



(RESEARCH ARTICLE)



## Field trial to monitor the efficacy of some commercial poultry disinfection on *Salmonella typhimurium* through cold fogging

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### Abstract

The recovered results showed that: the incidence of *Salmonella typhimurium* in the observed commercial egg-layer flocks; the isolation of *Salmonella typhimurium* from 5 flocks (33.7 %) out of 15 flocks, the mortalities rates at the end of 78 weeks of age was 16 % and also, the current egg-production, average egg weight, hen housed day, hen housed egg and percent peak of egg-production were: 68 %,59.2 gm, 68.4%, 318, 78% and 70%, respectively.

The efficacy of the most common disinfectants against *Salmonella typhimurium* was determined. The selected disinfectants were; Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M against *Salmonella typhimurium* isolates of the studied commercial egg- layers in Egypt (*hfq* gene of *Salmonella typhimurium*). It was observed that, *S. typhimurium* affected significantly only by Micro Sept M and Virkon'S, at low rate of application; the Log 10 of populations after 15 min exposure were 4.2, 4.4, respectively the recovered results showed also, that: Formalin, Phenol, Micro Sept M and Virkon'S treatment demonstrated a significant reduction in *Salmonella typhimurium* populations at high rate of application. It is evident from the results using formalin, phenols and QAC at concentration of 4% ,5% and 33.3 % by fogging other than spraying had increased action on the tested pathogens *S. typhimurium* after 15 min contact time (35.7%,42.8 % and 76.8%, respectively).

**Keywords:** *Salmonella typhimurium*; Egg -Layer Flocks; Performance; Disinfection; Method of Application

### 1. Introduction

35 *Salmonella* species were isolated from poultry houses (25%); (6%) and (4%) from cloacal swab; liver and litter, respectively. Average prevalence of *Salmonella* spp. was 11.33 % in open broiler houses (*Salmonella typhimurium*, *S. enteritidis* and *S. kentuckywerethe* [1]. In addition, *Salmonella* can occur in very low numbers in house and still infect chicks [2, 3], and the distribution in a solid is often uneven, so when only a minor fraction of the feed is sampled, *Salmonella* will often be undetected [4].

Airborne *Salmonella* infections between houses or farms are uncommon [5, 6], thus they are mainly introduced with any vehicle that comes into the poultry house. These vehicles either have to enter the poultry house (feed, the poultry itself, water, litter and people who attend to the poultry), or their introduction can be avoided (e.g. wild animals or equipment) by bio-security measures. Though it is difficult to document the infection source in every single case, it is generally agreed that many *Salmonella* infections are introduced either by contaminated feed or the poultry itself [2, 7, 6].

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Many studies have described the presence of various horizontally transmitted serotypes [8, 9, 4], whereas vertically transmitted serotypes such as *S. enteritidis* or *S. Typhimurium* are rarely found the reasons for this difference in occurrence are not known.

The lack of association of the serotypes might indicate the multifactorial epidemiology of Salmonella infections [10-12, 8], and/or it reflects differences in infectious doses and susceptibility in chickens [11-15,6]. Several studies have described Salmonella contamination of water, mainly due to contamination from sewage or sludge [16-18], but this infection source is not common in poultry, although it has been reported [17]. Day-old chicks infected with Salmonella from the hatchery are frequently an important Salmonella source on the farms [19- 21].

In recent years, with the continuous improvement in the intensification of livestock husbandry, the density of housed animals has increased. In addition, the spread of animal infectious diseases is accelerating, and various animal epidemics have emerged [22-29]. Thus, the disinfection of the poultry houses has become an important measure to prevent and control diseases. The commonly used chemical disinfectants for poultry houses include available chlorine, ozone, quaternary ammonium salt, and glutaraldehyde [30-32]. The different disinfectants used for large-scale disinfection of poultry houses operate via different mechanisms, and thus, their disinfection efficacies also differ [33, 32]. Effective sanitation strategies for poultry farms require an appropriate selection of the disinfectant based on the contaminants present and their sensitivity to the disinfectants.

