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The risk of Bacterial Contamination in Hen Eggs of Sulaimani Poultries.

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Abstract
To evaluate the risk of bacterial contamination and the penetrability of some pathogens through the shell, light brown fertile and brown infertile eggs were subjected to microbiological analyses. The benefit of using 70% ethanol as a proposed disinfectant was assessed also. Non disinfected infertile eggs showed higher contamination with both aerobic and anaerobic bacteria, anaerobic were higher (shells: 8.7x 10^2, albumen: 0.28 x 10^2, yolk: 0.97 x 10^2 cfu/gm or ml). Disinfection reduced contamination on shells (for fertile eggs: 85.1% aerobic and 54.4% anaerobic bacteria, for infertile: 65% aerobic and 47.7% anaerobic bacteria). There was no reduction in interior components but the isolation of bacteria from interior components may belong to a contamination prior to disinfection. *Pseudomonas aeruginosa* showed higher penetrability when tested artificially in all types of eggs followed by *Proteus vulgaris*, *Staphylococcus aureus* and *Escherichia coli* respectively regardless whether eggs were disinfected or not. Fertile and infertile eggs of Sulaimani poultries were within permissive hygienic quality.

Keywords: - eggs, contamination, bacteria.

Introduction
Although it has been assumed that avian eggs in general are germ free at oviposition, three routes of infection have been considered. The transovarian which resulted in yolk infection, oviducal resulted in vetelline membrane and\or albumen infection and trans-shell which resulted in translocation of bacteria from the outer to inner surface of the shell [1]. Some field studies concentrated on both rotting and pathogenic bacteria as contaminant during oviposition. Some of these studies achieved on hen ovaries surgically using enriched media to recover saprophytic bacteria that revealed in very low numbers only [2]. Studies on rotting in clean eggs stored for long periods concluded that well over 90% of hens eggs are microbiologically sterile at lay [3]. The contamination of egg shells is with a wide range of variation from a few hundred to tens of millions of bacteria per shell with an average of about 100,000 [4]. Such features as breed, housing, method of storage, marketing procedures may play a minor role of causing rotting, the genera of *Alcaligenes, Acinetobacter, Pseudomonas, serratia, Cloaca, Hafnia, Proteus*, and *Aeromonas* regarding the terms of new taxonomy, have shown to be frequently isolated from rotten eggs [5-7].

Sparks & Board [8] used electron option and appropriate microbiological techniques to study the bacterial penetration of egg shell at oviposition and demonstrated that the shell structure is vesicular within a few minutes of laying, so that there was a low incidence

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of experimentally penetration challenge with bacteria. During incubation of eggs, some water has to be lost from the egg in order to obtain a large air space, enough to sustain the embryo for the short time that it breaths with it's lungs [9]. Board [10] reviewed and stressed that the successful avoidance of egg contamination is by operation of egg-washing machines that depended upon the temperature of the wash and rinse water that being higher than the temperature of the egg. Hen and other bird eggs have a marked resistance to water due to the cuticle that covers the surface of the shell [10].

The egg albumen posses some antimicrobial defense mechanisms, such as its organization in the albumen us sac and the viscosity of its protein [4]. Chemical antimicrobial defense by lysozyme C, ovomucin, alkaline state (pH 9.5), potential chelating of ovotransferrin, other toxic components such as certain cations and vitamins made unavailable to organism by some proteins [11, 4]. It was appeared that the ovotransferrin plays an important role in preventing normal growth of the nascent vegetative cells that emerging from spores and germinating in egg white, particularly at the high pH of the white [12]. The shell acquire infection from all surfaces with which it makes contact, and the extent of infection is directly related to the cleanliness of these surfaces, and storage under very humid conditions (RH>98), the cuticle can be colonized and digested by *Pseudomonas* spp. [13]. The induction of bacterial growth leading to contamination of the albumen was subjected to some studies. Lock, *et al.* [14] presented evidence of a chemotactic response directing the movement of *Pseudomonas putida* and *Salmonella enteritidis* towards the surface of the yolk. The recent studies considered hen eggs as a source of food-born diseases included that caused by *Salmonella enteritidis* [15]. However, experimental chicken infection studies have also shown that much higher frequencies of egg contamination are occurred [16,17]. The present study was aimed to estimate the contamination of hen's table and hatching-eggs including shell, albumen and yolk with an experimentally contamination to evaluate the penetrability of some local bacterial pathogens into eggs of Sulaimani poultry using 70% ethanol as proposed disinfectant. In Sulaimani farms, the produced fertile eggs are of light brown shells laid by inbred hybrids ROSS and COBB 500. The hybrid Highline brown and hybrid Highline white 36 produce brown and white shells infertile eggs successively.

