonies and stained with Gram's stain for studying their shape, arrangement, ends and their staining affinity to G stain. The colonial appearance was also studied to investigate their structure, surface, edges, and color.

b. Biochemical identification: The isolated bacterial agents were subjected to different biochemical tests according to ref.[33]. Single colony of suspected bacteria was preserved into semisolid agar medium until used.

(3) Serotyping

The slide agglutination technique was performed. Isolated strains proofed to be either *E.coli* or *Salmonella* were serologically typed for somatic 'O' antigen using 8 polyvalent and 43 corresponding monovalent *E.coli* antisera "DENKA SEIKEN, Japan"according to ref.[24].

(4) Experimental infection

This experiment was planned to investigate the penetration ability of *S.enteritidis* through intact and cracked egg shells.

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TABLE 1 : Number, locality and breed of examined chicken embryos

Locality	Breed	No. of examined embryos		Total
		Early dead	Late dead	
Abo-Shehata	Native	35	47	82
Abo-Hassan		47	43	90
Abo-Khalifa	Foreign (Hubbard)	70	25	95
Total		152	115	267

TABLE 2 : Results of bacterial isolates from dead- in – shell chicken embryos at different localities

Breed	Locality	Stage	No of examined eggs	Positive bacterial isolation	
				No	%
Native	Abo-Shehata	Early	35	28	80
		Late	47	35	74.4
	Abo-Hassan	Early	47	26	55.3
		Late	43	21	48.8
Foreign (Hubbard)	Abo-khalifa	Early	70	35	50
		Late	25	12	48
Total			267	157	58.8

EXPERIMENTAL

A total of 50 fresh fertile eggs used to test the penetration of *S.enteritidis* of eggs shell. (According to 9).

The eggs were sterilized by 70% ethyl alcohol; 5 eggs were taken as random samples and examined for *S.enteritidis* and bacterial contaminants.

10 eggs were taken as control and manipulated the same as the tested eggs in all steps without contamination.

The *S.enteritidis* 24h peptone culture was done and the tested eggs[35] were soaked in it for 10 min. Then divided into groups each of 5 eggs (2 cracked eggs and 3 intact eggs in each group).

The contaminated eggs were examined every 24h for reisolation of *S.enteritidis*, under complete aseptic condition from each egg white, yolk and shell separately.

RESULTS

Collected samples

A total of 267 eggs were collected randomly from different hatcheries distributed in various localities in Ismailia Governorate.

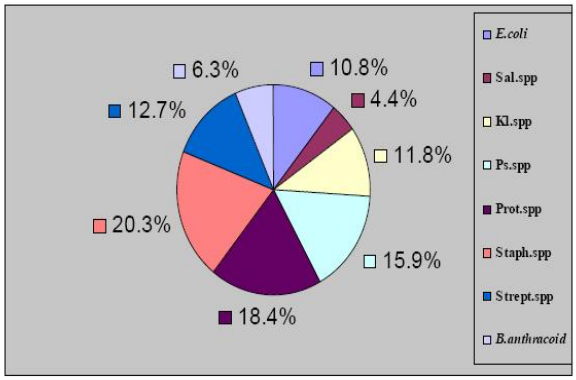

Figure 3 : Total percentage of bacterial isolates

TABLE 3 : Percentage of positive bacterial isolates at different localities

Locality	No. of examined eggs	Positive bacterial isolation	
		No	%
Abo-Shehata	82	63	76.8
Abo-Hassan	90	47	52.2
Abo-Khalifa	95	47	49.4
Total	267	157	

These samples were examined bacteriologicaly at different embryonic phases (early and late dead).

The results of bacteriological examination of all examined chicken embryos revealed 58.8% positive bacterial isolation. The rate of bacterial contamination was 63.9% in native breeds (balady hatcheries) (65.85% in early dead embryos and 62.2% in late ones), while it was 49.4% in foreign breeds (50% in early dead embryos and 48% in late one) as shown in (TABLE 2) (Figure 1).

