Veterinary Medical Journal – Giza
Faculty of Veterinary Medicine, Cairo University
(ISSN 1110 – 1423)
Accredited from National Authority for Quality Assurance and Accreditation
Giza, 12211 - Egypt

Microbial Infections Associated with Reduced Hatchability in Broiler Breeder's Eggs in Egypt

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Abstract

The present research work was conducted for the isolation and identification of the bacterial isolates causing failure of eggs to hatch. A total of 210 un-hatched broiler breeder's eggs were collected from different hatcheries located at Giza Governorate, Egypt. In this study 6 species of bacteria were isolated and identified as Staphylococcus aureus, Escherichia coli, Salmonella species, Mycoplasma gallisepticum, Pseudomonas and Klebseilla species. The highest incidence rate of Staphylococcus aureus was in dead in shells (20%), Salmonella species were the most prevalent organisms isolated from exploders eggs and eggs with mid embryonic deaths with an incidence of 56.6 % and 50 %, respectively. The highest isolation rate of E-coli was detected in Exploders (36.6%), while Mycoplasma gallisepticum was isolated from dead in shells and pipped eggs with an incidence rate of 6.6%. Klebsiella and Pseudomonas were isolated from exploders and pipped with incidence rate of 13.3% and 3.3%, respectively. The results of this study would be helpful for prevention and control of bacterial causes of un-hatched broiler breeder's eggs.

Keywords: hatchability, eggs, bacteria, embryonic deaths, exploders, pipped

Introduction

Despite the importance of egg hatchability on fecundity in birds, the mechanisms of embryo mortality remain poorly understood. Viability of avian eggs declines as the time to incubation onset increases (Stoleson and Beissinger 1999), and microbial infection likely represents an important mechanism of embryo mortality (Cook et al., 2003 & 2005 and Godard et al., 2007). Potentially pathogenic bacteria may be transferred to the surface of the eggshell from nesting females, or may originate in the environment, and they are able to enter the egg through pores in the shell and membrane (Ruiz-de-Castaneda et al., 2011). A variety of bacteria are commonly present on eggshells shortly after laying and increase in abundance thereafter (Godard et al., 2007). The presence of water on eggshells increases the abundance and diversity of bacteria and appears to play an important role in sustaining bacterial growth (D'Alba et al., 2010). In tropical environments, there is a positive relationship between trans-shell infection with humidity and temperature. Incubation inhibits bacterial growth and trans-shell penetration by reducing moisture on shells (Cook et al., 2005 and Shawkey et al., 2009). The most common infection of hatched eggs are Staphylococcus, Streptococcus, Klebsiella, Escherichia coli, Enterobacter, Citrobacter, Proteus, Salmonella, Pseudomonas spp. and Mycoplasma. Fungi have been also reported with dead-in-shell embryos (Al-Sadi et al., 2000).

Less commonly, vertical transmission from the nesting female may result in infection or mortality of embryos. For example, in chickens, there is no direct relationship between the presence of Salmonella Enteritidis on the eggshell and infection of egg contents, indicating that transmission occurs in the reproductive tract; infection of the albumen occurs as the egg passes through the oviduct (De Buck et al., 2004). Mycoplasma spp. are also vertically transmitted (Bencina et al., 1988). The present study aimed to identify different bacterial species causing embryonic deaths in broiler breeder's eggs in Giza Governorate, Egypt.

Materials and Methods

A total of 210 un-hatched broiler breeder's eggs were collected from different hatcheries located at Giza Governorate, Egypt. These eggs were brought from broiler breeders of 36 and 52 weeks old with reduced hatchability of 55 and 48%, respectively. These eggs were classified into infertile, early embryonic deaths, mid embryonic deaths, dead in shells, pipped, exploders and rotten eggs. The eggs were rapidly transported on ice to the lab for clinical and bacteriological examination.

Sterile swabs from entire side of egg shell as well as a loopful of thoroughly mixed whole contents of infertile eggs were exposed to bacterial isolation and identification. Extra embryonic fluid and embryo tissues were collected for detection of Mycoplasma gallisepticum.

