

Epidemiological Studies on the Bacterial Contamination of an Ostrich Hatchery and the Application of Control Measures

¹Y.F. Metaweia and ²A.A. El-Shibiny

¹Department of Hygiene, Animal Behavior and Management,
Faculty of Veterinary Medicine, Benha University, Benha Egypt

²Department of Food Sciences, Faculty of Environmental Agricultural Sciences,
El-Arish, Suez Canal University, El-Arish, Egypt

Abstract: Microorganisms contamination in hatcheries has serious impacts on the quality and growth performance of ostrich. This study was conducted to examine the bacterial contamination of an ostrich hatchery environment and hatching eggs. Additionally, the inhibitory effects of some commercial disinfectants on microbial contamination on hatchery floor were also investigated. Our results indicated that, there were significant differences ($P < 0.05$) among all microbial counts (Aerobic plate count, Enteribactercae, Coliform) isolated from both walls and floors (eggs receiving room, setters and Hatchers) and the floors of all sites were highly contaminated compared to the walls of all sites. Also, the floors and walls of Hatchers showed the highest microbial contamination, followed by the setters and lastly by the eggs receiving room. The overall prevalence of *E. coli* and *Salmonella* in all examined samples [infertile, dead in shell eggs, hatching eggs, air, floor and walls swabs from the hatchery and chicks dropping (288 samples)] were 11.5 and 6.3% respectively. The most predominant serotypes of *E. coli* were O126:K71 (24.2%), O86:K61 (18.2%) and O128:K67 (15.2%) while, the most predominant serotypes of *Salmonella* were *S. typhimurium* (44.4%) and *S. enteritides* (33.3%). Our results indicated that the disinfectants had a significant effect ($P < 0.05$) on microbial contamination and the best recommended concentration for effective control of microbial contaminants on Hatcher floor was 0.5% for Germicidan F1 and Viricidal Extra and 1.0% for Germicidan Iodes for less than 2 hours treatment. Our results recommended that, the control programs should be maintained at hatcheries and breeder farms and should include routine microbiological monitoring and practical sanitation disinfectants to reduce the occurrence of such pathogens on hatching eggs and hatcheries.

Key words: Ostrich Hatchery • Epidemiology • Contamination • Control Measures

INTRODUCTION

Hatcheries play a significant role in influencing the level of microbial challenge to hatchlings. In Egypt, hatchery collects hatching eggs from the breeder farms, incubates them and finally sells the newly hatched chicks to the commercial poultry farms. Good hygiene practices are very important to reduce the contamination with microorganisms in broilers. Ostrich meat and products can be sources for human infections and may get contaminated through handling, processing, cooking, packaging and storage. Such contamination with pathogenic microorganisms not only renders ostrich products unfit for human consumption but also increases

human risk [1]. The hatchery hygiene is depending on the health of parent stock and it is usually connected to the biosecurity measures. Those measures include the disinfecting and cleaning of the farm in addition to the avoidance of risk factors that can cause harm to the hatching eggs before and after they reach the hatchery [2]. However, the environment of a hatchery can be a source of problems such as the spread of a variety of pathogenic microorganisms that can cause diseases in a poultry farm [3-5]. The pathogenic microorganisms which can be isolated from hatching eggs can be easily distributed to other places through air movements during hatching and as a result all other chicks in the hatcher can be contaminated [3]. It was reported that microorganisms

such as *Salmonella* and *E. coli* that are found on hatching egg surfaces could be distributed all the way through the facility to affect other chicks within the hatchery and can cause infection in chicks younger than 1 week of age [6-8]. Studies have shown that, the nest clean eggs have a high hatch rate over the dirty eggs as indicated by late embryo mortality, most likely from an increase in bacterial invasion [8-9]. There are many factors are involved in the influencing of the succession of incubation process and also affect the hatchability of ostrich eggs such as the storage length of egg, the environmental conditions of pre-incubation, the egg size, the shell thickness and the incubation criteria such as; temperature, humidity and frequently of egg turning [10].

To produce hygienic poultry meat, it is very imperative to reduce the numbers of microorganisms in the breeder farm, in the hatchery, in the broiler farm and also in the chicken slaughter house. There are many problems are involved in ostrich production and the most common problem is the unacceptable high incidence of death in full developed embryo [11]. There are lots of standards have been set to evaluate the hatchery hygiene in order to measure the overall contamination by aerobic bacteria; the Coliform and fungi contamination of eggs, fluff, air and equipment; and also the contamination of the facility surfaces that are involved in the processing steps from the egg sorting room to the chick counting room [12]. Effective sanitation and disinfection programs are very important to control and reduce such contamination with pathogenic microorganisms in the hatchery and consequently reduce the human infection with them and to produce high quality chicks and increase the hatchability [13]. The efficacy of such sanitation programs may be increased through the examination of bacterial contamination of the air and the surface inside hatcheries [14].

This study aimed to study the bacterial contamination of an ostrich hatchery environment and the hatching eggs. It also aimed to isolate and identify both *E. coli* and *Salmonella* from hatchery samples. Additionally, the study of the inhibitory effects of some commercial disinfectants on microbial contamination on hatchery floor was also investigated.