The bacteriological examination proved that 267 samples gave 157 positive bacterial findings (58.8%) as 63 out of them from Abo-Shehata (76.8%), 47 from Abo-Hassan (52.2%) and 47 from Abo-Khalifa (49.4%) as shown in (TABLE 3 and Figure 2).

Identification of bacterial isolates from dead-in shell embryos

The examination of early dead chicken embryos (native breeds) revealed high incidence of *Staphylococcus* spp. (20.3%) followed by *Proteus* spp. (18.4%), *Pseudomonas* spp. (16.6%), *E.coli* (11.11 %), *Klebsiella* spp., *Streptococcus* spp., and *B.anthracoid* were found at same percentage (9.2%) for each and *Salmonella* spp. (5.5%).While those of late dead embryos revealed that the *Staphylococcus spp*. were

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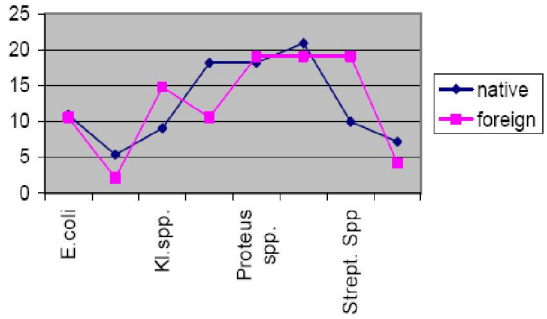

Figure 4 : Prevalence of bacterial isolates from native and foreign breeds

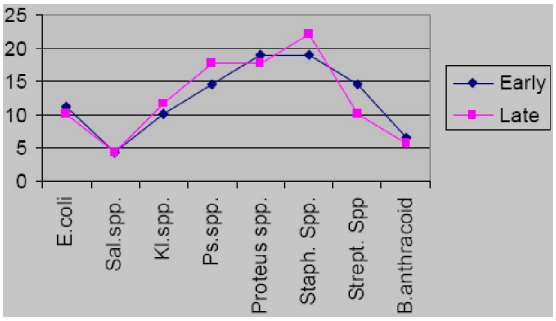

Figure 5 : Prevalence of bacterial isolates from early and late dead embryos

TABLE 4 : Prevalence of bacterial isolates among early and late dead-in-shell embryos

Breed	samples	No	Bacterial isolates		*E.coli*		*S.spp.*		*Kl.spp.*		*Ps.spp.*		*Pr.spp.*		*Staph.spp.*		*Strept. Spp.*		*B.anthracoid*	
	Type	82	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Native	Early	90	54	65.85	6	11.11	3	5.5	5	9.2	9	16.61	10	18.5	11	20.3	5	9.2	5	9.2
	Late	70	56	62.2	6	10.6	3	5.3	5	8.9	11	19.6	10	17.8	12	21.4	6	10.6	3	5.3
Foreign	Early	25	35	50	4	11.4	1	2.8	4	11.4	4	11.4	7	20	6	17.1	8	22.8	1	2.8
	Late	267	12	48	1	8.3	-	-	3	25	1	8.3	2	16.6	3	25	1	8.3	1	8.3
Total		No	157	58.8	17	10.8	7	4.4	17	10.8	25	15.9	29	18.4	32	20.3	20	12.7	10	6.3

the most prominent bacterial isolates with incidence of (21.4%), followed by *Pseudomonas spp.* (19.6%) then *Proteus spp.* (17.8%), *Streptococcus spp.* and *E.coli* (10.6%) for each of them, *Klebsiella* spp. (8.9%) and both of *Salmonella* spp. and *B.anthracoid* had (5.3%) for each (TABLE 4, 5 & 6 and Figure 3, 4 & 5).

The examination of early dead embryos (foreign breeds) revealed that *Streptococcus* spp. (22.8%), *Proteus* spp. (20%), *Staphylococcus* spp. (17.1%), each of *E.coli*, *Klebsiella* spp. and *Pseudomonas* spp. (11.4%) for each and *Salmonella* spp. and *B.anthracoid* have the same percentage for each (2.8%). While those of late dead embryos revealed that *Klebsiella* spp. and *Staphylococcus* spp. were (25%), followed by *Proteus spp.* (16.6%) and each of *E.coli*, *Pseudomonas* spp., *Streptococcal* spp. and *B. anthracoid* were (8.3%) (TABLE 4-6, Figure 4 & 5).

