Bacteriologic and pathologic studies on dead-in-shell chicken embryos

Hanan, M.A.; Hanan M. Ibrahim and Ghehan, N.A.

Serology, Bacteriology and pathology units
Animal Health Research Institute, Dokki, Giza and Zagazig

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Abstract

A total of 210 samples were collected from three chicken hatcheries in sharkia governorate for bacteriological examination. The samples were including 180 dead-in-shells embryos (60 from each hatchery) and 30 hatcheries swabs (10 from each one). The high prevalence of the isolates were obtained from hatchery (A) which used Virkon® S 1% (25 strains) ,followed by hatchery (B) which used formaldehyde(19 strains) then hatchery (C) used virocid (18 strains). The majority of isolates are *E.coli* (16 strains) followed by *Staphylococus aureus,* (10 strains) *Pseudomonas aeruginosa (*9 strains), *Klebsiella pneumoniae* (8 strains), *Citrobacter freundii* (6 strains), *Coryne spp* (3 strains), *Salmonella enterica subsp enterica serovar Gallinarium*(3 strains), *Salmonella serovar Birkenhead* (2 strains), *Salmonella serovar Virchow*(1strain). *Klebsiella oxytoca* (2 strains), *serratia marcescens* (2strains). At the present work, seven different serogroups were identified among the recovered 16 *E. coli* isolates. The recovered serogroups from hatchariy (A) were O157 (1), O166 (1), O78 (1), O6 (1) and two untypable strains. Meanwhile the recovered serogroups from hatchariy (B) were O167 (1), O78 (2), O146 (1), and one untypable strain. One serogroup O78, O18 and one untypable strain were identified in hatchariy (C). Specimens from the liver and intestine of the dead embryos were examined microscopically for histopathology. Macroscopically, Liver and intestine were showing different degree of congestion and liver was enlarged showed numerous greyish or whitish necrotic foci according to the isolated bacterial group. Microscopically, the liver showed congestion, ischemic necrosis and /or fatty change while intestine revealed catarrhal enteritis, ulcerated or necrotic mucus coagulative necrosis. Isolated bacteria were tested for their efficient to commercial preparations of Virkon® and Virocid® disinfectants. Bacterial isolates were exposed to disinfectant dilution recommended by the manufacture for 10, 30 and 60 min exposure periods before sub culturing to broth medium. Virkon® concentration had lead to unsatisfactory results in the present work. On the other hand Virocid® disinfectant showed high efficiency against examined bacteria at recommended dilutions and exposure time. It can be concluded that virocid may be effective disinfectant for using in the hatchery at a lower cost.

Key words: *chicken hatcheries, Virkon® , Bacterial isolates*

Introduction

Every unhatched chick represents a financial loss which can make the difference between profit and loss for the commercial hatchery **(poultry world 2008).**The presence of microorganisms in the hatchery is directly related to deficiencies in hygiene which can result in elevated first-week chick mortality and depressed

growth rate **(Agabou 2009).** Several researchers have emphasized the importance of yolk sac infection as a cause of mortality, even in the hatchery, which could increase the number of so-called dead-in-shell eggs **(Dzoma and Dorrestein, 2001; Walker *et al*., 2002)**. Faecal contamination of eggs is considered to be the most important source of yolk sac infection **(Rajesh *et al*., 2001).** Bacteria may be acquired *in ova* if the hen has oophoritis or salpingitis or *via* contamination following artificial insemination **(Montgomery *et al*., 1999).** Yolk sac infections can also result from translocation of bacteria from the chick's intestine or from the bloodstream **(Saif *et al*., 2003).**The relative occurrence of different bacterial species in three local hatcheries recorded by **(Mirza et al., 1999)** as following, *E.coli* 52.54%, *paratyphoid Salmonella* 12.7%.*Salmonella Gallinarum11.86%, Salmonella Pullorum5.93%, Streptococcus faecales5.93%, Bacillus subtilis4.2%, Pseudomonas aeruginosa3.39% and Proteus mirabilis3.39%.* It appears that a rigorous cleaning and disinfecting program is a necessity for hatcheries. The construction of new cabinets and the purchase of large capacity and high technology machines do nothing if good hygienic measures are not undertaken. Selection, Care and Cleaning of Hatching Eggs must be taken in consideration .In the selection of a successful incubator room, factors such as heating, humidity, ventilation, and sanitation should all be considered. Eggs used for hatching should be clean and stored in clean containers in a sanitary egg holding room. Eggs contaminated with bacterial organisms usually do not hatch well and this poor quality is reflected in the chicks that do hatch.

Gross pathological changes and microscopical examination at necropsy were variable according to the isolated bacterial strains. Thus, studying the pathological finding of dead in shell chicken embryos constitutes an important diagnostic and confirmative technique.

Disinfecting is a fundamental process in poultry hatchery where facing a wide variety of pathogen such as bacteria, fungi, viruses and protozoa. There is a normal practice of fumigation of hatching egg just after laying and receiving eggs. There is no question about the efficacy of fumigation as dry disinfectant. It is a cheaper disinfectant but has carcinogenic effect. As a result most of the developed country has imposed ban on it.