The current study was conducted to Evaluate some commercially available disinfectants against *Salmonella typhimurium* through different field experiments and by different methods of application (Low & high rate of spray and cold fogging).

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## 2. Material and methods

### 2.1. Isolation of *Salmonella typhimurium* and its effect on performance.

The current study was a survey conducted to isolate and identify as well as to estimate the effects of the *Salmonella typhimurium* infection on performance of commercial egg -layer hens (egg production parameters, mortality rate).

### 2.2. Egg-layer flocks

15 voluntary commercial egg-layer flocks (Hy-line) were visited between October 2019 and January 2021. Individual cloacal and tracheal swabs were collected from 300-layer hens. The mean flock size was 10,000 birds were kept for eggs production.

### 2.3. Nonstructural properties of the house

Housing System - Floor system on litter; Capacity- 10.000 hens; Density m<sup>2</sup> / bird -7.5 - 8 and Ventilation system -Natural + Fans.

Lighting system included of 16 h light and 8 h dark. Measurements were conducted during spring-summer months. Birds had free access to food and drink. They received all necessary vaccinations except for *Salmonella typhimurium*.

### 2.4. Flock Management

- Vaccination program: Merck vaccine, New Castle vaccine, Gambro vaccine, Infectious Bronchitis, Infectious Laryngotracheitis, Avian Influenza., Pox vaccine.
- Feeding program: The flocks were fed standard diets for commercial -egg layers with contents of necessary nutrients balanced in accordance with the Hyaline recommendations company.
- Disinfection program Cleaning and Disinfection (Preliminary and formalin fumigation).

### 2.5. Samples collection

Cloacal and Tracheal swab samples were collected from the commercial layer flocks (Triple swabs). Swab samples, the total of 600 samples (tracheal and cloacal were collected from the commercial layer flocks. Samples were collected aseptically and transferred immediately into sterile Petri-dishes. The samples were then brought to the laboratory. Various bacteriological and biochemical examinations were carried out to collected samples. Case history and the performance of each flock were recorded.

## 2.6. Detection of *Salmonella* spp.

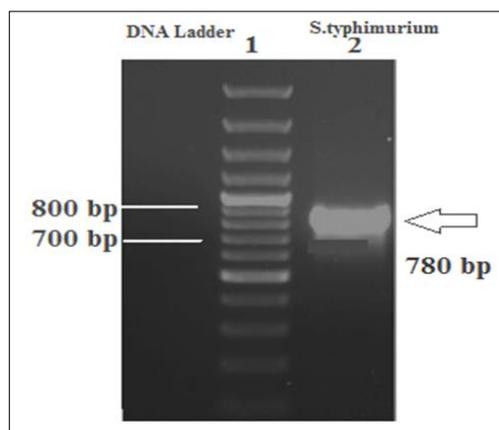
Samples were sent on ice to the Laboratory, stored overnight, and tested the following day. Testing for *Salmonella* spp. was performed according to the standardized methods currently used in the European Union (ISO 6579: 2002 for ISO 6579: 2002/Amd.1: 2007 for fecal samples. Briefly, swab samples were added to 10 ml BPWW, and incubated for 37°C ± 1°C for 18 h ± 2 h for pre-enrichment. *Salmonella* spp. isolation buffered with peptone water or tetrathionate (TT) are used as the enrichment broth, and brilliant green agar, and xylose lysine deoxycholate (XLD) agar are used as selective media [Singh et al., 2010; Abd-ElGhany et al., 2014; Nazer et al., 2006] [34-36].