MATERIALS AND METHODS

Ostrich Hatchery: The present study was carried out in an ostrich hatchery located at El-Kassaseen, Ismailia Province. It is about 100 meters far from an ostrich farm. The hatchery's dimensions are 17×8×3.5 meter and it

contains 6 setters (the capacity of each setter is 120 eggs), 2 hatcher (the capacity of each Hatcher is 40 eggs) and a multi-stage incubator (USA made). The setter's temperature and relative humidity were 36.5°C and 25% respectively for 39 days, while the temperature and relative humidity of the hatcher were 36.0°C and 30% respectively for 3 days (total 42 days). Rotating of eggs was carried out every hour and candling of eggs was carried out at 14th, 21st, 28th and 35th days of incubation. A pre-settled electronic control unit was attached to each machine to demonstrate the required and actual value of temperature, humidity and turning as well as light indicator. Walls and roofs of all machines were made of sheets of sandwich panels of very highly insulating material. The floor of the whole hatchery was constructed with a layer of concrete cement and covered with special heavy duty smooth tiles. Eggs sanitation was carried out once daily at the end of each day by dry cleaning of dirty eggs by using a piece of cloth then all eggs were sprayed by Virucidal extra® at a concentration of 0.25% and stored in eggs receiving room (same outdoor temperature and RH%) for 5-7 days before they transported to the setter.

Sampling: Two hundreds and eighty eight samples were collected during summer season, 2011 after three visits at one month interval from an ostrich hatchery. The samples included infertile and dead in shell eggs (30 of each); eggshell swabs from hatching eggs before and after sanitation, eggs from setters (30 of each), air, wall and floor swabs (36 of each) from eggs receiving room, setters and hatcher. Additionally, 30 chicks dropping samples were collected from chick boxes inside the hatcher [15-18].

Microbial Counts on Walls and Floors of Hatchery Environment and Hatching Eggshells:

- Aerobic plate count and Coliform count were carried out according to ICMSF [19].
- Enterobacteriaceae count was carried out according to AOAC [20].

Isolation and Identification of *E. coli* and *Salmonella* from Different Samples:

- Isolation and identification of *Salmonella* was carried out according to Andrews and Hammack [21].
- Isolation and identification of *E. coli* was carried out according to the procedures mentioned by Mackfaddin [22].

- Serological identification of both *E. coli* and *Salmonella* were carried out according to Edwards and Ewing [23] at Food Analysis Lab. (Fac. Vet. Med. Benha Univ., by Prof. Dr. Mohamed Ahmed).

Microbial Contamination Control on Hatchery Floor by Using Some Commercial Disinfectants

Commercial Disinfectants:

Germicidan F1®: (Glutraddehyde 22.5%, formaldehyde 16.7% and Quaternary ammonium compounds 2.5%) (German, imported by Khayrat El-Nile Co., Egypt).

Virucidal Extra®: (Potassium Peroxymonosulfate 23%, Sodium Dichloroisocyanurate 5% (3.1% Available Chlorine). Bio Agri Mix, UK.

Germicidan Iodes®: (Active iodine 2% and phosphoric acid 15%), (German, imported by Khayrat El-Nile Co., Egypt).

All tested commercial disinfectants were diluted with sterile tap water and applied at different concentrations (0.25, 0.5 and 1.0%) on contaminated surfaces (concrete) by spraying at rate of 0.5 liter/m². The neutralizer of choice was letheen broth for Germicidan F1 and 0.5% sodium thiosulphate for Virucidal Extra and Germicidan Iodes according to MacCkinon [24].

The Procedures of Trial: The aerobic pale count on naturally contaminated hatchery floor was determined before the application of different concentrations of commercial disinfectants. After 15, 30, 60 and 120 minutes of contact times, the viable bacteria per cm² were picked up by sterile moistened cotton swabs and inserted in tubes of each contained 9 sterile saline plus 1 ml of neutralizer and transported to the lab in an ice box to determine the aerobic pale count (APC), then the percentage (%) of reduction of microbial count was calculated. The procedures were carried out according to Ahmed [17].

Statistical Analysis: Results were analyzed by software program according to Selvin [25].

RESULTS AND DISCUSSION

Microbial counts on walls and floor of hatchery environment were examined (Table 1). The results clarified that the highest aerobic plate count, Enterobacteriace count and Coliform count