Marica et al., (2011) studied the influence of the applied different disinfectants procedures used for reduction of number of microorganisms in chicks hatching. Best hatching results were achieved at sanitary treated hatching eggs in poultry house and hatchery with formaldehyde steaming.

The present study aims to measure and identify the bacterial contaminations within three chicken hatcheries in Sharkia Governorate which lead to decrease the rate of hatchability and increase the number of dead in shell embryo of chickens. Also to examine the effect of different sanitizers on the bacterial isolates from hatcheries and to find out a disinfectant alternative to fumigation for more safe use at hatchery.

Material and method

1- Collection of the samples:

A total of 210 samples were collected from three different hatcheries in Sharkia governorate for bacteriological examination. The examined hatcheries used different types of disinfectants, hatchery (A) used Virkon® S 1%, hatchery (B) used formaldehyde (35ml formalin 40% + 17.5 g potassium permanganate) and hatchery (C) used virocid. The samples were 180 dead-in-shell embryos (60 from each hatchery) and 30 hatchery swabs (10 from each one).

Table (1). Type and number of collected samples.

Type of samples	Dead in shell embryo (180)			hatcheries swabs (30)			Total (210)
	Hatchery A	Hatchery B	Hatchery C	Hatchery A	Hatchery B	Hatchery C	
No of samples	60	60	60	10	10	10	210

1- Bacteriological examination.

Dead in shell embryo. The egg shells were accurately disinfected with ethyl alcohol 70% and flamed then dried and broken. The embryo was removed with sterile forceps and put in sterile Petri dish then opened to expose the internal organs. With sterile dry swabs, impression smears were taken from yolk contents, liver, lung and intestine.

Hatchery samples. The samples for determination of the extent of microbial contamination of surfaces, prior to disinfection were obtained directly after hatching and cleaning of the hatcheries. The samples for establishing the extent of microbial contamination of surfaces after disinfection were collected after cleaning and disinfection of the hatcheries .

At each hatchery, 5 swabs were taken immediately at the hatching day and 5 swabs were taken directly after disinfection of hatchery. Samples were collected at five different places in setters and the hatchers rooms. At each hatchery, the swabs were taken by rubbing with sterile nutrient broth moistened swabs on the corners and wall joints.

All collected swabs from dead in shell embryos and hatchery samples were inoculated onto Muller tetrathionate broth and nutrient broth media and incubated at 37°C for 24 h. Sub culturing was carried out onto the following media: nutrient agar, blood agar, XLD agar and MacConkey agar and incubated aerobically at 37° C for 48 hrs **Cruickshank *et al.*, (1975).**

2- Biochemical identification of bacterial isolates.

The identification of the isolated colonies was performed using standard bacteriological and biochemical procedures as described by **Swayne *et al*. (1998)** and **Quinn *et al*. (2002).**

3-Serological identification.

Serological identification of Salmonella was carried by slide agglutination technique according to **Minor and Popoff (2000)**. Serological identification of *E.coli* was carried out according to **Edwards and Ewing (1972).**

4-Histopathological examination.

Specimens from the liver and intestine of the dead embryos were collected and fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained by HE and then examined microscopically for histopathology (**Bancroft and Gamble 2008**).

5-Experimental Design

Evaluation of disinfectants against isolated bacteria:

Preparation of bacterial isolates.

The strains of *Staphylococcus aureus, E. coli O78, E. coli O157, Salmonella enterica subsp enterica serovar Birkenhead, Salmonella serovar Virchow* and *Salmonella serovar Gallinarium* isolated during this study were used to assess disinfectant efficacy. Retained bacterial samples were plated on tryptic soy agar and grown for 24 hr at 36 C under normal atmosphere. Pure fresh culture was inoculated into 10 ml of 1% peptone broth at a sufficient quantity to reach 65% transmission (˚480nm) without additional incubation time. This corresponds to approximately10^8cfu/ml. Disinfectant dilutions were prepared daily.

Preparation of disinfectant solution.

1. Virkon® S disinfectant, Powder contains 20.4% potassium peroxymonosulfate & 1.5% sodium chloride. The manufacturer's recommendations call for a 1:100 dilution and a 10-min exposure time. 1% solution was preperd by mixing one part Virkon to 100 parts of water make up fresh 1%Virkon solution.

2. VIROCID disinfectant solution active ingredient was Benzalkonium Chloride, Didecyl Dimethyl Ammonium Chloride and Glutaraldehyde. The manufacturer's recommendations dilution was 0.25-0.5% (1:400 - 1:200) by spraying or foaming 30-90 seconds once per hour or once every two hours.

The tow disinfectants were used and mixed with sterilized, distilled, deionized water at dilutions below and above the manufacturer's recommendations. Virkon® S was used at dilutions of 1:50, 1:100, and 1:200. VIROCID® was used at dilutions of 1:100, 1:200, 1: 400 and 1:500.

Ten micro liters of inoculated broth was transferred to 10 ml of each disinfectant dilution. At 10, 30, 60 minutes post inoculation 1 µl was transferred from the inoculated disinfectant to 4 ml of 1% peptone broth (subculture tubes). Subculture tubes were incubated for 48 hr at 37°C under normal atmosphere and checked for growth. Growth was determined by visual observation of cloudiness of the broth or pellet formation at bottom of tube. Samples showing growth in any tube were repeated in duplicate. Samples showing growth in multiple dilution levels of one or more disinfectant type were identified **(Eric *et al.*, 1996)**.Identification was made using API 20E.