## 2.7. Identification of *S. typhimurium* by PCR.

The isolates of *Salmonella typhimurium* strains were culture by growing in LB broth at 37°C overnight. Hektoen enteric agar (HEA) media was used to isolate pure colonies of the bacteria through streaking a loopfull of the broth culture. Further cultures were confirmed by conventional biochemical methods (Cardona-Castro et al., 2002) [37]. The genomic DNA was isolated from the culture as per the standard protocol (pure link genomic DNA isolation kit, Invitrogen, USA). The integrity of the DNA was also checked by running on 1% w/v agarose gel. Polymerase chain reaction (PCR) was carried out using gene-specific primer (Table 1). The primers of *hfq* gene (309 bp) of *Salmonella typhimurium* (Behera et al., 2015) [38]. The PCR product was checked by electrophoresis on 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) under UV transilluminator.

**Table 1** Designed primers of *hfq* gene of *Salmonella typhimurium*

Agent	Gene	Primer	Reference
Forward primer	<i>hfq</i>	5' GGAAGGATCCATGGCTAAGGGGCAATCT 3'	Behera et al., 2015
Reverse primer	<i>hfq</i>	5'GCGCGTCGACTTATTCAGTCTCTTCGCTGTC 3'	



**Figure 1a** Polymerase chain reaction (PCR) for detection of *S. typhimurium*  
Lane 1 DNA Ladder 1000 bp, Lane 2: PCR product of isolates

## 2.8. Evaluate some commercially available Disinfectants

*Salmonella Typhimurium* culture was growing in brain-heart infusion broth and incubated overnight at 37°C. The overnight culture was used to inoculate fresh brain-heart infusion broth for 2 additional 24-h transfers.

On the day of the trial, 1 mL of the overnight culture was diluted with of BPD to create a 10<sup>6.8</sup> cfu/ml concentration. Both the overnight culture and diluents were serially diluted in BPD and spread plated on XLT4 agar and incubated 24 h at 37°C to verify cell counts.

## 2.9. Bacterial Inoculation into plots of the floor

For each experimental plot the inoculums (*S. synhymenium*; 10<sup>7</sup> per ml) were applied via pipette, and the inoculation rate of 40 ml was chosen due to its ability to create a good surface coverage. Whereas the positive control plots received 40 mL/plot of distilled water.

## 2.10. Experimental test

Experimental test units were 1-ft<sup>2</sup> floor plots randomly blocked with a 1-ft<sup>2</sup> space between each experimental plot. The treatments consisted of 8 different disinfectants, which included: Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M and a control.

Each disinfectant was prepared according to the manufacturers' recommendations using distilled water (Formalin 4 % (v/v) , Phenol 5 % (v/v), Diluted 1: 3 ,Halamid diluted 1: 18, Virkon S 1% (w/v) potassium peroxymonosulfate and sodium chloride in H<sub>2</sub> o, Micro Sept M 1: 5 (for spraying)).

## 2.11. Application of the disinfectants (Payne et al., 2005[39])

Six treated plots for each bacterial pathogen and for each experimental trial, received each tested disinfectant alone. Two untreated plots, receiving no disinfectant, served as the negative control group.

- Low rate application (coarse spray) 55 ml/plot. The rate was chosen due to its ability to create a good surface coverage.
- High rate of application (fine spray) 125 ml/plot. The rate of 125 ml was chosen because it correlated to a common disinfectant usage level of 500 gal/16,000 ft<sup>2</sup> [39].
- Cold fogging 125 ml/plot was chosen because it correlated to a common disinfectant usage level. Disinfectant Fog Machine of nano-atomizer adjustable fogger.

### 2.11.1. Sampling

In the trial (a 5 × 2 factorial design), none-half of the plots for each disinfectant were sampled 15-min post-application with the remaining half sampled 6-h and 24-h post-application. Surface samples were taken using cellulose drag sponges contained in sterile whirl pack bags [40] that were hydrated with 20 ml of laboratory prepared Butterfield's phosphate diluents (BPD) [41] prior to sampling. Sponges were aseptically removed from each bag by the string and used to sample the surface of the plot. A 1: 10 dilution was then prepared by placing each sponge into sterile bottles containing 180 ml of BPD. Samples were immediately stored in a cooler with ice packs and transported to the laboratory.

### 2.11.2. Counting

All samples were shaken vigorously and then cultured to determine plate counts of *bacteria* (*Salmonella typhimurium*).