Results of bacterial spp. isolated from dead-in-shell embryos

The isolated *Klebsiella* spp. comprised of *Kl.pneumoniae* (3.1%)-of the total positive bacterial isolates- and *Kl.ozaenae* (7.6%). But the isolated *Pseudomonas* spp. comprised of *Ps.aeruginosa* (11.4%) and *Ps.fluorescens* (4.4%). While *Proteus* spp. were *Pr.vulgaris* (7.006%) and *Pr.mirabilis* (11.4%). On the other hand *Staphylococcus* spp. were *Staph.aureus* (4.4%) and *Staph.epidermidis* (15.9%). Finally *Streptococcus* spp. was *Strept.faecalis* (12.7%) (TABLE 5).

(a) Results of *E.coli* serogrouping

The serogrouping of the isolated *E.coli* strains were O6[2], O27[1], O44[1], O125[1], O126[1], and O164[1] and 10 untyped strains.

(b) Results of *Salmonella* serotyping

The serotyping of isolated *Salmonella* strains were *S.enteritidis*[1], *S.infantis*[1], *S.arizonae*[2], *S.pullorum-gallinarum*[1] and *S.cerro*[2] as shown in (TABLE 8).

(c) The penetration ability of *S.enteritidis* to the egg shells

The tested *S.enteritidis* could penetrate the intact egg shells and was reisolated from egg contents (egg white and egg yolk) after 72hr from artificial contamination but could penetrate the cracked ones after 24hr only. Control groups gave negative results.

DISCUSSION

Great attention was paid towards poultry production during the last two decades. Chicken meat is considered the cheapest animal protein to satisfy the increasing population demands. Problems associated with hatching eggs have great economic importance particularly those of microbial origin[29].

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TABLE 5 : Results of bacterial spp. isolated from dead-in-shell embryos

Bacterial isolates	Native		Foreign		Total	
	Early	Late	Early	Late	No.	%
Kl.pneumoniae	1	2	1	1	5	3.1
Kl.ozaenae	4	3	3	2	12	7.6
Ps.aeruginosa	7	7	3	1	18	11.4
Ps.fluorescens	2	4	1	-	7	4.4
Pr.vulgaris	2	3	5	1	11	7.006
Pr.mirabilis	8	7	2	1	18	11.4
Staph.aureus	2	2	2	1	7	4.4
Staph.epidermidis	9	10	4	2	25	15.9
Strept.faecalis	5	6	8	1	20	12.7

% was calculated according to the total No of bacterial isolates

TABLE 6 : Prevalence of bacterial isolates from 172 native and 95 foreign breeds

Bacterial Isolates	Native		Foreign		Total
	No.	%	No.	%	
E.coli	12	10.9	5	10.6	17
S.spp.	6	5.4	1	2.1	7
Kl.spp.	10	9.09	7	14.8	17
Ps.spp.	20	18.18	5	10.6	25
Proteus spp.	20	18.18	9	19.1	29
Staph. Spp.	23	20.9	9	19.1	32
Strept. Spp	11	10	9	19.1	20
B.anthracoid	8	7.2	2	4.2	10
Total isolates	110		47		157

% was calculated according to the total No of bacterial isolates

Hatchability is the most essential measure for the reproductive efficiency of domestic birds, depending on several factors either environmental, management or due to infectious agents. Bacterial contamination appeared to be one of the infectious agents having tremendous effect on the survival of embryos and final hatchability rate[45,20].

The present study was planned to distinguish the possible bacterial agents which may be incriminated in lowering hatchability by causing dead-in-shell embryos.

The bacteriological examination of early and late dead-in-shell chicken embryos of both native and foreign breeds revealed 58.8% positive bacterial isolation. Similar result (60%) was reported by ref.[28].