Results and Discussion

An assessment of the level of contamination in the examined samples indicated an average level of contamination of (29.5%). A total of 62 bacterial spp were isolated from all examined samples as shown in Table (2). The high prevalence of the isolates were obtained from hatchery (A) which used Virkon® S 1% (25 strains), followed by hatchery (B) which used formaldehyde (19 strains) then hatchery (C) used virocid (18 strains). From these samples, 19, 16, and13 bacterial isolates were recovered from dead in shell embryos in hatcheries A, B and C respectively. Meanwhile 6, 3 and 5 bacterial isolates were recovered from hatchery swabs in hatcheries A, B and C respectively.

These results were similar to those reported by **Al-Sadi *et al.* (2000)** who isolated Potential pathogenic bacteria from the three hatcheries and they were in the order of 38, 26, and 25 isolates, respectively. Also **Jordan (1990)** concluded that embryonic mortality at the late stage (16-21) of incubation accounts of more than 60% of the total embryonic mortality and is more closely related to the bacterial infection.

Table (2). Total number of bacterial isolates from the collected samples.

	Hatchery A		Hatchery B		Hatchery C		total
Type of samples	D. S.E *	Hatchery swabs	D. S.E	Hatchery swabs	D. S.E	Hatchery swabs	
No of samples	60	10	60	10	60	10	210
No of bacterial Isolates (%)	19 (31.7%)	6 (60 %)	16 (26.7%)	3 (30%)	13 (21.6%)	5 (50%)	62 (29.5%)
Total No of isolates	25		19		18		62

D. S.E *: Dead in shell embryo

In the present work, three hatcheries used different disinfectants were examined. The high prevalence of the isolates were obtained from hatchery A which used Virkon® S 1% (25 strains), followed by hatchery B which used formaldehyde(19 strains) then hatchery C used virocid (18 strains). Data obtained in table (3) represented the relative occurrence of different bacterial spp. in the three examined hatchery samples. The results indicate that the majority of isolates are *E.coli* which represented the main isolate [16(25.8%)strains] followed by *Staphylococcus aureus,* [10(16.1%) strains] *Pseudomonas aeruginosa* [9(14.5%) strains], *Klebsiella pneumoniae* [8(12.9%) strains], *Citrobacter freundii* [6(9.7%) strains], *Coryne spp* [3(4.8%) strains], *Salmonella serovar Gallinarium* [3 (4.8%) strains], *Salmonella serovar Birkenhead* [2 (3.2%) strains], *Salmonella serovar Virchow* [1 (1.6%) strain], *Klebsiella oxytoca* [2 (3.2%) strains], *and serratia marcescens* [2 (3.2%) strains].

In the present work the majority of isolates are *E.coli* which represented the main isolate (16 strains) . *E.coli* was highly prevalence in hatchery (A) followed by (B) then (C). *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolation were nearly equal in all hatcheries while *Klebsiella pneumoniae* was highly prevalence in hatchery (A) followed by (C) then hatchery (B) meanwhile *Klebsiella oxytoca* was recovered only from hatchery (C) .on the other hand, *Citrobacter freundii* was equally isolated from hatchery (A) and (B) and not recovered from hatchery (C). *Coryne spp* was recovered from hatchery (B) and (C) not from hatchery (A). Isolates of *Salmonella serovar Gallinarium* were obtained from hatchery (A), *Salmonella serovar Birkenhead* from hatchery (B) and *Salmonella serovar Virchow* from hatchery (C). *serratia marcescens* isolates was obtained from (A) and (C) and not recovered from (B).

This result agree with that of **Al-Sadi *et al.* (2000)** who stated that bacteria which isolated from dead in shell embryos, arranged in order of decreasing frequency, included *Escherichia coli, Pseudomonas* spp., *Shigella* spp., *Staphylococcus* spp., *Proteus* spp., *Streptococcus* spp. and *Salmonella* spp. However**, Enany *et al.*, (1989)** could isolate *Staph. aureus* from dead in shell embryos at a prevalence of 3.3%. Moreover, *Staph. aureus* contamination is very important which cause of arthritis in chicks and early chick mortalities **Abd El-Latif**,(**1995)** reported that the prevalence of *Staph. Aureus* was 18.6% while **Said, (1988)** isolated in a rate of 3.9%. **Orajaka and Mohan (1985)** and **White *et al.*, (2003)** recoded that *Staphylococcus aureus* is a very ubiquitous microorganism associated with omphalitis, yolk sac and liver infections in first week dead chicks and in-shell dead embryos.