Bacterial isolation and identification

Samples were inoculated aerobically onto brain heart infusion broth, microaerophilic into Frey's broth (El-Jakee et al., 2008) and anaerobically onto cooked meat medium (El-Jakee et al., 2014). A loopful of broth culture was streaked on Mannitol salt agar, Blood agar, MacConkey agar, Sabouroud agar, Neomycin blood agar and Frey's agar medium. The plates were incubated aerobically, microaerophilic and anaerobically for detection of aerobic, anaerobic bacteria and Mycoplasma. The suspected isolates were subjected to detect biochemical and serological activities according to Koneman et al. (1992) and Quinn etal. (2002).

Typing of the isolates by PCR

DNA was extracted from the isolates by heat block. PCR procedure was carried out using primers mentioned in Table (1). Screening of PCR products by agarose gel electrophoresis in comparison with 100 bp– 1.5kb DNA ladder (Qiagen) was done according to Sritharan and Barker (1991).

Table (1): Oligonucleotide primers used for genotyping of the isolates:

Detection	Primer designation	Nucleotide Sequence	Amplified Product size (bp)	Annealing temperature	References
Mycoplasma gallisepticum	MG-14F	5'GAGCTAATCTGTAAAGTTGGT-C-3'	185 bp	55°C for 30 seconds	OIE, 2008
	MG-13R	5'GCTTCCTTGCGGTTAGCAAC3'			
Salmonella species	invA	5'GTGAAATTATCGCCACGTTCGGGC-3'	284 bp	64°C for 30 seconds	Kamelia et al.,2014
		5'-AATCATCGCACCGTCAA AGG AAC G-3'			
E.coli	16 SrDNA ECO-f	5'GACCTCGGTTTAGTTCACAGA-3'	585 bp	60°C for 1 minute	Croci et al., 2003
	16 SrDNA ECO-r	5'CACACGCTGACGCTGACCA-3'			
S. aureus	S. aureus Nuc 1 –	5'GCGATTGATGGTGATACGGTT-3'	279 bp	55°C for 60 seconds	El-Jakee et al., 2013
	S. aureus Nuc 1 –	5'AGCCAAGCCTTGACGAACTAAAGC-3'			
Pseudomonas	16S rRNA PA-GS-F	5'-GACGGGTGAGTAATGCCTA-3'	618bp	53 °C for 30 sec	Spilker et al. 2004
	16S rRNA PA-GS-R	5'-CACTGGTGTTCCTTCCTATA-3'			
Klebsiella	Gyra A F	CGC GTA CTA TAC GCC ATG AAC GTA	441 bp	55° for 1 min	Yogesh and Kevan 2011
	Gyra A R	ACC GTT GAT CAC TTC GGT CAG G			

Results

Bacterial isolation and identification

Microbiological examination of all types of eggs revealed neither fungal nor anaerobic population. On the other hand, different isolates appeared on the media as yellow fermenter colony on Mannitol salt agar, lactose fermenter colony (pink), late fermenter colony and non-lactose fermenter colony (yellow) onto MacConkey agar medium while, Frey 's agar medium showed typical fried egg colony. The isolates were identified biochemically as S.aureus, E.coli, Salmonella species, Mycoplasma gallisepticum, Pseudomonas and Klebsiella spp.