($9.4 \times 10^6 \pm 1.6 \times 10^6$, $1.2 \times 10^6 \pm 2.9 \times 10^5$, $4.0 \times 10^5 \pm 1.2 \times 10^5/16 \text{ cm}^2$ respectively) were detected on the floor of hatchers, while the lowest microbial counts were detected on the floor of eggs receiving room ($8.1 \times 10^5 \pm 5.7 \times 10^4$, $9.6 \times 10^4 \pm 1.8 \times 10^4$, $2.5 \times 10^4 \pm 3.0 \times 10^3/16 \text{ cm}^2$ respectively). In addition, the highest numbers of aerobic plate count, Enterobacteriace count and Coliform count ($2.2 \times 10^6 \pm 6.0 \times 10^5$, $2.4 \times 10^5 \pm 5.9 \times 10^4$, $4.4 \times 10^4 \pm 7.8 \times 10^3/16 \text{ cm}^2$ respectively) were detected on the walls of hatcher, while the lowest microbial counts were detected on the walls of eggs receiving room ($3.1 \times 10^5 \pm 3.7 \times 10^4$, $2.8 \times 10^4 \pm 5.8 \times 10^3$, $1.1 \times 10^4 \pm 2.3 \times 10^3/16 \text{ cm}^2$ respectively). The statistical analysis of data showed that there were significant differences ($P < 0.05$) among all microbial counts on walls and floors of all tested places (eggs receiving room, setters and hatchers) and this may be attributed to the transported hatching eggs, the equipments and facilities [2, 12]. The results also indicated that the floors of the hatchers showed the highest microbial contamination, followed by the floors of the setters and lastly the floors of the eggs receiving room. It was clear that the floors of all sites were highly contaminated compared to the walls. The high microbial contamination in both hatcher and setter may be attributed to many factors including the temperature and humidity of setter and hatchers which are suitable for the growth and the multiplication of microorganisms. The present microorganisms on hatching eggs may be quickly disseminated throughout the setter and hatcher by air circulation, in addition to the irregular cleaning and disinfection of setter and hatcher. These results are quite similar to the results obtained by Kim and Kim [2] who isolated very high levels of aerobic bacterial contamination on the surface of the equipment and facilities. Moreover, the hatcher was contaminated with large amount of dust, chick fluffs and hatching wastes which contain large numbers of microorganisms. On the other hand, the low microbial counts on walls and floors of the eggs receiving room is due to the regular cleaning and disinfection by Virucidal extra (0.25%) between egg patches since the room is used to store the sanitized eggs. Whenever there are impressive sanitary conditions in hatcheries, the contamination levels will be very low [13, 26].

Results in Table (2) clarified that the highest aerobic plate count, Enterobacteriace count and coliform count ($1.5 \times 10^6 \pm 3.6 \times 10^5$, $4.1 \times 10^4 \pm 7.4 \times 10^3$ and $2.9 \times 10^4 \pm 6.8 \times 10^3/16 \text{ cm}^2$) were detected on eggshells before sanitation, while the lowest microbial counts ($1.4 \times 10^5 \pm 4.9 \times 10^4$, $5.3 \times 10^3 \pm 9.8 \times 10^2$ and $1.7 \times 10^3 \pm 3.1 \times 10^2/16 \text{ cm}^2$) were detected on eggshells after sanitation. Our results agreed

Table 1: Microbial counts (Mean ± SE) on walls and floor/ 16 cm² of hatchery environment (N= 12)

Microbial counts	Walls			Floor		
	Eggs Receiving Room	Setter	Hatcher	Eggs Receiving Room	Setter	Hatcher
Aerobic plate count	3.1±10 ⁵ ^a 3.7×10 ⁴	6.3×10 ⁵ ^b ±9.1×10 ⁴	2.2×10 ⁶ ^c ±6.0×10 ⁵	8.1×10 ⁵ ^d ±5.7×10 ⁴	2.4×10 ⁶ ^e ±5.7×10 ⁵	9.4×10 ⁶ ^f ±1.6×10 ⁶
Enterobacteriaceae C.	2.8×10 ⁴ ^a ±5.8×10 ³	6.2×10 ⁴ ^b ±9.3×10 ³	2.4×10 ⁵ ^c ±5.9×10 ⁴	9.6×10 ⁴ ^d ±1.8×10 ⁴	2.8×10 ⁵ ^e ±6.8×10 ⁴	1.2×10 ⁶ ^f ±2.9×10 ⁵
Coliform count	1.1×10 ⁴ ^a ±2.3×10 ³	2.0×10 ⁴ ^b ±2.4×10 ³	4.4×10 ⁴ ^c ±7.8×10 ³	2.5×10 ⁴ ^d ±3.0×10 ³	5.1×10 ⁴ ^e ±8.6×10 ³	4.0×10 ⁵ ^f ±1.2×10 ⁵

Values with different letters in the same raw are significantly different at P<0.05

Table 2: Microbial counts (Mean ± SE) on eggshells/ 16 cm² of hatching eggs (N=15)

	Before sanitation	After sanitation	From setter
Aerobic plate count	1.5×10 ⁶ ± 3.6 ×10 ^{5a}	1.4×10 ⁵ ± 4.9×10 ^{4b}	4.6×10 ⁵ ± 1.2×10 ^{4c}
Enterobacteriaceae C.	4.1×10 ⁴ ± 7.4×10 ^{3a}	5.3×10 ³ ± 9.8×10 ^{2b}	1.2×10 ⁴ ± 2.5×10 ^{3c}
Coliform count	2.9 ×10 ⁴ ± 6.8×10 ^{3a}	1.7×10 ³ ± 3.1×10 ^{2b}	8.2×10 ³ ± 2.8×10 ^{3c}

Values with different letters in the same raw are significantly different at P<0.05

Table 3: Overall prevalence of *E. coli* and *Salmonella* in samples collected from ostrich hatchery (N= 288)