Shalaby and Abd El-Hamid (1987) isolated *E. coli* in prevalence of 44.5% from hatching eggs**. Enany *et al.*, (1989)** reported that *E. coli* was isolated from dead in shell embryos at a rate of 17.5%, Also they said that *Klebsiella pneumoniae* has been reported as one of the bacteria infecting the yolk sac and causing embryos and chicks mortalities during their first week of life. **Karaman, (1980); El-Atrebe, (1982); Shalaby and Abd El-Hamid, (1987) and Said, (1988)** isolated *K. pneumoniae* from dead in shell embryos with various prevalences .The difference in the rate of isolation may be attributed to the heavy contamination of the eggs after laying and the improper handling and storage of the hatching eggs. **Orajaka and Mohan (1985)** and **White *et al.*, (2003)** reported that K. *pneumoniae* was one of the bacteria infecting the yolk sac,causing embryos and chicks mortalities during their first week of life .

In addition **Thermote, (2006)** stated that Pseudomonas *aeruginosa* is an opportunistic pathogen that can invade and colonize fertile and embryonated eggs causing the in-shell death of embryos and newly hatched chicks**. El-Atreby, (1982)** isolated *P. aeruginosa* from dead in shell embryos at a prevalence of 2.7%. In addition, **Karaman, *et al.* (1980)** isolated *P. aeruginosa* from dead in shell embryo and non fertile eggs in prevalence of 17.2**%. Shalaby and Abd El-Hamid, (1987)** isolated it from hatch-

ing eggs at a prevalence of 4.9%, while **Enany *et al.*, (1989)** isolated *P.aeruginosa* from hatching eggs and dead chicken embryos at a prevalence of 1.75%.

Moreover, **Lecoanet (1992a) and (1992b)** found that the presence of *Salmonella spp* in the fertile eggs may result in embryonic deaths (at the 6th day, but especially after the 15th day of incubation) and abnormal hatching **Lecoanet (1992b)** .Infected chicks may die with peaks in mortality between the 4th and the 5th day and by the 15th day. In a study conducted by **Cox *et al.* (1990),** the isolation rate of *Salmonella spp* was 71% on shell fragments. **Barbour and Nabbut (1982)** recovered Salmonellae from shell contents and shell surfaces of hatching eggs with the same prevalence (1.24%) from farm A. They recovered Salmonella from shells of hatching eggs (2.48%) and hatching egg contents (0.35%) from farm B in Saudi Arabia. However, Salmonella species could be isolated from dead in shell embryos **(Karaman, 1980; Shala by and Abd El Hamid, 1987; Enany *et al.*, 1989; Abd El-Latif, 1995)** with various rates. Without a doubt, effective cleaning and disinfection programmes are vital in the poultry hatchery. These programmes control key organisms, such as *Salmonella* spp., *Pseudomonas* spp., *Proteus* spp., *E. coli, Staphylococcus* spp., *Streptococci* spp. and *Aspergillus* spp **.Magwood & Marr (1964),** and concentrate on four key areas of concern: the egg, surfaces which can contaminate the egg, air-borne contaminants, and movable equipment and personnel.

Table (3). Type of bacterial isolates from the collected samples.

Type of bacteria	Hatchery A		Hatchery B		Hatchery C		total	
	NO	%	NO	%	NO	%	NO	%
Staphylococus aureus	3	12	4	21	3	16.7	10	16.1
Coryne spp	-	-	1	5.3	2	11.1	3	4.8
E.coli	8	32	5	26	3	16.7	16	25.8
Citrobacter freundii	3	12	3	15.8	-	-	6	9.7
K. pneumoniae	4	16	1	5.3	3	16.7	8	12.9
K.oxytoca	-	-	-	-	2	11.1	2	3.2
Pseudomonas *aeruginosa*	3	12	3	15.8	3	16.7	9	14.5
serratia marcescens	1	4	-	-	1	5.6	2	3.2
Salmonella Gallinarium	3	12	-	-	-	-	3	4.8
Salmonella Birkenhead	-	-	2	10.5	-	-	2	3.2
Salmonella Virchow	-	-	-	-	1	5.6	1	1.6
total	25	100	19	100	18	100	62	100

Results in table (4) revealed that, before disinfection 5, 3 and 5 isolates were obtained from hatcheries (A), (B) and (C) respectively while after disinfection one isolate was detected in hatcheries (A) only. Coryne *spp, serratia marcescens* and *Salmonella serovar Birkenhead* were not reported in all hatcheries samples. *Staphylococus aureus* and *Citrobacter freundii* were recovered from hatchery (A) and (B) before disinfection (one from each). One *E.coli* isolate was recovered from the three hatcheries before disinfection and one from hatchery (A) after disinfection. One isolate of *Klebsiella pneumoniae* was obtained from hatchery (A)

and (B) before disinfection. One isolate of *Salmonella serovar Gallinarium* was obtained from hatchery (A) before disinfection. Meanwhile, one isolate of *Klebsiella oxytoca, Pseudomonas aeruginosa* and *Salmonella serovar Virchow* was recovered from hatchery (C).