Genotyping

PCR profile confirmed that S.aureus (Amplified product 279 bp), E.coli (Amplified product 585 bp), Salmonella species (Amplified product 284 bp), Mycoplasma gallisepticum (Amplified product 185 bp) and Pseudomonas spp (Amplified product 618bp) and Klebsiella spp. (Amplified product 441bp) are responsible for different form of eggs affection. The Incidence of isolates in different form of eggs affection are illustrated in table (2). It is cleared that the highest incidence rate of S. aureus was in dead in shells (20%), followed by infertile and rotten eggs (13.3%), while it was not detected from eggs of early embryonic mortalities and pipped ones as shown in figures (1). that the highest isolation rate of E. coli was detected in Exploders (36.6%), followed by early embryonic deaths (33.3%) and dead in shell eggs (30%), while the lowest isolation rate was detected in infertile eggs (20%) as revealed in figures (2). Salmonella spp were the most prevalent organisms isolated from exploders eggs and eggs with mid embryonic deaths with an incidence of 56.6 % and 50 %, respectively, and from infertile and rotten eggs with an incidence of 40 %. The lowest isolation rate was detected in pipped and dead in shell eggs with an incidence of 33.30% (figures 3). Mycoplasma gallisepticum was isolated from dead in shells and piped eggs with an incidence rate of 6.6%, followed by

infertile and rotten eggs with an incidence of 3.3%. While, Mycoplasma gallisepticum is not detected from Early & Mid embryonic mortalities as well as from exploders as illustrated in figure (4). Pseudomonas spp was isolated only from pipped eggs with an incidence rate of 3.3%, while Klebsiella spp was isolated only from exploders eggs with an incidence rate of 13.3% (figures 5).

Table (2): The frequency of isolates among the examined unhatched eggs

Types of eggs	No of eggs	S.aureus	E.coli	Salmonella Spp.	Mycoplasma gallisepticum	Pseudomonas Spp.	Klebsiella Spp.
Infertile	30	4 (13.3%)	6 (20%)	12 (40%)	1 (3.3%)	0 (0%)	0 (0%)
Early embryonic deaths	30	0 (0%)	10 (33.3%)	11 (36.6%)	0 (0%)	0 (0%)	0 (0%)
Mid embryonic deaths	30	2 (6.6%)	7 (23.3%)	15 (50%)	0 (0%)	0 (0%)	0 (0%)
Dead in shell	30	6 (20%)	9 (30%)	10 (33.3%)	2 (6.6%)	0 (0%)	0 (0%)
Pipped	30	0 (0%)	8 (26.6%)	10 (33.3%)	2 (6.6%)	1 (3.3%)	0 (0%)
Exploders	30	2 (6.6%)	11(36.6%)	17 (56.6%)	0 (0%)	0 (0%)	4 (13.3%)
Rotten eggs	30	4 (13.3%)	8 (26.6%)	12 (40%)	1 (3.3%)	0 (0%)	0 (0%)