Samples/ site	No. of samples	<i>E. coli</i>		<i>Salmonella</i>		
		No of +ve	%	No of +ve	%	
Eggs contents	Infertile	30	4	14.8	2	6.7
	Dead in shell	30	5	16.7	3	10.0
Eggshells	Before sanitation	30	4	13.3	3	10.0
	After sanitation	30	1	3.3	1	3.3
	From setter	30	3	10.0	2	6.7
Hatchery environment	Air	36	3	8.3	1	2.8
	wall	36	4	11.1	2	5.6
	floor	36	6	16.7	3	8.3
	Chicks droppings	30	3	10.0	1	3.3
Total	288	33	11.5	18	6.3	

with those reported by Abouzeid and Ashour [27] who found that APC and EPC on hens' eggs were 2.0×10⁵ and 2.3×10³ / shell respectively. On the other hand, Moustafa [28] found that Aerobic plate count and Coliform count on eggshell before sanitation were 8 ×10³ and 3 ×10³/shell respectively, while after sanitation the microbial counts were 45 ×10¹ and 20 ×10¹/shell respectively. Statistical analysis of our data clarified that there were significant differences (P < 0.05) among all microbial counts on the surface of eggshells before and after sanitation as well as between eggs after sanitation and those collected from the setter. Our results indicated that eggs sanitation significantly reduced the microbial contamination on eggshells but not completely eliminate microorganisms. Moreover, hatching eggs were recontaminated from the setter environment by air circulation. Nearly similar results were reported by Metawea [29] who found that the aerobic plate count on eggshell of broiler breeder hatching eggs were decreased after fumigation followed by gradual increase during the incubation to reach the maximum level on dead in shell eggs. Furthermore, other researchers [30] found that, the sanitation of ostrich eggs before incubation improved the hatchability percentage.

On the other hand, Gentry and Quarles [31] reported that the number of contaminating bacteria on hatching eggs was decreased during 22 day of incubation to be as low as 2% of initial count. Therefore, cleaning and disinfection of hatchery compartments, efficient eggs sanitation and control the flow inside the hatchery (air, eggs, chicks, works and wastes) are considered important factors to reduce the microbial contamination through out the hatchery. Moreover, the high microbial counts on eggshells before sanitation may be attributed to the bad hygiene application in breeder farm (contaminated nest materials, eggs not frequently collected, sanitation too late after collection) or/and to the eggs contamination during collection, transportation and storage {same outdoor temperature and relative humidity (32°C and 45%)}. The data presented in Table (3) clarified that the overall prevalence of *E. coli* and *Salmonella* in all examined samples (288) were 11.5 and 6.3% respectively. The prevalence of *E. coli* and *Salmonella* in infertile eggs were 14.8 and 6.7% respectively, while in dead in shell were 16.7 and 10% respectively and these results were in agreement with those obtained by Jahantigh [32]

who detected *E. coli* in 2 out of 12 (10%) dead in shell embryos of ostrich and were quite similar to the results of Zaki *et al.* [33] who found that the prevalence rates of both *E. coli* and *Salmonella* in dead in shell ostrich eggs were 10 and 7% respectively. Additionally, Moursi and Husein [34] detected *E. coli* and *Salmonella* in 5.4 and 24.2% in infertile ostrich eggs respectively, while in dead in shell they were 8.3 and 21.6% respectively. Moreover, Metawea [35] detected *Salmonella* in 8 and 11% of examined infertile and dead in shell broiler breeders' eggs respectively. Higher prevalence rates were reported by Metawea [29] who found that, the prevalence of *E. coli* in infertile and dead in shell broiler breeder eggs were 33.3 and 41.6% respectively. On the other hand, Jahantigh [32] reported that all examined dead in shell ostrich embryos were *Salmonella* negative. The fertility and hatchability of hatching eggs in our study were less than 60% and this mainly attributed to the microbial contamination of hatching eggs from breeder flocks and/or hatchery environment. These results indicated that such pathogens are incriminated in embryo mortalities and reduction of hatchability [36]. Moreover Gonzalez *et al.* [11], Mushi *et al.* [37] and Dzoma [38] found that the storage length of egg, egg size, the environmental conditions of pre-incubation, shell thickness and the incubation criteria such as; temperature, humidity and frequently of egg turning affect the fertility and hatchability of hatching eggs of ostrich.

E. coli and *Salmonella* were detected on eggshells of hatching eggs and the highest prevalence rates (13.3 and 10% respectively) were detected on eggshells before sanitation while the lowest prevalence rates (3.3% of each) were detected on eggshells after sanitation. Nearly similar results were obtained by Metawea [35] who found that the prevalence rate of *Salmonella* in examined broiler breeder hatching eggs (eggshells) before and after 19 days of incubation were 2 and 5% respectively. Higher prevalence was reported by Metawea [29] who detected *E. coli* in 25% of examined hatching eggs (eggshells) after 19 days of incubation while it was isolated from 19.4% of hatching eggs after sanitation. Also, Nour and Ali [39] detected *E. coli* in 94 out of 200 chickens hatching eggshells after 19 days of incubation. While, Moustafa [40] has detected *Salmonella* in 15-27.2% of examined chicken hatching eggs (shells) and this variation may be attributed to the breed of chicken. On the other hand, Cabssi *et al.* [41] and Oliveiro *et al.* [42] did not isolate *Salmonella* in unhatched ostrich eggs. Our results indicated that the bad hygienic measures in breeder farm during collection, handling, sanitation and storage of

hatching eggs in addition to bad hygienic measures inside the hatchery are the primary factors in detecting both *E. coli* and *Salmonella* on hatching eggs before and during different stages of incubation. In general, eggs do not contain any bacteria when they are laid and become contaminated afterwards by the dropping, sandy litter, nest and used equipments and this faecal contamination of the surface of ostrich eggs initiate the penetration of organisms through shell and shell membrane, particularly if the shell is scratched [32, 42]. Moreover, Davis and Christensen [43] found that *E. coli* and *Salmonella* Spp. are commonly transmitted through ostrich eggs.