This results were agree with that of **Chen, *et al.* (2002)** who recorded that among hatcheries, 13 to 29% were contaminated with Salmonella spp. in Taiwan. Also **Agabou (2009)** concluded that the presence of microorganisms in the hatchery is directly related to deficiencies in hygiene which can result in elevated first-week chick mortality and depressed growth rate. Bacteriological analyses of the air in two broiler hatcheries in the province of Constantine (North-East of Algeria) showed the presence of a large number of highly pathogenic bacteria including *Salmonella spp*, *E. coli, Pseudomonas aeruginosa and Staphylococcus aureus. E. coli* was isolated from the yolk (in large numbers). According to **Friend and Franson (1999),** *Salmonella spp* can persist for 4 to 5 years within the hatchery. **Cox *et al.* (1990)** described the egg shells as the major source of *Salmonella spp* spread in hatcheries. In a study conducted by, the isolation rate of *Salmonella spp* was 71% on shell fragments. **Cason *et al.* (1994)** found that during an experiment, a hatchability rate of 86% of fertile eggs heavily infected with *Salmonella serovar Typhimurium*. All the hatchers room (especially the air) was tested positive to this bacterium and a significant number of control chicks hatched in the same hatchers (about 44% of checked chicks) were contaminated.

Table (4). Type of bacterial swabs isolates from hatcheries samples before and after disinfection.

Hatchery (No of samples)	Hatchery A swabs (10)		Hatchery B swabs (10)		Hatchery C swabs (10)		Total (30)
	B.D	A.D	B.D	A.D	B.D	A.D	
Coryne spp	-	-	-	-	-	-	-
Staphylococus aureus	1	-	1	-	-	-	2
E.coli	1	1	1	-	1	-	4
Citrobacter freundii	1	-	1	-	-	-	2
serratia marcescens	-	-	-	-	-	-	-
Klebsiella pneumoniae	1	-	-	-	1	-	2
Klebsiella oxytoca	-	-	-	-	1	-	1
Pseudomonas aeruginosa	-	-	-	-	1	-	1
Salmonella serovar Gallinarium	1	-	-	-	-	-	1
Salmonella serovar Virchow	-	-	-	-	1	-	1
Salmonella serovar Birkenhead	-	-	-	-	-	-	-
TOTAL	5	1	3	-	5	-	14

B.D: before disinfection A.D: after disinfection

Seven different serogroups were identified among the recovered 16 E. coli isolates as shown in table (5). The recovered serogroups from hatchariy (A) were O157 (1), O166 (1), O78 (1), O6 (1) and two untypable strains. Meanwhile The recovered serogroups from hatchariy (B) were O167 (1), O78 (2), O146 (1), and one untypable strain. One serogroup O78, O18 and one untypable strain were identified in hatchariy (C).

These results were similar to those reported by **El-Atrebe, (1982)** who mentioned that the prevalent strains of *E. coli* were O78 and O125. Also, **Abd El-Galil, *et al.* (1984)** reported that the most prevalent serotypes of *E. coli* were O125, O78, O11 and O55. In addition, **Shalaby and Abd El-Hamid (1987)** found that the most prevalent serotypes were O78, O128 and O114. This difference in serotypes of isolated *E. coli* might be due to the locality and to the environmental condition of isolation.

Table (5). Serotyping of *E.coli* isolates from the collected samples.

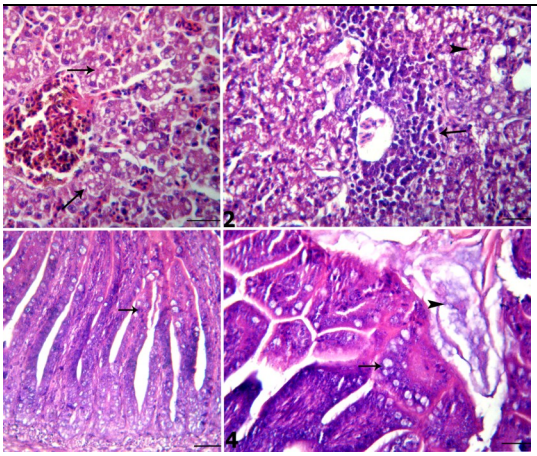
	Hatchery A		Hatchery B		Hatchery C		total
Type of samples	D. S.E D. S.E	Hatchery swabs	D. S.E	Hatchery swabs	D. S.E	Hatchery swabs	
E.coli isolates no	6	2	4	1	2	1	16
E.coli Antigenic structure	O157 (1) O166 (1) O78 (1) O6(1) Untypable (2)	O157(1) O146(1)	O167(1) O78(1) O146(1) untypable(1)	O78(1)	O78(1) O18(1)	untypable (1)	

Pathological Findings.

Histopathological finding of dead in shell embryos revealed that, liver and intestine of dead in shell embryos evok different kinds of lesions according to the isolated bacterial strains.

Klebsiella-Isolated Group.

Macroscopically, the liver was slightly enlarged and markedly congested and the intestine was slightly dilated with grayish or mucoid material. Microscopically, the **liver** showed severe congestion of the hepatic blood vessels and sinusoids besides diffuse hepatocyte vacuolations (**Fig 1**). The portal areas and rarely the interstitium were infiltrated with mixed population of mononuclears and heterophils (**Fig 2**). Small areas of necrosis were rarely dispersed throughout the hepatic parenchyma. The wall of gallbladder was edematous and thickened. The **intestine** revealed mild catarrhal enteritis which represented by an increase of epithelial cells desquamation and goblet cells metaplasia with basophilic material (mucus) inside the intestinal lumen (**Figs 3 and 4**). Few round cells infiltrations were noticed in the submucosa. The muscular coat was edematous and focally hyalinized. This was agreeing with **Wilson (1994).**



Figs (1-4): Klebsiella-Isolated Group:

1-Liver shows congestion of hepatic blood vessel (arrowhead) and vacuolations in the hepatocytes (arrows).