Materials and methods

Ten hen eggs of each group: Light brown fertile and browns infertile were collected at the day of laying from five different farms of west Sulaimani city. The eggs were divided into two subgroups each of five eggs. The first subgroup was disinfected by 70% ethanol using dusting method. The second group was left with no disinfection, both groups were kept in a room temperature for seven days, which is the mean period of maintenance in local market. Shell and interior components, including albumen and yolk, were separately analyzed for both aerobic and anaerobic bacterial contamination then total bacterial count was performed by estimating colony forming units (cfu) per gram shell or one ml albumen and yolk. Isolation of some expected contaminants included *Bacillus*

spp., *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus* was also achieved.

An experimentally penetration for two groups of ten fertile and infertile eggs was achieved. One group disinfected by dusting70% ethanol then each egg put in a single sterile petri dish containing 25 ml of 24h age nutrient broth (oxoid) culture of each of known bacterial contaminant. Plates were incubated at 35°c for 7 days. The mean total number of each of the contaminants per gram shell and one ml interior part including albumen and yolk collectively was counted by analyzing a homogeneous sample of each egg separately.

The Bacteriological analyses were achieved according to the Bacteriological analytical manual [18] and Bergy's manual [19]. Each complete egg was cracked aseptically, the shell, albumen, yolk or albumen and yolk collectively were separated in sterile beakers. Samples were mixed using sterile glass pearls and rods. Serial ten-fold dilutions of each sample in saline were prepared. 0.1ml from the suitable dilution was spread on plate count agar (mast diagnostics) for calculation the aerobically total count and on anaerobic agar (plate count agar with 2g/l sodium thioglycollate) incubated anaerobically using anaerobic jar for anaerobic total count. The following procedures were used to detect the counts of natural and experimentally contamination: Nutrient agar (mast diagnostics) for isolation of *Bacillus* spp., which identified furtherly by gram staining and endospore formation under phase contrast microscope after 48h incubation. *Escherichia coli* was detected as lactose fermenters on Eosin-methylene blue agar (oxoid) and identified as gram negative non endospore-forming bacilli, catalase positive, oxidase negative, indole producers. 5% sheep blood agar for isolation of *Proteus vulgaris* confirmed as swarmed colonies and gram negative, catalase positive, oxidase negative, non lactose fermenter on Mackonkeys agar (oxoid) and urease fermenter on Christesen's urea agar (Fluka). Citrimid agar (BDH) used for isolation of *Pseudomonas aeroginosa* which identified further as oxidase positive, Salmonella-Shigella agar (Fluka) used for detection of *Salmonella* and *shigella* spp. *Salmonella* spp. was differentiated on Bismuth sulfate agar (oxoid). Mannitol salt agar (oxoid) for detection of *Staphylococcus aureus* that confirmed by coagulase test. χ^2–square analysis was used to compare the mean total penetrated bacterial count among different species [20].

Results

Table (1) showed the mean number of bacterial contamination in the different parts of all egg samples. Shell appeared to be the higher contaminated in infertile eggs for all cases. The mean number of anaerobic bacteria was higher than that of aerobic in all cases. Albumen of non disinfected fertile eggs showed higher contamination by aerobic and anaerobic bacteria. No isolation of both aerobic and anaerobic bacteria contaminants from disinfected fertile eggs was detected. The same result appeared with aerobic bacteria from non disinfected infertile eggs. Yolk of disinfected fertile eggs appeared to be with no contamination by either aerobic or anaerobic bacteria, also the yolk of infertile eggs showed no contamination by aerobic bacteria with

Table (1): The mean total count as cfu x 10^2 per gram shell and ml albumen and yolk of aerobic and anaerobic bacteria contaminated eggs naturally.

sample	Fertile eggs				Infertile eggs			
	aerobic bacteria		anaerobic bacteria		aerobic bacteria		anaerobic bacteria	
	non-disinfected	disinfected (% reduction)	non-disinfected	disinfected (% reduction)	non-disinfected	disinfected	non-disinfected	disinfected (% reduction)
shell	1.35	0.2(85.1)	1.36	0.62(54.4)	3.89	1.36(65)	8.7	4.9(43.7)
albumen	1.2	NG	1.0	NG	NG	0.05	0.28	0.03
yolk	0.4	NG	0.56	NG	NG	0.03	0.97	NG

NG: no growth.

non disinfected eggs, and no anaerobic bacteria isolated with disinfected eggs. Disinfection reduced aerobic bacteria on shells with higher percents (85.1% for fertile and 65% for infertile) but lesser anaerobic.

There was no isolation of the expected contaminants that searched for which were *Bacillus* spp., *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus* from all egg samples (results not showed in tables). Penetration of bacteria each alone experimentally showed no significant differences among egg groups but differences were apparent among bacterial species (table, 2).

Penetration of *P.aeruginosa* was significantly higher than other species in all treatments followed by *P. vulgaris* while it was appeared that *E. coli* is lower penetrability even than non motile *S. aureus* (table, 2).