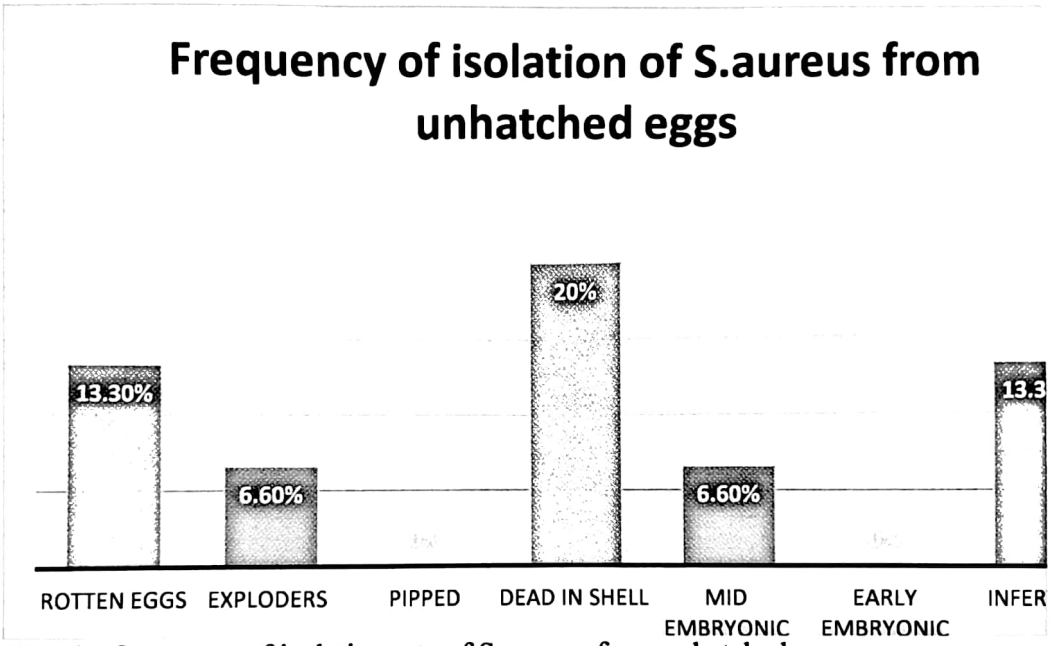

Figure (1): The frequency of isolation rate of S. aureus from unhatched eggs.

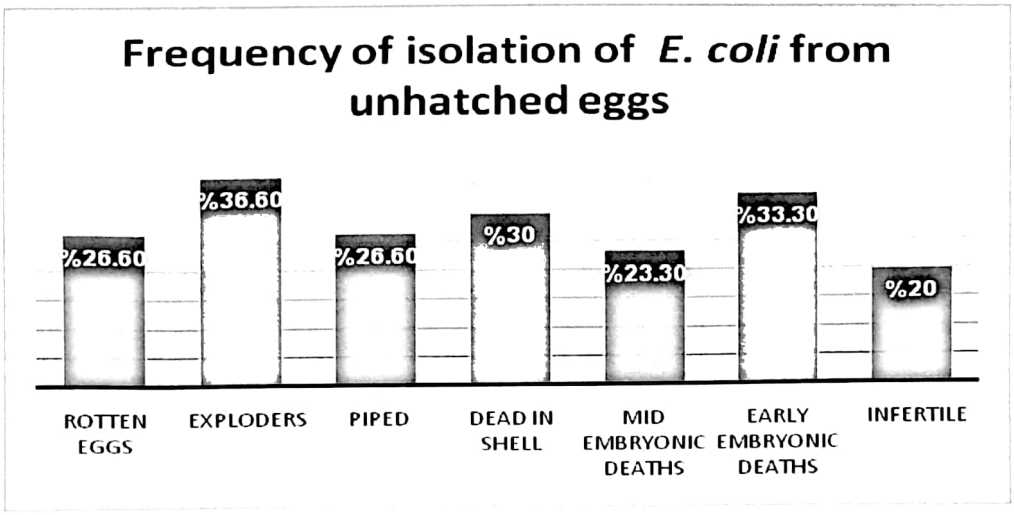

Figure (2): The frequency of isolation rate of E. coli from unhatched eggs.