- Petri-film was used in accordance with the manufacturer's instructions to determine Salmonella counts. Serial dilutions of BPD were made, and 1 mL was transferred onto the appropriate Petri-film.

Counting of *Salmonella* was determined in accordance with the US Department of Agriculture and the Food Safety Inspection Service Microbiology Laboratory Guidebook with the addition of the drag swab as a medium for sample collection. Briefly, BPD samples were incubated at 37°C for 24 h, and then 0.5 mL was transferred into 10 mL of tetrathionate broth for salmonella, and 0.1 mL was transferred into 10 mL of Rappaport Vassiliadis [42] broth followed by a 24-h incubation at 42°C. Both broths were then streaked onto xylose lysine tergitol 4 (XLT4), brilliant green sulfa, and modified lysine iron agar plates and incubated at 37°C for 24 h. Suspect colonies were inoculated onto triple sugar iron agar and lysine iron agar slants and incubated at 37°C for 24 h. Negative controls were used for all plating procedures to ensure that the media had been properly sterilized.

## 2.12. Statistical analysis

Data were converted to log<sub>10</sub> values prior to analysis. Individual plots were the experimental units. Disinfectant and exposure time were the main effects for factorial analysis of the field trials. For the trials, disinfectants were compared using a 1-way ANOVA. Variables having a significant *F*-test were compared and were considered to be significant at *P* < 0.05.

$$\text{Percent Reduction} = \frac{A - B}{A} \times 100$$

Where: A is the number of microorganism before treatment: is the number of microorganism

After treatment.  $\log_{10} \left( \frac{A}{B} \right)$

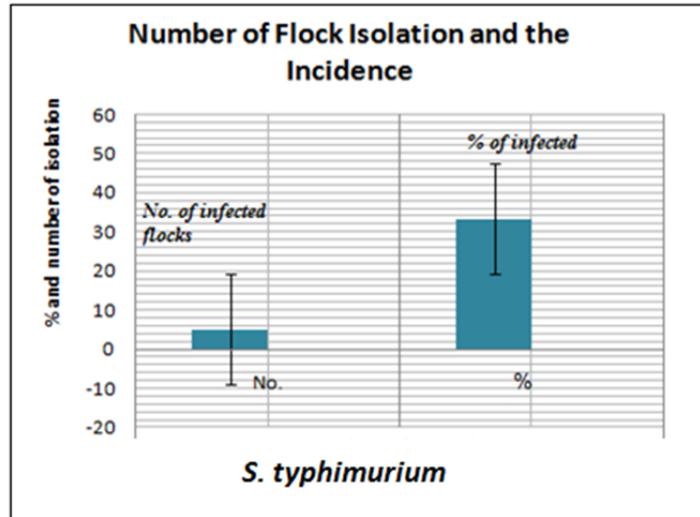
B

### 3. Results

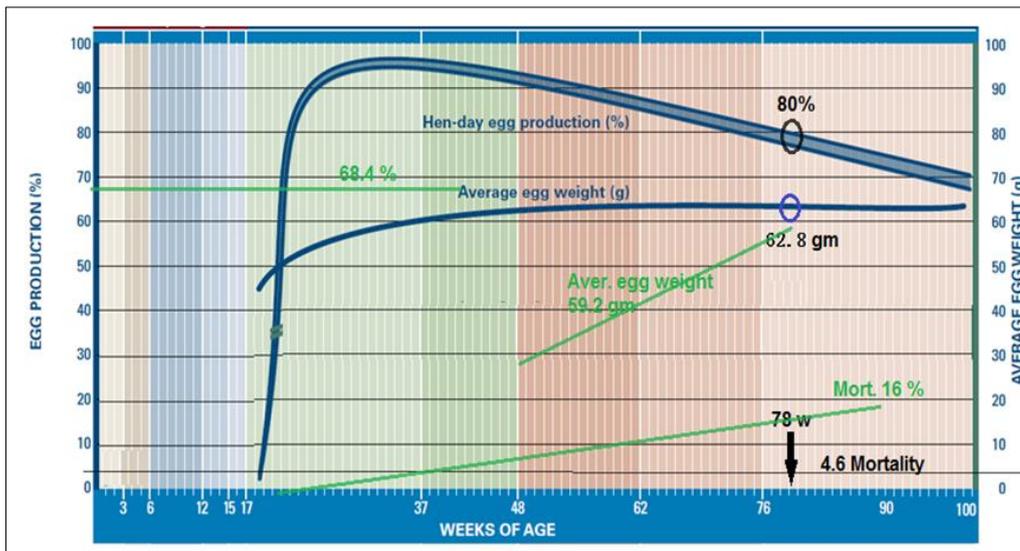
**Table 2** The incidence of *S. typhimurium* in observed commercial egg-layer flocks