Discussion

The shell probably receives its first load of microorganisms when passing through the cloaca and during the time until the egg is used [4]. The contamination during handling and storage may come from environment through air and dust and by hands of handlers during packaging; shell contamination increases also with exposure to dirty conditions. The high number of bacteria on shells of non disinfected eggs may render the contamination, starting from oviposition until the time of analyzing results, regarding that the eggs in Sulaimani poultries are not subjected to washing or disinfection. The contamination of disinfected eggs was almost come from the environment during storage. It was obviously appeared that anaerobic bacteria were more dominants on shells either disinfected or non disinfected egg groups, fertile and infertile.

Table (2): The mean total count as cfu x 10^2 per ml interior egg component (albumen and yolk collectively) of some bacterial species penetrated shells experimentally.

organism	Fertile eggs*		Infertile eggs**	
	Non Disinfected	disinfected	Non disinfected	disinfected
E. coli	5.1	4.6	5.2	4.4
P. vulgaris	51	48	48	50
p. aeruginosa	488	480	484	492
S. aureus	6.2	5.8	6.3	6.0

*The bacterial mean total count of non disinfected and disinfected fertile eggs are dependent with respect to different species ($p<0.05$).
** The bacterial mean total count of non disinfected and disinfected infertile eggs are dependent with respect to different species ($p<0.05$).

The explanation of this phenomenon is not related to the ability of anaerobes to live on shell that exposed to oxygen but may be due to the ability of facultatively anaerobes that dominated shell to prefer anaerobic incubation. The higher reduction of aerobic bacteria on shells by disinfection confirms the high incidence of anaerobe isolation from interior parts of eggs. This may explain that the interior components of eggs may supply anaerobic conditions too. It was mentioned that egg shells are predominated by the facultatively anaerobeic *Micrococcus* spp. and the gram negative bacteria, these bacteria can not tolerate the dryness of shells while they are the principal contaminants of rotten eggs [4]. So far it is not clear whether fertility of eggs can reduce the number of contaminants on shell as came in the present results, this may be due to the quantity of exposure to the storage environment. So that further studies must be done to evaluate the effect of fertility on the number or the growth of contaminants on egg's shell. The water content of the egg and the cuticle covers the shell may also have an effect on the dehydration and dissolving organics, makes evaporation more rapidly than non disinfected shells. Milakovic-Novak and Prukner [21] revealed that hatching eggs treated with formaldehyde reduced contamination by *Salmonella* from 1.08% to 0.009%. Ethanol and formaldehyde have a similar action on bacteria. The same researchers showed that egg shells and contents of table eggs were significantly less contaminated with bacteria than those of hatching eggs. It was also mentioned that, during incubation for hatching, some water has to be lost physiologically [9]. This may explain the low number of shell contaminants on fertile egg shells than infertile ones especially with disinfected shells. Some of non disinfected eggs showed no growth in their albumen and yolk while contaminants appeared in those of disinfected, this may belonged to an old contamination before disinfection. The low contamination of albumen and yolk as all egg groups showed suggest the low penetrability of bacteria during storage conditions. Albumen has some prevention potential against bacteria, which reduce the risk of contamination.

The problem is that when bacteria reach yolk, they vegetate well as particularly anaerobes showed especially in infertile eggs. This lead to the explanation that yolk of infertile eggs may supply appropriate conditions for growth of anaerobes that reaches yolk.

It was appeared that the penetrations of all species tested have the same chance in disinfected and non disinfected of both fertile and infertile eggs (table, 2). There were obvious differences with respect to the isolation of each species alone from interior contents of all types of eggs in spite of that previous work revealed penetration of egg shells and egg content in hatching eggs was higher than table eggs [22]. The higher isolation of gram negative bacteria was almost due to the presence of lysozyme in albumen, which inhibit gram positive more than gram negative bacteria. Motility have also an important effect on penetration but the marked sign is that *S. aureus* was isolated more than *E. coli* in spite of that *E. coli* is the more frequent contaminant of eggs from oviposition till using of eggs in food and industry. With respect to *P.aeruginosa,* it has an ability to dissolve cuticle when humidity available that leading to higher frequency of penetration [13]. However, there is evidence available of chemotactic effect directing *P. aeruginosa* toward yolk [14]. *P. aeruginosa* is a known contaminant. The artificially penetration of *S. aureus* at the conditions of the experiment may explained by its ability to dissolve the cuticle, this permit routes to penetrate passively. *E. coli* may or may have not a little ability to dissolve cuticle in spite of that *E. coli* have the higher chance to contaminate eggs in normal condition but not artificially. From this study it was appeared that eggs in sulaimani farms are within permissive hygienic quality according to USDA standard bacteriological quality [23]. The disinfecting of shells by 70% ethanol was effective in reducing bacterial contamination of eggs during maintenance in market but it was with no effect on artificially inoculated eggs. It was appeared that the growth of bacteria in eggs inoculated artificially was higher when storage in room temperatures as shown in this study. However, minimal or no growth occurs when inoculated eggs refrigerated at 4°C [24].