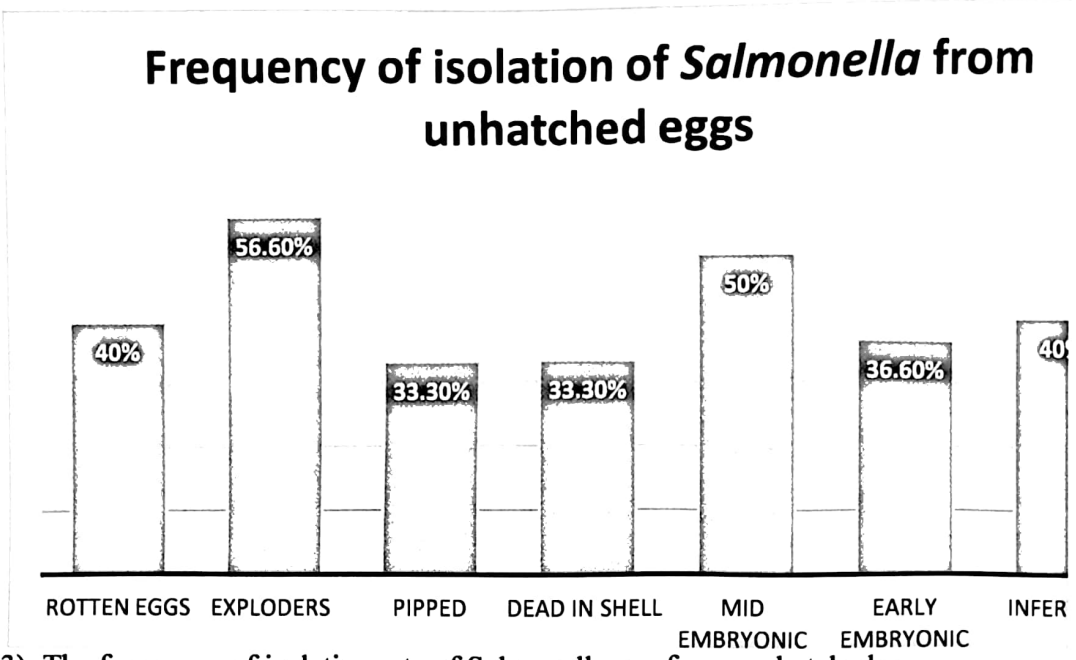

Figure (3): The frequency of isolation rate of Salmonella spp from un-hatched eggs.

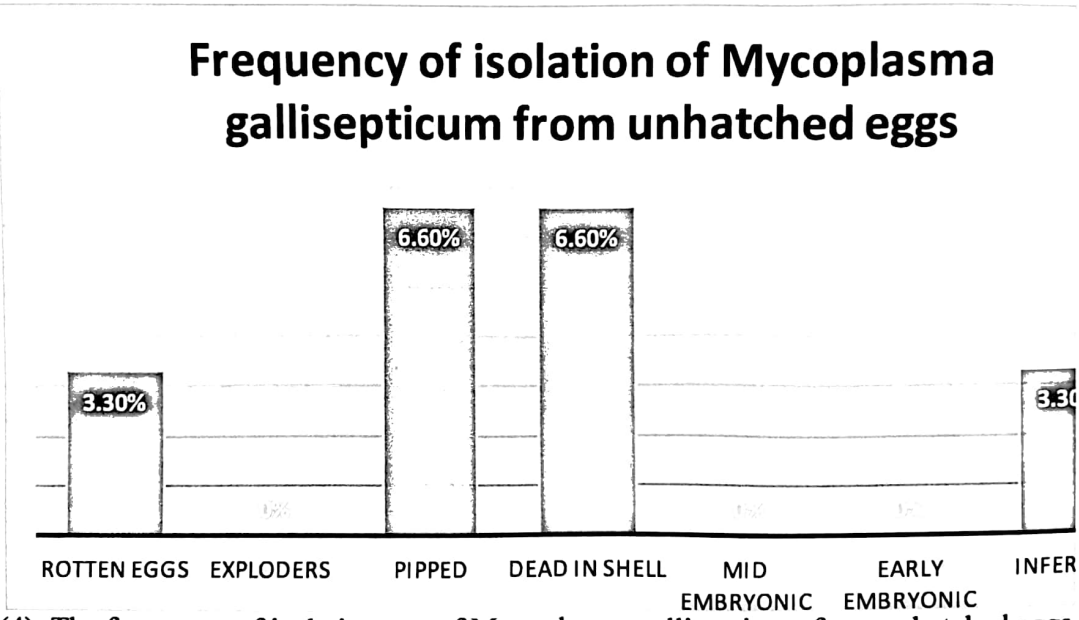

Figure (4): The frequency of isolation rate of Mycoplasma gallisepticum from unhatched eggs.