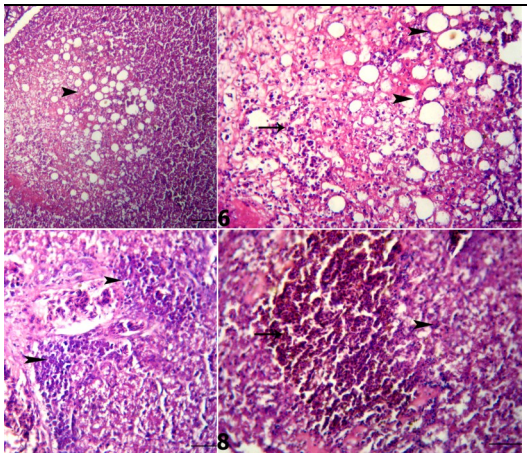
2-Liver shows portal area with mixed population of mononuclears and heterophils (arrow).

3-Intestine shows an increase of goblet cells (arrows).

4-Intestine shows basophilic mucus in the lumen (arrowhead).

Salmonella-Isolated Group:

Macroscopically, the chicks were thin or emaciated than the others. Omphalitis and peritonitis were rarely evident. The liver was yellow or pale or with multiple necrotic foci (1-2 mm in diameter) and with hemorrhagic borders. The gallbladder was overdistended with bile and surrounded by a narrow green zone. The intestine showed hyperemic mucosa and yellowish white contents. The yolk sac did not completely absorbed and intestinal yellow core was also detected. Microscopically, the **liver** showed severe fatty change and multifocal necrosis of hepatocytes with infiltration of heterophils mixed with few lymphocytes and macrophages randomly scattered throughout the parenchyma (**Figs 5 and 6**). These infiltrates were invaded to the adjacent portal areas. Sometimes, the portal areas were expanded by congested blood vessels, leukocytic infiltration and fibrous connective tissue proliferation (**Fig 7**). Extensive hemorrhages were noticed among the degenerated hepatocytes (**Fig 8**). Vacuolar and hydropic degenerations were also visualized. The **intestine** showed ulcerated or necrotic mucosa and the intestinal villi were completely denuded from the lining epithelium. Such mucosa was infiltrated with lymphocytes and heterophils. The intestinal lumina were stuffed with the sloughed mucosa and their desquamated epithelia (**Figs 9 and 10**). The muscular coat was edematous and the serosal blood vessels were congested (**Fig 11**). The yolk sac revealed insipissated yellowish material with numerous granular basophilic bacterial colonies (**Fig 12**). **Rao HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed?term=Rao%20V%5BAuthor%5D&cauthor=true&cauthor_uid=3303205" *et al* (1987)** recorded similar result.

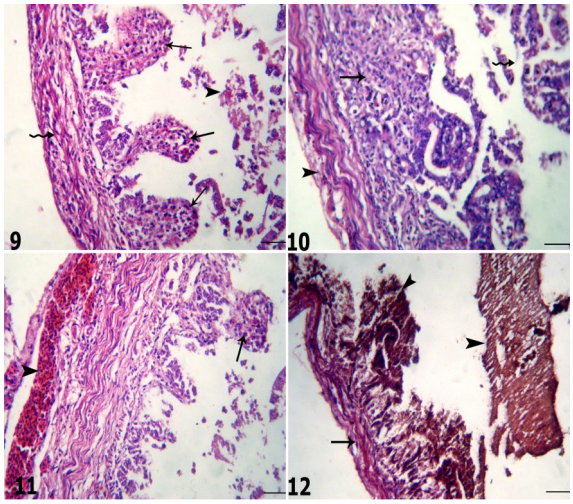


Figs (5-12): Salmonella-Isolated Group:

5 and 6-Liver shows fatty change (arrowhead), coagulative necrosis and heterophils infiltration (arrow).

7-Liver shows expanded portal area with congested blood vessels (arrow) and leukocyte aggregation (arrowheads).

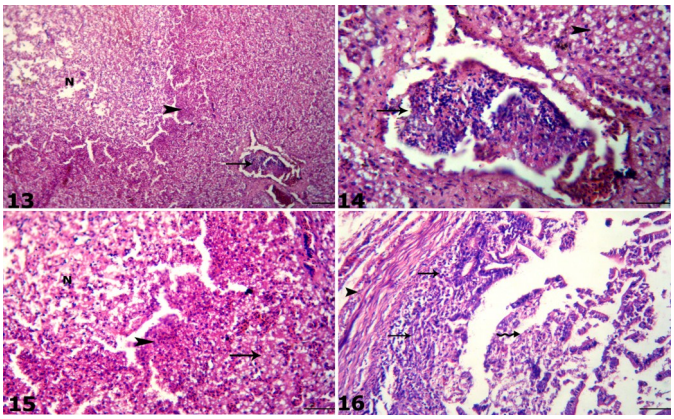
8-Liver shows extensive hemorrhage (arrow) and degenerative changes in the hepatocytes (arrowheads).