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ئافاتی پیسبوون بە بەکتیریا لە هێلکەی مریشکی پەلەوەرگەکانی سلێمانی.

بەهرۆز مەحموود ئەمین جاف
بە شی بایۆلۆجی/کۆلێجی پەروەردە/ زانکۆی سلێمانی/ هەرێمی کوردستان، عیراق.

پوختە

شی کردنەوەی مایکرۆبایۆلۆجی لەسەر هێلکەی قاوەیی کاڵی پیتراو و قاوەیی نەپیتراو بە نەنجام درا بۆ خەملاندنی پیسبوون بە بەکتیریا و تاقیکردنەوەی توانای پیارۆ بۆ هەندێك لە بەکتیریا نەخۆشخەرەکان بۆ ناوهێلکە لەگەڵ بەکارهێنانی ٧٠ % ئیسانۆڵ وەکو پاکژکەرەوەیەکی پێشنیازکراو. دەرکەوت کە هێلکەی پیتراو و پاکژنەکراو زیاترین پیسبوونی بە بەکتیریای هەوایی و نا هەوایی بەخۆوە بینیوە کە زیاتر بە ناهەوایەکان (لە تۆکڵ:٨,٧ ×١٠^{٢} ، لە سپێنە:٠,٢٨ ×١٠^{٢} و لە زەردینە:٠,٩٧ ×١٠^{٢}یەکەی دروست کردنی کۆلۆنییەك بۆ گرام یان میللیلیتر). پاکژکردن، پیسبوونی سەر تۆکڵەکانی کەم کردەوە بەشێوەیەکی دیار (هێلکەی پیتراو:٨٥,١ % بەکتیریای هەوایی،٥٤,٤ % ناهەوایی، بۆ بەکتیریای نەپیتراو: ٦٥% بەکتیریای هەوایی،٤٧,٧ % ناهەوایی) بەڵام بەشێوەیەکی کەمتر لە سپێنە و زەردینە. کە رەنگە جیاکردنەوەی بەکتیریا لە سپێنە و زەردینە هۆکەی ئەگەرێتەوە بۆ پیسبوونی پێش پاکژکردنەوە. دەرکەوت کە بەکتیریای *Pseudomonas aeruginosa* زیاترین توانای پیارۆی هەیە بە تاقیکردنەوەی دەستکرد لە هەموو جۆرە هێلکەکان بەبێ جیاوازی ئەگەر پاکژکراو یان نەکراو بێت، دوای ئەو، بەکتیریای *Proteus vulgaris* و *Staphylococcus aureus* و *Escherichia coli* بوون یەك لە دوای یەك. هەموو جۆرە هێلکەکان لە نێو جۆرێتی تەندروستی رێگا پێدرا.

آفة التلوث البكتيري لبيض الدجاج في حقول دواجن مدينة السليمانية.

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الخلاصة

أجريت التحليلات المايكروبايولوجية على البيض الملقح البني الفاتح و غير الملقح البني لغرض تقييم آفة التلوث البكتيري وقابلية بعض الممرضات البكتيرية على النفوذ إلى داخل البيضة إختباريا بالإضافة إلى استخدام ٧٠٪ ايثانول كمطهر مقترح. أظهر البيض غير الملقح وغير المطهر تلوثا أعلى بالبكتريا الهوائية و اللاهوائية إذ كانت غير الهوائية أعلى (في القشرة:٨,٧ ×١٠^{٢} ،في البياض:٠,٢٨ ×١٠^{٢} و في المح:٠,٩٧ ×١٠^{٢}وحدة تكوين مستعمرة لكل غرام أو ملليلتر). لقد اختزل التطهير التلوث على القشرة (في البيض الملقح: ٨٥,١ %بكتريا هوائية و ٥٤,٤% لا هوائية، في البيض غير الملقح: ٦٥ ٪ بكتريا هوائية و٤٧,٧ ٪ لاهوائية) و لكن بشكل أقل في البياض و المح مما يدل على إن عزل البكتريا من البياض و المح يعود إلى تلوث سابق للتطهير. أبدت بكتريا*Pseudomonas aeruginosa* قابلية أعلى على النفوذ عندما اختبرت صناعيا في جميع أنواع البيض بغض النظر عما إذا كانت مطهرة أم لا، تلتها بكتريا *Proteus vulgaris* ثم *Staphylococcus aureus* ثم *Escherichia coli*. كانت كل أنواع البيض ضمن النوعية الصحية المسموح بها.

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