The incidence	No. of infected flocks	Percent
<i>S. typhimurium</i>	5	33.3 %

\*Number of infected flocks = 5 (33.3 %), \* Number of studied flocks = 15



**Figure 2** The incidence of *S. typhimurium* in observed commercial egg-layer flocks: The results showed that: *S. typhimurium* were isolated from 5 commercial egg-layer flocks (33.3 %) out of 15 flocks



**Figure 3** The results revealed that, there were a significant difference ( $P < 0.05$ ) among the control flocks and the infected ones by *S. typhimurium*. In mortality rate (4.6, 16%), current percent egg-production (81, 68), average egg weight (62.8, 59.2, gm), hen housed day (80 %, 68.4 %), hen housed egg (351.7 –362.4, 318,) and percent peak of egg-production (95–97 %, 78 %).

**Table 3** Effect of *Escherichia coli* infection on egg production and mortality

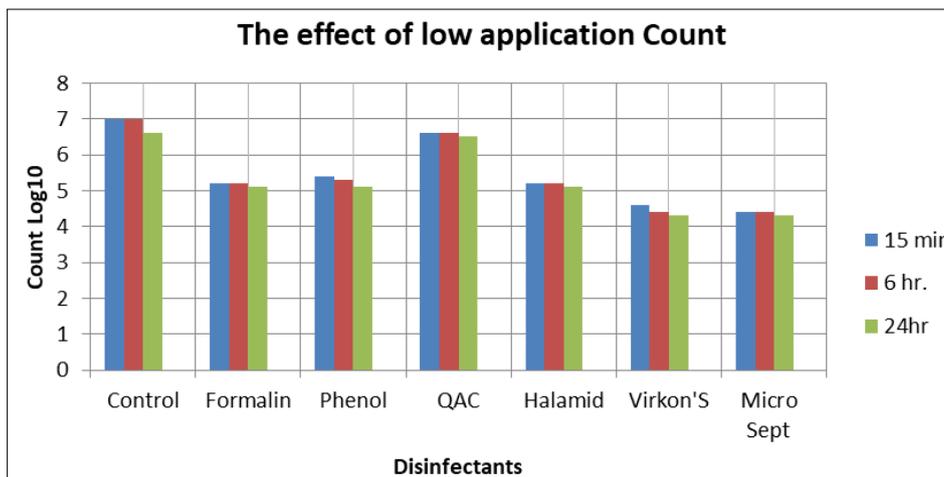
Performance Pathogens	Egg production at 78 W Average					Aver egg production	Mort
	Current %	Aver. egg Weight.	Hen housed day	Hen Housed egg	Percent Peak	Cycle of production	At 78 W Aver %
Control	78	62.8	80 %	351	95%	86 %	4.6
<i>S. typhimurium</i>	68	59.2	68.4%	318	78%	70%	16%

**3.1. Effect of rate of application and exposure time of the disinfectant on *Salmonella typhimurium* of poultry floor.**