E. coli and *Salmonella* were also detected in hatchery environment and the highest prevalence rates (16.7 and 8.3% respectively) were detected on the floor followed by the walls (11.1 and 5.6% respectively) then the chick droppings (10.0 and 3.3% respectively). Whereas, the lowest prevalence rates of *E. coli* and *Salmonella* (8.3 and 2.3% respectively) were detected in air samples and this can be reduced by using biofilters to reduce the health hazard [44]. Similar results were reported by Moustafa [45] who found that the prevalence of *E. coli* on walls and floors of inner chambers of hatching machines were 4.3 and 13.6% respectively while air samples were *E. coli* free. Also, Metawea [35] detected *Salmonella* in examined air, walls, floors and chicks dropping samples collected from poultry hatchery with prevalence rates 5.0, 1.7, 8.3 and 6% respectively. Higher prevalence rates were recorded by Metawea [29] who isolated *E. coli* from air, walls and floor swabs of hatchery compartments and found that the prevalence rates were 37.5, 29.16 and 45.8% respectively. This variation may be attributed to the levels of hygienic measures applied in the hatcheries and breeder farms, levels of biosecurity applied and the levels of microbial contamination on hatching eggs. Our results agreed with those reported by Sheldon and Brake [3] who found that the environment of poultry hatcheries was highly contaminated with a variety of microorganisms that cause diseases in chick population and such microbial contaminants can easily spread through employees activity, air currents and recycled into the setters and hatching by the ventilation system. Moreover, the commercial hatcheries may become contaminated with microorganisms from various sources [18].

Our results indicated that the hatchery environment may be contaminated with *E. coli* and *Salmonella* pathogens from different sources such as; microorganisms on hatching eggs, the circulation of air inside the hatchery, the movement of workers throughout

Table 4: Distribution of isolated strains of *E. coli* and *Salmonella* from ostrich hatchery (N=288)

Strains of <i>E. coli</i> / <i>Salmonella</i>	Eggs shells		Hatchery environment				Total				
	Infertile eggs	Dead in shell	Before Sant.	After Sant.	From setter	Air	Wall	Floor	Chick droppings	No	%
O126:K71 (B16)	1	1	1	1	-	1	1	1	1	8	24.2
O86:K61 (B7)	1	1	1	-	-	1	1	1	-	6	18.2
O128:K67 (B12)	-	1	-	-	1	1	1	1	-	5	15.2
O111:K58 (B7)	1	-	1	-	-	-	1	1	-	4	12.1
O55:K59 (B5)	-	1	1	-	-	-	-	-	1	3	9.1
O26:K60 (B6)	1	-	-	-	-	-	-	1	-	2	6.1
O114: K90	-	-	-	-	1	-	-	-	-	1	3.0
Untypable	-	1	-	-	1	-	-	1	1	4	12.1
Total	4	5	4	1	3	3	4	6	3	33	100.0
<i>S. typhimurium</i>	1	1	1	1	1	1	1	1	-	8	44.4
<i>S. enteritidis</i>	1	1	1	-	-	-	1	1	1	6	33.3
<i>S. chester</i>	-	-	1	-	-	-	-	-	-	1	5.6
<i>S. muenster</i>	-	-	-	-	1	-	-	-	-	1	5.6
Untypable	-	1	-	-	-	-	-	1	-	2	11.1
Total	2	3	3	1	2	1	2	3	1	18	100.0

Table 5: Effectiveness of some commercial disinfectants to control microbial contamination on hatchery floor (The initial count on hatchery floor before disinfectant application was 3.2×10^5 / cm²)

Disinfectants	Conc. %	APC and % of reduction after application of disinfectant /time per min.							
		15		30		60		120	
		APC/cm ²	% of Reduction	APC/ cm ²	% of Reduction	APC/ cm ²	% of Reduction	APC/ cm ²	% of Reduction
Germicidan F1 (glutaldehyde) 22.5%	0.25	1.7×10^5	45.2	1.1×10^5	66.3	6.0×10^4	81.4	2.8×10^4	91.2
	0.50	5.3×10^4	83.3	1.2×10^4	96.1	Nil	100	Nil	100
	1.0	Nil	100	Nil	100	Nil	100	Nil	100
Viricidal Extra (3.1% chlorine)	0.25	2.0×10^5	38.1	1.7×10^5	46.3	1.2×10^5	61.9	8.6×10^4	73.1
	0.50	1.1×10^5	64.3	5.9×10^4	81.5	1.8×10^4	94.3	Nil	100
	1.0	2.3×10^4	92.6	Nil	100	Nil	100	Nil	100
Germicidan Iodes (2% active iodine)	0.25	2.3×10^5	25.1	2.0×10^5	38.2	1.8×10^5	44.6	1.4×10^5	55.3
	0.50	2.0×10^5	35.9	1.3×10^5	57.0	8.6×10^4	73.1	3.7×10^4	88.3
	1.0	1.4×10^5	56.3	9.0×10^4	71.9	2.0×10^4	93.6	Nil	100

the hatchery without restriction, the visitors from rearing, grower and breeder farms, the used vehicles for hatching eggs transportation in addition to the nearby hatchery ostrich farm and other large animal farms which considered as important sources of such pathogens into the hatchery. Moreover, the detection of *E. coli* and *Salmonella* in ostrich droppings may be attributed to the vertical transmission of such pathogens (mainly *Salmonella*) and/or contaminated ostrich chick boxes from previous patch as well as from hatchery environment due to the improper sanitation.