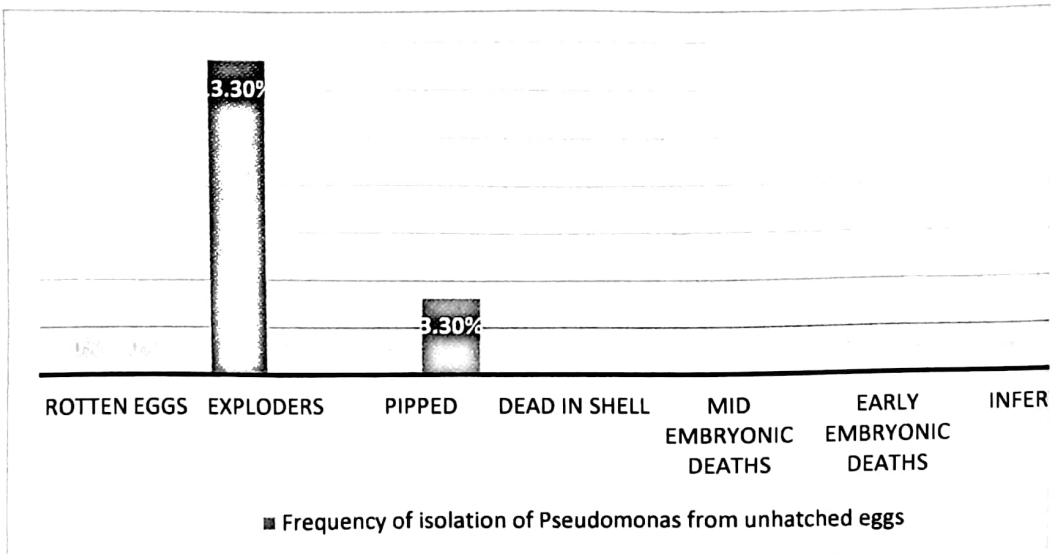

Figure (5): The isolation rate of Pseudomonas and Klebseilla spp. from unhatched eggs.

Discussion

Incubation facility industry is considered as one of the significant strides in poultry generation cycles, great sanitation and low bacterial pollution assume an essential part in enhancing hatchability and diminishing the execution of brought forth chicks.

The present research work was conducted for the isolation and identification of the bacterial isolates causing failure of eggs to hatch. In this study 6 species of bacteria disengaged and distinguished were S. aureus, Escherichia coli, Salmonella, Mycoplasma gallisepticum, Pseudomonas and Klebseilla spp. The results of isolation are in agreement with the findings of Al-Sadi et al. (2000), Ciocîrlan (2008) and Azmy (2010).

The results of bacterial isolation revealed that the highest incidence rate of S. aureus was in dead in shells (20%), followed by infertile and rotten eggs (13.3%), while it is not detected at eggs of early embryonic mortalities and pipped ones. These results are higher than those recorded by EL-Hariry (2012) and AL-Khalaf et al. (2010) who isolated S. aureus from the infertile eggs and dead in shells with an incidence of 5.83 % & 5.89%, respectively. Enany, et al. (1989) could isolate S. aureus from dead in shell embryos at a prevalence of 3.3%. S. aureus contamination is very important cause of arthritis in chicks and early chick mortalities (Abd El-Latif, 1995).

The results of bacterial isolation revealed that Salmonella spp were the most prevalent organisms isolated from exploders eggs and eggs with mid embryonic deaths with an incidence of 56.6 % and 50 %, respectively, and from infertile and rotten eggs with an incidence of 40 %. These results were in complete agreement with Calnek et al. (1991) who described salmonellae and coliforms to be the major contaminating bacteria of hatching eggs. Salmonella species could be isolated from dead in shell embryos (Karaman, 1980; Shalaby and Abd El Hamid, 1987; Enany et al., 1989; Abd El-Latif, 1995) with various rates. The variance in the pervasiveness of salmonella species isolated from dead in shell embryo may be due to the quality of used eggs and the health status of parent flocks.

With regard to isolation of Salmonella spp. in eggs with early embryonic deaths, its percentage was 36.6% which was nearly agree with the result reported by Sharada et al., (1999), who recorded a percentage (30.5%) but higher than the result obtained from Abd El-Galil et al. (1995) who recorded it in 6%.