9 and 10-Intestine shows coagulative necrosis in the intestinal villi which became denuded from the epithelia and infiltrated with leukocytes (arrows).
11-Intestine shows necrotic mucosa (arrow) and congested serosal blood vessel (arrowhead).
12-Yolk sack shows edematous wall and bacterial colonies in the insipissated yolk material (arrowheads).

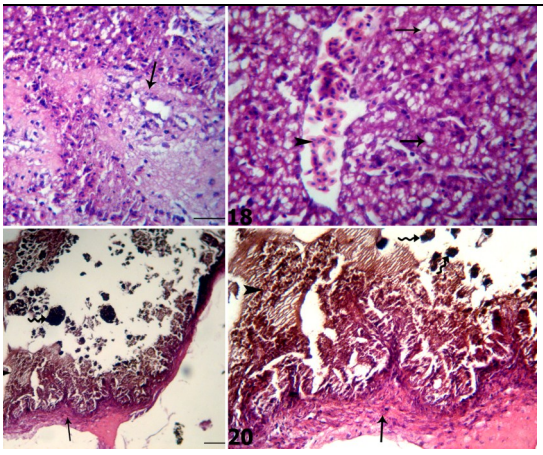
E. coli-Isolated Group.

Macroscopically, the liver showed numerous grayish or whitish necrotic foci (spotty liver). Sometimes, it became slightly enlarged and congested. Whitish cheesy material was seen on the hepatic surface and peritoneum. The intestine was apparently normal except segmental mucosal congestion. Our results agreed with **Mohamed (2004)** Microscopically, the **liver** revealed large irregular areas of ischemic necrosis and thrombosis (**Figs 13 and 14**). Such necrosis was of coagulation type and surrounded by a thick zone of cellular infiltration (**Fig 15**). Fibrinous exudates were seen on the hepatic capsule. Fatty change and other retrogressive changes were observed besides congestion of some hepatic blood vessels. The **intestine** showed mild catarrhal enteritis which represented by sloughing of the lining epithelium in the lumen, mucinous degeneration and few heterophils infiltration in the submucosa (**Fig 16**). The muscular coat was slightly edematous. Our resuts were agreed with that of **Aly (1989), Eisa (1998) and Khalid(1990).**



Figs (13-16): E. coli-Isolated Group.
13 and 14-Liver shows ischemic necrosis (N) and thrombosis (arrow).
15-A higher magnification of fig 13 to show the necrotic area separated from the normal by heterophils infiltration (arrowhead).
16-Intestine shows catarrhal enteritis with sloughing of the lining epithelia and leukocyte infiltrations (arrows).

Pseudomonas-Isolated Group.
Macroscopically, the liver was normal in size and congested. Few pinpoint whitish necrotic foci were also observed. Persistent yolk sac containing watery yellowish material was seen with hyperemic wall. The intestine was apparently normal. Microscopically, the **liver** showed small areas of coagulation necroses presented by pyknosis and disappearance of the nuclei (**Fig 17**). Diffuse vacuolations in the hepatocytes were also detected besides congested blood vessels (**Fig 18**). The **intestine** revealed catarrhal enteritis with inflamed yolk sac. The latter was dilated with yellowish content and surrounded with thick hyperemic wall. Few round cells and bacterial colonies were mixed with the yolk sac-content (**Figs 19 and 20**). ***en.cqqant.com***



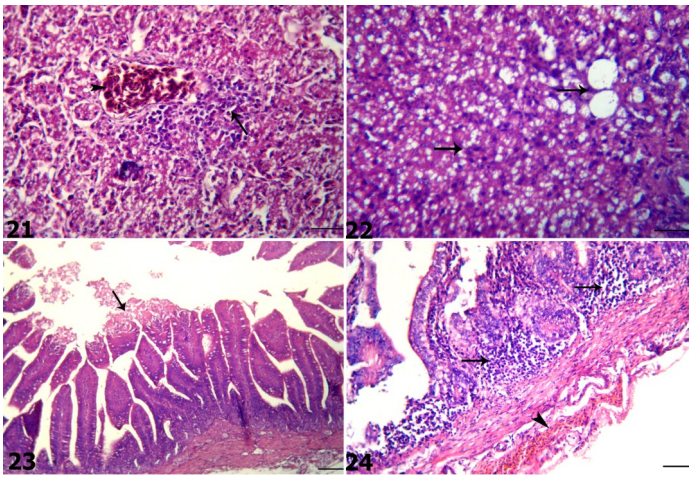
Figs (17-20): Pseudomonas-Isolated Group:

17-Liver shows coagulative necrosis (arrows).

18-Liver shows congested blood vessel (arrowhead) and degenerative changes in the hepatocytes (arrows).

19 and 20-Yolk sac shows yolk material mixed with leukocytes and bacterial colonies (arrowheads) besides thickening its wall (arrow).