**Table 4** The effect of low rate of application and exposure time on *Salmonella typhimurium* of poultry floor

Mic Time	<i>S. typhimurium</i> Count		
	15 min	6 hr.	24hr
Control	7	7	6.6
Formalin	5.2 <sup>b</sup>	5.2 <sup>b</sup>	5.1 <sup>b</sup>
Phenol	5.4 <sup>b</sup>	5.3 <sup>b</sup>	5.1 <sup>b</sup>
QAC	6.6 <sup>b</sup>	6.6 <sup>b</sup>	6.5 <sup>b</sup>
Halamid	5.2 <sup>b</sup>	5.2 <sup>b</sup>	5.1 <sup>b</sup>
Virkon'S	4.6 <sup>a</sup>	4.4 <sup>a</sup>	4.3 <sup>a</sup>
Micro Sept M	4.4 <sup>a</sup>	4.4 <sup>a</sup>	4.3 <sup>a</sup>

A–b Column values with different superscripts differ significantly ( $P < 0.05$ ). 55-ml application rate per plot (surface coverage). 2n = 12 plots per disinfectant in the floor.



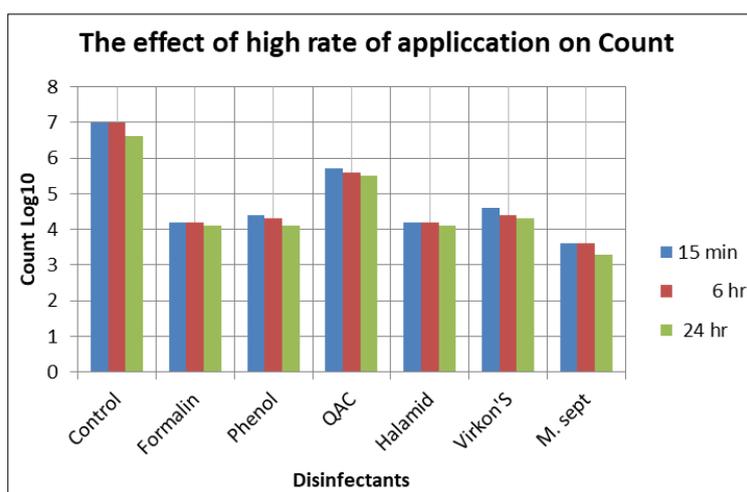
**Figure 4** The effect of low rate of application and exposure time on *Salmonella typhimurium* of poultry floor

**Table 5** The effect of high rate of application and exposure time on *Salmonella typhimurium* of poultry floor house

Mic	<i>S. typhimurium</i> Count
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Time \ Disinfectant	15 min	6 hr.	24hr
Control	7	7	6.6
Formalin	4.2 <sup>a</sup>	4.2 <sup>a</sup>	4.1 <sup>a</sup>
Phenol	4.4 <sup>a</sup>	4.3 <sup>a</sup>	4.1 <sup>a</sup>
QAC	5.7 <sup>b</sup>	5.6 <sup>b</sup>	5.5 <sup>b</sup>
Halamid	4.2 <sup>a</sup>	4.2 <sup>a</sup>	4.1 <sup>a</sup>
Virkon'S	4.6 <sup>a</sup>	4.4 <sup>a</sup>	4.3 <sup>a</sup>
Micro- Sept M	3.6 <sup>a</sup>	3.6 <sup>a</sup>	3.3 <sup>a</sup>

a–b Column values with different superscripts differ significantly ( $P < 0.05$ ). 125-ml application rate per plot (common usage level of 500 gal/16,000 ft<sup>2</sup>). 2n = 12 plots per disinfectant in the floor