The rate of bacterial contamination in late dead embryos was 62.2% and 48% in native and foreign breeds; respectively (TABLE 4). This result was agreed with ref.[28,29] as they reported 60% and 35.47%; respectively.

In the present study 48% positive bacterial isolation was reported in late dead embryos (foreign breed). System of hatching, egg management and hygienic measures adopted in parent flocks may influence the rate of bacterial contamination[49].

Among total bacterial isolates (as shown in TABLE 4, 5 & 6), *E.coli* prevalence was 11.11% in early dead embryos (native breed). Nearly similar result 13.04% was reported by ref.[29] in early embryos deaths. Higher prevalence 17.5%, 19.4% and 21.9% were reported by ref.[1,2,14]; respectively in early embryonic deaths; while in late embryonic deaths *E.coli* prevalence was 10.7%. Nearly similar incidence 13.4% was proved by ref.[29]. Lower percentage 6.3% was recorded by ref.[20]. Higher incidence was reported by ref.[8,19,22,41] 19.9%, 20%, 44.5% and 39.5%; respectively.

In case of early dead embryos (foreign breed) *E.coli* prevalence was 11.4%. This result was in agreement with ref.[14] who reported 15%. While in late dead embryos in the present investigation showed *E.coli* prevalence was 8.3%. Higher prevalence was proved by ref.[4,29,30], in a percentage of 24.7%, 25% and 25.6%; respectively. These findings were in agreement with those of ref.[35] who also isolated *E.coli* predominantly from dead-in-shell embryos, although the percentage of *E.coli* isolates varied between different authors. The variation in the percentage of *E.coli* isolates may be partly related to the prophylactic and therapeutic use of certain antibiotics, vaccination against respiratory viruses, and improved hatchery sanitation.

With regard to *Salmonella* spp. the represented data showed that its incidence was 5.5% in early embryonic deaths (native breed). Similar prevalence was 6% and 6.3% reported by ref.[1,2]. Lower incidence 4% was proved by ref.[14]. While the prevalence in late embryonic deaths was 5.3%. Lower incidence of 3.3% and 3.2% was recorded by ref.[19,41].

On the other hand *Salmonella* spp. percentage of early embryonic deaths (foreign breeds) was 2.8%. This result was confirmed by ref.[14] who reported 2% incidence of *Salmonella* spp. in Hubbard breed in early dead embryos. Moreover[18] isolated *Salmonella* in a percentage of 0.6% from late dead embryos.

Salmonella spp. prevalence of late dead embryos (foreign breed) in the present study was negative. This result agreed with those reported by ref.[29,30].

The prevalence of *Klebsiella* spp. was 9.2% in early

TABLE 7 : Prevalence of bacterial isolates from early 152 and 115 late dead embryos

Bacterial Isolates	Early		Late		Total
	No.	%	No.	%	
E.coli	10	11.2	7	10.2	17
S.spp.	4	4.4	3	4.4	7
Kl.spp.	9	10.1	8	11.7	17
Ps.spp.	13	14.6	12	17.6	25
Proteus spp.	17	19.1	12	17.6	29
Staph. Spp.	17	19.1	15	22.05	32
Strept. Spp	13	14.6	7	10.2	20
B.anthracoid	6	6.7	4	5.8	10
Total isolates	89		68		157

% was calculated according to the total No of bacterial isolates

dead embryos (native breed). This result was agreed with ref.[1] who reported 11.5%. But this result was differing from those of ref.[13,19] who recorded 3.2%, and 12.3%; respectively. While *Klebsiella* spp. incidence of late dead embryos was 8.9%. This result was confirmed by ref.[12,29] who reported 9.3% and 8.7%; respectively.

On the other hand the prevalence of *Klebsiella* spp. of early dead embryos (foreign breed) was 11.4%. This result in accordance with ref.[30] who recorded 14.13%. While in late dead embryos was 25%. This result was in agreement with ref.[29] who reported 28.3%.