The highest isolation rate of E-coli was detected in Exploders (36.6%), followed by early embryonic deaths (33.3%) and dead in shell eggs (30%). The result of bacterial isolation revealed that E. coli was recovered from dead-in-shell embryos in a percentage of 30% which agreed with Shalaby & Abd El-Hamid (1987) who recorded its percentage 39.5% but different from Raji et al. (2007) who reported it at 4.7% and 7.5% from Simtu farm and NAPRI, respectively.On the other hand, this percentage was lower than the result obtained by Sharada et al. (1999) who recorded its percentage 52.54%. The variation in the percentage of E. coli isolates might be partly related to the prophylactic and therapeutic usage of certain antibiotic, vaccination against respiratory viruses and improved hatchery sanitation.

Abd El-Galil et al. (1995) studied bacterial causes of lowering hatchability and early embryonic chicken death in Balady hatcheries. They reported that E. coli was the most common isolate for both fertile eggs (26.4%) and dead in shell embryo (21.9%). Al-Sadi et al. (2000) isolated E. coli, Proteus and Salmonella microorganisms from dead in shell embryos.

The predominance of Salmonella spp. was the highest, followed by E. coli. This may be attributed to multi-factorial causes such as poor sanitary conditions, malpractices of egg storage and predominance of open production systems which exposes poultry to unhygienic conditions. Other exposure factors included poor disease control and prevention practices, untreated water sources and feeding scavenging on contaminated feeds and water (FAO, 2009). As a consequence, hatching eggs are exposed to heavy microbial contamination, which, in turn influence hatchability. This is supported by other studies (Bailey et al. 1994 and Foley et al. 2008).

Mycoplasma gallisepticum was isolated from dead in shells and pipped eggs with an incidence rate of 6.6%, followed by infertile and rotten eggs with an incidence of 3.3%.Valks and Burch (2002) said that mycoplasma caused late embryo mortalities and reduced hatchability by about 5-6%. Aviagen technical team mentioned that Mycoplasma causes air sacculitis in pipped embryos and increases late dead embryos.

Klebsiella spp was isolated only from exploders eggs with an incidence rate of 13.3%.Our results are nearly similar to that found by Sarakbi et al. (1981) who isolated K. pneumoniae from embryonated chicken eggs with prevalence of

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الملخص العربى

العدوى الميكروبية المرتبطة بانخفاض القدرةعلى التفريخ فى بيض أمهات التسمين

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***مهندس زراعى

تم تجميع عدد 210 بيضة أمهات التسمين لم تفقس من مفرخات مختلفة في محافظة الجيزة، مصر. لمحاولة عزل والتعرف على العزلات البكتيرية التى تسبب انخفاض نسبه الفقس فى بيض امهات التسمين. في هذه الدراسة تم عزل 6 أنواع من البكتيريا هى الاستافيلوكوكس اوريس، الايشريشيا كولاي، السالمونيلا ، الميكوبلازما جاليسبتكم، السودوموناس والكلبسيلا. وكان أعلى معدل عزل الاستافيلوكوكس اوريس في البيض الفاطس (20٪)، وكانت معزولات السالمونيلا الأكثر انتشارا فى البيض المنفجر والبيض مع الوفيات الجنينية بمنتصف فترة التحضين بنسبة 56.6٪ و 50٪ على التوالي. كان أعلى معدل عزل الايشريشيا كولاي في البيض المنفجر (36.6٪)، في حين تم عزل الميكوبلازما جاليسبتكم من البيض الناقر و البيض الفاطس بمعدل اصابة 6.6٪. كما تم عزل الكليبسيلا والسودوموناس فقط من البيض المنفجر والبيض الناقر بمعدل حدوث 13.3٪ و 3.3٪ على التوالي. إن نتائج هذه الدراسة ستكون مفيدة للوقاية والسيطرة على البكتيريا المسببة للبيض اللذى لم يفقس من امهات التسمين.

الكلمات الداله: نسبه الفقس-البيض-البكتريا-النفوق الجنينى-البيض المنفجر-البيض الناقر