The data illustrated in Table (4) clarified that the most predominant serotype of *E. coli* was O126:K71 (24.2%), followed by O86:K61 (18.2%), O128:K67 (15.2%), O111:K58 (12.1%), O55:K59 (9.1%), O26:K60 (6.1%), O114: K90 (3%) and finally 4 untypable strains (12.1%). While, the most predominant serotype of *Salmonella* was *S. typhimurium* (44.4%) followed by *S. enteritides*

(33.3%), *S. muenster* and *S. chester* (5.6% each) and finally 2 untypable strains (11.1%). Many other researches isolated the same serotypes in addition to more serotypes from ostrich, poultry and their environment [1, 34-35, 46-53]. Our results indicated that all isolated strains of *E. coli* and *Salmonella* were previously detected in other poultry species by many researchers which clarified that ostrich has not specific pathogens and the main sources of both pathogens were hatching eggs. Furthermore, air and hatching eggs may import strains of both pathogens into the hatchery in addition to the inter-transmission of *E. coli* and *Salmonella* isolates between eggs and hatchery environment.

The effectiveness of some commercial disinfectants to control microbial contamination on hatchery floor was investigated. The results in Table (5) clarified that Germicidan F1 and Viricidal Extra were powerful enough to eliminate the microorganisms on the contaminated floor

within 60 and 120 minutes respectively when they used at 0.5% concentration. Alternatively, Germicidan Iodes eliminated the numbers of those pathogens when it is used at 1.0% concentration within 120 minutes. Moreover, Germicidan F1, Viricidal Extra and Germicidan Iodes eliminated 91.2, 73.1 and 55.3% of microbial contaminants on the floor respectively when they are used at 0.25% concentration within 120 minutes. Our results indicated that the recommended concentration for effective control of microbial contaminants in hatchery environment was 0.5% for Germicidan F1 and Viricidal Extra and 1.0% for Germicidan Iodes (for less than 2 hours). Moreover, the most powerful disinfectant was Germicidan F1 followed by Viricidal Extra and then Germicidan Iodes. This effect may be attributed to both chlorine (Viricidal Extra) and iodine (Germicidan Iodes) releasing agents where their antimicrobial activities were greatly reduced in the presence of organic matter (dust, chick fluffs, hatchery wastes and salts in tap water used for the dilution of disinfectant). On the other hand, the germicidal power of Germicidan F1 (glutaldehyde) is less effective in the presence of such organic matter. Similar results were reported by other researchers [29, 35, 54]. In addition, Abd El-Aal [55] found that dlutraldehyde was able to reduce the numbers of tested organisms (gram +ve and -ve) to log 4 when it is used at 0.5% concentration within 30 minutes. Moreover, Kadria *et al.* [53] found that glutaldehyde and iodine releasing agents inhibited 100% of tested organisms (*Salmonella*, *Staph.* and *Yersinia*) when they are used at 0.5 and 1% concentrations respectively within 10 minutes. On the other hand, Angelillo *et al.* [56] revealed that 2% glutaldehyde was able to kill the vegetative bacteria within 1-2 minutes. Moreover, Metawea [35] found that the minimum inhibitory concentration (MIC) of virocid, halamid and iofaster against *Salmonella* were 0.13, 1 and 2% respectively. These differences may be attributed to the technique used for evaluating the efficiency of disinfectant, type and strain of pathogen as well as type and concentration of disinfectant and contact time.

Our results concluded that strict application of eggs and hatchery hygiene through cleaning and disinfection is very important to control microbial contamination and to increase both fertility and hatchability. Chen *et al.* [13] found that the absence of bacterial growth control in hatcheries may result in the production of poor-quality chicks in addition to mortality increase, feed efficiency decrease and poor flock uniformity. Moreover, El-Arid [57] recommended that, incubator disinfection with Virkon S 5% spray will increase the hatchability. We recommend that, both public health and animal health officials in

addition to the industry partners should develop guidelines and programs to reduce pathogens transmission. Those control programs should be maintained at hatcheries and breeder farms and should include routine microbiological monitoring and practical sanitation components to reduce the occurrence of such pathogens on hatching eggs and in hatcheries.