The prevalence of *Kl.pneumoniae* in this study was 3.1%. This result was lower than 5.7% which was recorded by ref.[37]. But higher incidence 7.9% was recorded by ref.[39]. While *Kl.ozaenae* was 7.6%.this result was confirmed by ref.[41] who recorded 7.1%.

Regarding of the prevalence of *Pseudomonas* spp. was 16.6% in early dead embryos (native breed). Although somewhat higher percentage of *Pseudomonas* spp. 18.3% was reported by ref.[2]. But lower percentage 11.1% was reported by ref.[1]. While in late dead embryos was 19.6%. This percentage nearly similar with 11% and 11.6% which proved by ref.[28,29]; respectively.

Pseudomonas spp. prevalence of early dead embryos (foreign breed) was 11.4% in the present investigation. Lower incidence 2% was reported by Enany et al., (1989). While in late dead embryos was 8.3%.This result was confirmed by ref.[30] who reported 9.78%. Higher incidence 20.3% was reported by ref.[29].

Regarding to *Ps.aeruginosa* prevalence in this study was 11.4%.this result was agreed with ref.[1,28,37] who recorded 11%, 10.7% and 10.4%; respectively. While the prevalence of *Ps.fluorescens* in this study was 4.4%. This result was confirmed by ref.[19] who reported 3.4%.

The prevalence of *Proteus* spp. was 18.5% in early dead embryos (native breed). Although somewhat lower incidence 15.5% and 16.6% was recorded by ref.[1,14]; respectively. While in late dead embryos was 17.8%. The obtained result was in accordance with ref.[20] who reported 16.9%. Some what higher incidence 24.6% was proved by ref.[29]. Lower incidence (14.1%) was proved by ref.[37].

The incidence of isolation of *Proteus* spp. from early dead embryos (foreign breed) in the present study was 20%, while ref.[14] reported a prevalence of 17% from early dead embryos in Hubbard breed. While in late dead embryos was 16.6%. This result was differed from those of ref.[29,30] who recorded 7.6% and 5.1%; respectively.

The difference in the rate of isolation may be attributed to the heavy contamination of the eggs after lying as well as improper handling and storage of the hatching eggs.

On the other hand; *Pr.vulgaris* percentage in this study was 7.006%. This result was confirmed by ref.[19,30] who recorded 7.60% and 8.20%; respectively. But higher incidence 15.5% was recorded by ref.[14]. While *Pr.mirabilis* prevalence was 11.4%. This result was agreed with ref.[8,22] who recorded 11.8% and 12.54%; respectively.

Among total bacterial isolates, *Staphylococcus* spp. was 20.3% in early dead embryos (native breed). This result nearly similar to those of ref.[29] who reported 17.4%. Some what higher results were recorded by ref.[1,2] who reported 15.2% and 14.6%; respectively. While in late dead embryos was 21.4%. This result was confirmed by ref.[13] who isolated *Staphylococcus spp.* in a percentage of 20.17% in commercial eggs. Some what lower findings 11.8% and 14.5% were reported by ref.[29,37].

Concerning early dead embryos (foreign breed) was 17.1%. This result was differed from those of ref.[14] who recorded 2%. While in late dead embryos the prevalence of *Staphylococcus* spp. was 25%. This result was agreed with the record of 27.17% which reported by ref.[30]. Lower incidence 12.8% was recorded by ref.[29].

The prevalence of *Staph.aureus* in this work was 4.4%. This result was confirmed by ref.[19] who reported

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TABLE 8 : Serotypes of *Salmonella*e isolated from dead-in-shell shell embryos

Salmonella serotype	No. of samples	O-group	O-Antigens	H-Antigens	
				Phase 1	Phase 2
S.enteritidis	1	D_1	1,9,12	g,m	(---)
S.arizonae	2	K	18	r	z
				/,v	z
S.pullorum-gallinarum	1	D_1	9,12	_	_
S.infantis	1	C_1	6,7	r	1,5
S.cerro	2	K	18	z_4-z_{23}	--
Total	7				

4%. Nearly similar incidence 5.7% was recorded by ref.[25]. While *Staph.epidermidis* was 15.9%. Higher incidence 25.5%was recorded by ref.[4]. But lower incidence 3.25% was reported by ref.[14].