Staph and Corynebacterium-Isolated Groups.
Macroscopically, only few sporadic cases were identified with omphalitis and rarely unabsorbed yolk sac. The liver was apparently normal or slightly congested. The intestine was normal except congested duodenal mucosa and the wall of the yolk sac was thick. Microscopically, the **liver** showed congestion of portal blood vessels and heterophils infiltration (**Fig 21**). Fatty change (**Fig 22**) and hydropic degeneration were focally detected particularly around the congested blood vessels. The **intestine** revealed small areas of coagulative necrosis at the tips of intestinal villi (**Fig 23**). The intestine of some cases showed lymphohistiocytic infiltrations in the lamina propria and submucosa besides congested serosal blood vessels (**Fig 24**). These results were partially agreed with **Hill et al (1989).**



Figs (21-24): Staph and Corynebacterium-Isolated Groups:
21-Liver shows portal area with congested blood vessel (arrowhead) and heterophils infiltration (arrow).
22-Liver shows fatty change (arrows).
23-Intestine shows coagulative necrosis at the tips of intestinal villi (arrow).
24-Intestine shows leukocytes infiltration in the submucosa (arrows) and congested serosal blood vessel (arrowhead).

Experimental Design:
The evaluation of Virkon® S and VIROCID® disinfectants against isolated bacteria was showed that all tested bacteria except salmonella serovars were grown after 10 min while disinfectant application results after 30min and 60 min were recorded in table (6).

Table (6). Disinfectant efficacy against the selected bacteria after 30 and 60 min contact time.

disinfectant	Virkon® S						VIROCID®					
Duration	30min			60 min			30min			60 min		
Concentration	1/50	1/100	1/200	1/50	1/100	1/200	1/100	1/200	1/500	1/100	1/200	1/500
Staph aureus	+	+	+	+	+	+	-	-	+	-	-	-
E.coli O87	+	+	+	+	+	+	-	+	+	-	-	-
E.coli O157	+	+	+	+	+	+	-	+	+	-	-	-
S.Gallinarium	+	+	+	-	-	+	-	-	+	-	-	-
S.*Virchow*	+	+	+	-	-	+	-	-	+	-	-	-
S.Birkenhead	+	+	+	-	-	+	-	-	+	-	-	-

* (-) is equivalent to no microbial growth and (+) is equivalent to microbial growth

The human health risks of formaldehyde fumigation are a cause of great concern. Use of formaldehyde is prohibited in some countries. Human exposure should be avoided, and gas masks and protective clothing are essential **ANON. (1984).** At this study we try find out a disinfectant alternative to fumigation for more safe use at hatcheries. The recorded results in (Table 6) showed that all of the selected bacteria were resistant to Virkon® S within the first 30 minutes of the contact time. However, VIROCID® dilution 1:100 was able to inhibit the growth of all tested isolates during that period of time. After 60 min contact time, all examined bacteria were inhibited by VIROCID® at all dilutions while , most of the selected bacteria were still resistant to virkon-s except Salmonella serovar Gallinarium, Salmonella serovar Virchow and Salmonella serovar Birkenhead at dilutions 1:50 and 1: 100.
Disinfectant preparations, concentrations and exposure time need to be carefully determined. The manufacturer's recommendations call for Virkon-s was 1:100 dilutions and a 10-min ex-

posure time. This concentration had led to unsatisfactory results in the present work. On the other hand VIROCID® disinfectant showed high efficiency against examined bacteria at recommended dilutions and exposure time.

This result was agree with that of **Mustafa et al (2009)** who tested Five commercially available disinfectants against 7 selected bacterial, fungal and viral isolates. The obtained results indicated that, most of the tested disinfectant products were effective at the manufacturer recommended level within 30 min contact time when tested in the absence of organic matter meanwhile Virkon-s could not demonstrate satisfactory results. Also, **Herńndez et al (2000), Miguel et al., (2001), Spielholz, (1998)** and **Youseif et al. (2001)** who concluded that1% Virkon is effective only against vegetative bacteria, yeasts and viruses, and should therefore be considered a low-level disinfectant.

Moreover, **Willinghan et al. (1996)** sampled three commercial chicken hatcheries for environmental bacteria and tested isolated bacteria for resistance to commercial preparations of quaternary ammonia, phenolic, and glutaraldehyde liquid disinfectants.They found that approximately 8% of the isolates were resistant to disinfectant concentrations at and above the manufacturers recommended dilution and time of exposure. Meanwhile **Gasparini et al. (1995)** showed that Virkon has a high concentration coefficient (mean value of k: 0.374/ min) and a wide range of action and affect wide rang of bacteria.

Conclusion:

- Presence of many pathogenic bacteria in hatcheries indicated that the hygienic measures remains unsatisfactory so, cleaning and disinfecting program is very important for hatcheries.
- Isolated bacteria from hatcheries have variations in sensitivity to the commonly used disinfectants and may be referred to prolonged use of this disinfectant.
- It was clear that disinfectants should be used subsequent to the cleaning and removal of most of organic material on the surfaces subjected to sanitation.
- Most of examined bacteria needed increased contact times with the disinfectant or may need higher disinfectant concentration.
- VIROCID® disinfectant showed high efficiency against examined bacteria at recommended dilutions and exposure time incomparable with Virkon-s which recorded unsatisfactory results in the present work.
- Fumigation is a cheaper disinfectant widely used but it has carcinogenic effect. Thus an alternative way for disinfectant must be applied.
- It can be concluded that VIROCID® may be effective disinfectant for using in the hatchery at a lower cost.

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