REFERENCES

1. Ouf, J.M., R.A. Elbassouny and E. S. Shabana, 2009. Microbial criteria of ostrich meat products. *J. Vet. Med. Ass.*, 69(3): 175-183.
2. Kim, J.H. and K.S. Kim, 2010. Hatchery hygiene evaluation by microbiological examination of hatchery samples. *Poult. Sci.*, 89: 1389-1398.
3. Sheldon, W. and J. Brake, 1991. Hydrogen peroxide as alternative hatching eggs disinfectant. *Poult. Sci.*, 70(5): 1092-1098.
4. Scott, T.A. and C. Swetnam, 1993. Screening sanitizing agents and methods of application for hatching eggs. 2. Effectiveness against microorganisms on the egg shell. *J. Appl. Poult. Res.*, 2: 7-11.
5. Gaffga, N.H., C. Barton Behravesh, P.J. Ettestad, C.B. Smelser, A.R. Rhorer, A.B. Cronquist, N.A. Comstock, S.A. Bidol, N.J. Patel, P. Gerner-Smidt, W.E. Keene, T.M. Gomez, B.A. Hopkins, M.J. Sotir and F.J. Angulo, 2012. Outbreak of Salmonellosis linked to Live Poultry from a Mail-Order Hatchery. *N. Engl. J. Med.*, 366(22): 2065-2073.
6. Chute, H.L. and M. Gershman, 1978. A new approach to hatchery sanitation. *Poult. Sci.*, 40: 568-571.
7. Padron, N.M., 1990. Salmonella typhimurium penetration through the eggshell of hatching eggs. *Avian Dis.*, 17: 574-581.
8. Berrang, M.E., N.A. Cox, J.F. Frank and R.J. Buhr, 1999. Bacterial penetration of the eggshell and shell membranes of the chicken hatching egg: a review. *J. Appl. Poult. Res.*, 8: 499-504.
9. Buhr, R.J. and J.M. Mauldin, 1994. Automated spray sanitizing of broiler hatching eggs 2. Hatchability of nest clean and dirty eggs. *J. Applied Poult. Res.*, 3: 226-233.
10. Badley, A.R., 1997. Fertility, hatchability and incubation of ostrich (*Struthio camelus*) eggs. *Poult. Avian. Biol. Rev.*, 8: 53-76.
11. Gonzalez, A.D., F.M. Salterlee and G.G. Gadd, 1999. Factors affecting ostrich hatchability. *Poult. Sci.*, 78: 1257-1262.

12. Jordan, F., M. Pattison, D. Alexander and T. Faragher, 2001. Hatchery. Pages 35-42 in Poultry Disease. 5th ed. W.B. Saunders, London, UK.
13. Chen, S.J., T.E. Lee, E.M. Wang, T.J. Cho and C.H. Wang, 2002. Monitoring the hygiene of chicken hatcheries in Taiwan during 1999-2001. J. Microbiol. Immunol. Infect., 35: 236-242.
14. Brake, J. and B.W. Sheldon, 1990. Effect of a quaternary ammonium sanitizer for hatching eggs on their contamination, permeability, water loss and hatchability. Poultry Sci., 69: 517-525.
15. Furuta, K. and S. Maruyama, 1981. Bacterial contamination of eggs during incubation and hatching and fluffs of newly hatched chicks. British Poultry Sci., 22: 247-254.
16. Soucy, K., C.J. Randall and R.A. Holly, 1983. Microbiological monitoring of hatchery sanitation. Poultry Sci., 62: 298-309.
17. Ahmed, A.M., 1990. Evaluation of disinfection process in modern poultry farms. M.V.Sc. Thesis (animal Hygiene) Fac. Vet. Med., Cairo Univ.
18. Willingham, E.M., J.E. Sander, S.G. Thayer and J. Wilson, 1996. Investigation of bacterial resistance to hatchery disinfectants. Avian Dis., 40(3): 510-512.
19. ICMSF, 1996. Microorganisms in food. 1- Their significance and methods of Enumeration. 2nd Ed. Univ. of Toronto Press, Toronto, Canada.
20. AOAC, 1990. Official Methods of Analysis, 931. 15th Ed. Public AOAC, P.O. Box: 540. Benjamin Franklin Station, Washington, DC.
21. Andrews, W.H. and T.S. Hammack, 1998. "Salmonella". Bacteriological analytical manual, 8th Ed. Revision A, Chapter 6. Hall. Publ. Comp. Limited, London, U.K.
22. Mackfaddin, C., 1980. Biochemical tests for identification of medical bacteria. 2nd Ed. Williams and Wilkins Naltmore, London.
23. Edwards, P. and W. Ewing, 1972. Identification of Enterobacteriaceae. 3rd Ed. Burgers Publishing Company, USA.
24. MacCkinon, I.H., 1974. The use of inactivators in the evaluation of disinfectants. J. Hyg. Comb., 73: 180, printed in Great Britain.
25. Selvin, W., 1996. Statistical analysis of epidemiological data, 2nd Ed. Oxford Univ., pp: 44-78.
26. Samberg, Y. and M. Meroz, 1995. Application of disinfectants in poultry hatcheries. Rev. Sci. Tech., 14: 365-380.
27. Abouzeid, A.M. and D.A. Ashour, 2002. Microbial contamination of Hens eggs. J. Egypt. Vet. Med. Asso., 62(6): 157-164.
28. Moustafa, G.Z., 2009. Field study to evaluate the biosecurity program currently used in some commercial Egyptian poultry farms. J. Egy. Vet. Med. Ass., 69(4): 169-181.
29. Metawea, Y.F., 2000. Some epidemiological studies on Escherichia coli in poultry farms. M.V.Sc. Thesis, Fac. of Vet. Med., Moshtohor, Benha University.
30. Husein, M.M., G.G. Medani and A.A. Amin, 2002. Some sanitary studies on hatchability of ostrich eggs. J. Egypt. Vet. Med. Ass., 63(1): 103-111.
31. Gentry, R.F. and C.L. Quarles, 1972. The measurement of bacterial contamination on egg shells. Poultry Sci., 51: 930-933.
32. Jahantigh, M., 2010. Bacteriological study of dead in shell embryos of ostrich. Iranian J. Vet. Res., Shiraz Un., 11(1)30: 88-90.
33. Zaki, M.S., A.M. Byomi and M.M. Hussin, 2004. Hatchability of ostrich (*Struthio camelus*) Eggs in relation to different breeding system. Minufiya Vet. J., 3(2): 349-265.
34. Moursi, M.K. and M.M. Husien, 2005. Microbial causes of reduction of hatchability in ostrich farms and some trials of control. SCVMJ, VIII(2): 25-38.
35. Metawea, Y.F., 2003. Epidemiological studies on Salmonellae in poultry farms fresh. Ph D. Thesis. Fac. of Vet. Med., Moshtohor, Benha University.
36. Agabou, A., 2009. Air-borne contaminations in two broiler hatcheries in the North East of Algeria. Vet. World, 2(2): 49-50.
37. Mushi, E.Z.M.G. Binta, R.G. Chabo and O. Galetshipe, 2008. Problems associated with the artificial incubation and hatching of ostrich (*Struthio camelus*) eggs in Botswana. Res. J. Poultry Sci., 2(2): 21-26.
38. Dzoma, B.M., 2010. Some factors affecting fertility and hatchability in the farmed ostrich: A Review. J. Animal and Vet. Advanc., 9(2): 229-239.
39. Nour, A.A. and M. Ali, 2012. Investigation of three *E. coli* serogroups isolated from fertile eggs of commercial chicken eggs in the Southern Region of Syria. Assiut Vet. Med. J., 58(132): 328-337.
40. Moustafa, G.Z., 1999. Studies on some hygienic aspects related to low hatchability in broiler breeders eggs. Ph D. Thesis (Animal Hygiene), Fac. Vet. Med., Cairo Univ.