The variation in the prevalence of isolated bacteria in the present study and others could be attributed to the rate of the bacterial egg contamination that might be influenced by measures of biosecurity and management adopted in parent flocks, hatching eggs and the type of hatchery.

The prevalence of *Streptococcus* spp. was 9.2% in early dead embryos (native breeds).This result was confirmed by ref.[13] in commercial eggs and ref.[29] who reported 9.86% and 8.6%; respectively. While in late dead embryos was 10.6%. This result was in agreement with ref.[28,29] who reported 12.2% and 11.6%; respectively. While in early and late dead embryos (foreign breed) were 22.8% and 8.3%; respectively. Lower incidence 5.14% was reported by ref.[29] in late dead embryos in foreign breeds.

Strept.faecalis prevalence in this work was 12.7%. This result was confirmed by ref.[3] who recorded 12.8%. This result was differed from those of ref.[15] who reported 1.3%.

Regarding to the prevalence of *Bacillus anthracoid* was 6.3% from total bacterial isolates. This result was in agreement with ref.[28] who recorded 5.2%.

The present investigation revealed that the isolated *E.coli* serogroups were O6[2], O27[1], O44[1], O125[1], O126[1], and O164[1]. This result nearly similar to those reported by ref.[12,15,29,36] from dead-in-shell chicken embryos, dead-in-shell turkey embryos and dead-in-shell quail embryos respectively.

The present work revealed that the isolated *Salmonella* serotypes were *S.enteritidis*[1], *S.arizonae*[2], *S.infantis*[1], *S.pullorum-gallinarum*[1] and *S.cerro*[2]. This result substantiates what has been reported by ref.[21] who isolated *S.enteritidis*, *S.cerro*, and *S.infantis* from eggshells and egg contents. Also ref.[5] isolated *S.enteritidis* and *S.infantis*. Regarding to *S.arizonae* which was isolated by ref.[19,40,41] while *S.pullorum-gallinarum* was isolated by ref.[11-13,23,41,42,48] but *S.cerro* was isolated by ref.[38] and *S.infantis* was reported by ref.[31] from commercial chicken layer flocks and ref.[32] from commercial chicken broiler flocks.

Transmission of avian arizonosis (AA) through eggs has been reported by many workers suggests that transovarian transmission can occur. *S.arizonae* from fecal contamination have a penetration pattern through the shell and shell membranes of chicken eggs very similar to that of *S.typhimurium* when incubated at 37°C, resulting in frequent presence of the organisms in chicken and turkey eggs. Fecal contamination may spread the infection from other animal species to poultry.

AA is transmitted in the incubator and brooder by direct contact and through contaminated feed and water[43].

With a meticulous vision one way conclude that the strategic polices to ensure must be planned to guarantee lowering of early embryonic death and increasing the rate of egg hatchability in balady hatcheries.

In the present study the tested *S.enteritidis* could penetrate the intact egg shells into the egg contents after 72hr from artificial contamination but could penetrate the cracked ones after 24hr only from artificial contamination. This result was confirmed by ref.[17,27,44,46]. But this result was different from those of ref.[16] who reported that *S.enteritidis* grew preferably in albumen of cracked eggs than intact eggs. Also this result was disagreed with ref.[34] who could not prove the penetration of *S.enteritidis* through eggshell experimentally. Although the shells of about 1% of commercial eggs are contaminated with *Salmonella* contamination of internal contents of eggs with *Salmonella* is rare event. Also ref.[6] isolated *Salmonella* from egg contents in a percentage of (0.35%).

Nearly similar result reported by ref.[10] that isolated *S.enteritidis* in incidence of (2.9%) from egg contents.

The differences in penetration ability may be attributed to the difference in the individual shell porosity, moisture, temperature, specific gravity of eggs and bacterial number[7].

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