41. Cabssi, C.S., S. Taddei, G. Predari, F. Ghidini, E. Schiano and G. Cavirani, 2004. Bacteriologic findings in ostrich (*Struthio camelus*) eggs from farm with reproductive failures. *Av. Dis.*, 48: 716-722.
42. Oliveiro, C.F., L.S. Sonia, O.T. Adriano and B.J. Angelo, 2009. Search for Salmonella spp. in ostrich productive chain of Brazilian Southeast region. *Trop. Anim. Health Prod.*, 41: 1607-1614.
43. Davis, G.S. and V.L. Christensen, 1995. Egg shell conductance and other functional qualities of ostrich eggs. *The Ostrich News*, 8(80): 60-68.
44. Chmielowiec-Korzeniowska, L. Tymczyna, C. Skórska, J. Sitkowska, G. Cholewa and J. Dutkiewicz, 2007. Efficacy of a novel biofilter in hatchery sanitation: I. Removal of airborne bacteria, dust and endotoxin. *Ann. Agric. Environ. Med.*, 14: 141-150.
45. Moustafa, G.Z., 1995. Effect of hatchery sanitation on the hatchability of breeders eggs. MVSc. Thesis (Animal Hygiene), Fac. Vet. Med., Cairo Univ.
46. Khafagy, A.R. and A.M. Kamel, 2001. Microbial contamination and other hatching problems causing dead in shell in ostrich (*Struthio camelus*) eggs during artificial incubation. *Vet. Med. J. Giza*, 49(3): 401-411.
47. Ali, A. and E. Youssef, 2003. Bacteriological studies and biochemical parameters of respiratory infection of ostrich. *Vet. Med. J. Giza*, 51(2): 189-203.
48. Effat, A. and M. Moursi, 2003. Studies on diarrhea in ostrich in Ismailia province with reference to cloacal prolepsis. *J. Egypt. Vet. Med. Ass.*, 63(6): 271-287.
49. Metawe, Y.F. and T. Abd El-Ghaffar, 2004. Epidemiological studies on Salmonellae in poultry feed mills. *Benha Vet. Med. J.*, 15(2): 129-141.
50. Ali, A.R. and H.S. Ibrahim, 2004. Bacteriological and serological studies on *S. enteritidis* of ostriches. *J. Egypt. Vet. Med. Ass.*, 64(1): 247-261.
51. Wong, T.L., C. Nicol, R. Cook and S. MacDiarmid, 2007. Salmonella in uncooked retail meats in New Zealand. *J. Food Prot.*, 70(6): 1360-1365.
52. Khater, D.F. and A.A. Abdel-Twab, 2009. Bacterial quality of ostrich meat and Edible offal. 3rd international Sci. Conf., Fac. Vet. Med. Benha Univ., pp: 632-645.
53. Kadria, G., H.A. Samaha and A.M. Hassan, 2009. Determination of the efficiency chemical disinfectants against some microbes isolated from air and water of poultry farms. *J. Egy. Vet. Med. Ass.*, 69(2): 143-155.
54. Dosoky, R.M.A.H. Hafez and A.S. Sotohy, 2000. Evaluation of some commercial disinfectants against some pathogens in presence of interfering substances. *Assiut Vet. Med. J.* 43(86): 147-158.
55. Abd El-Aal, S.F., 2008. Determination of the bactericidal activity of chemical disinfectants against bacteria in dairies according to the DVG-guidelines. *Hyg. Med.*, 33(11): 463-471.
56. Angelillo, I.F., A. Bianco, C.G. Nobile and M. Pavia, 1998. Evaluation of the efficacy of glutaraldehyde and hydrogen peroxide for disinfection of dental instruments. *Lett. Appl. Micro.*, 27(5): 292-296.
57. El-Arid, T.A., 2009. Studies on some bacterial causes of embryonic death in ostrich. MVSc Thesis (poultry diseases), Fac. Vet. Med. Zagazig Univ.