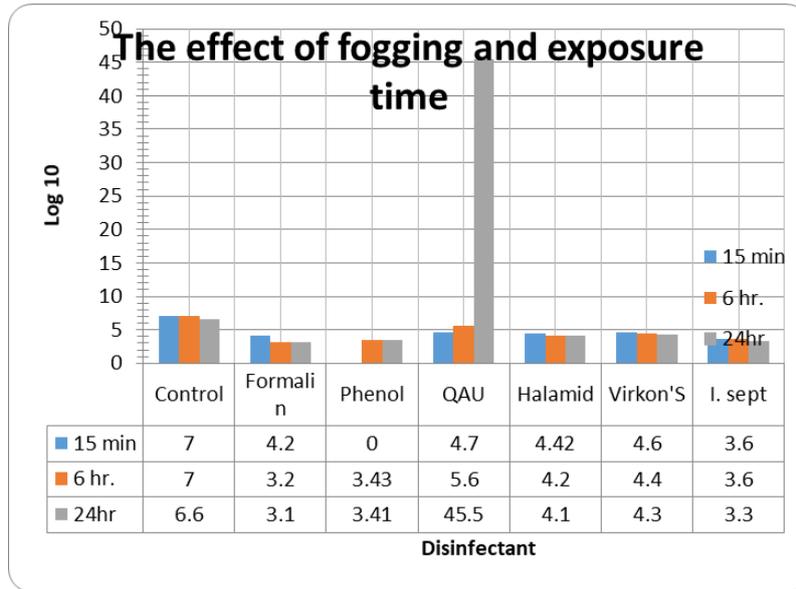


**Figure 5** The effect of high rate of application and exposure time on *Salmonella typhimurium* of poultry floor

**Table 6** The effect of fogging application and exposure time on *Salmonella typhimurium* of poultry floor

Mic Time	<i>S. typhimurium</i> Count		
	15 min	6 hr.	24hr
Control	7	7	6.6
Formalin	4.2 <sup>a</sup>	3.2 <sup>a</sup>	3.1 <sup>a</sup>
Phenol	4.4 <sup>a</sup>	3.43 <sup>a</sup>	3.41 <sup>a</sup>
QAC	4.7 <sup>a</sup>	5.6 <sup>b</sup>	5.5 <sup>b</sup>
Halamid	4.42 <sup>a</sup>	4.2 <sup>a</sup>	4.1 <sup>a</sup>
Virkon'S	4.6 <sup>a</sup>	4.4 <sup>a</sup>	4.3 <sup>a</sup>
Micro-Sept M	3.6 <sup>a</sup>	3.6 <sup>a</sup>	3.3 <sup>a</sup>

a–b Column values with different superscripts differ significantly ( $P < 0.05$ ). A 125-ml application rate per plot (common usage level of 500 gal/16,000 ft<sup>2</sup>). 2n = 8 plots per disinfectant. Control: *S. typhimurium* = 7

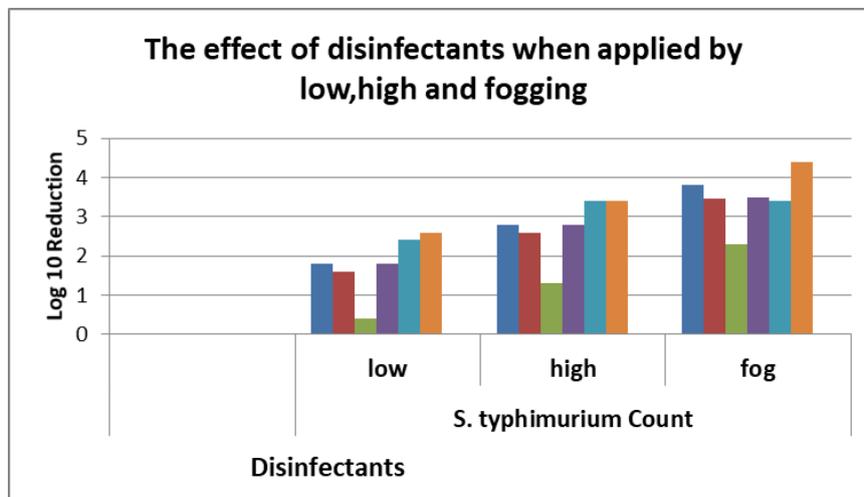


**Figure 6** The effect of fogging application and exposure time on *Salmonella typhimurium* of poultry floor

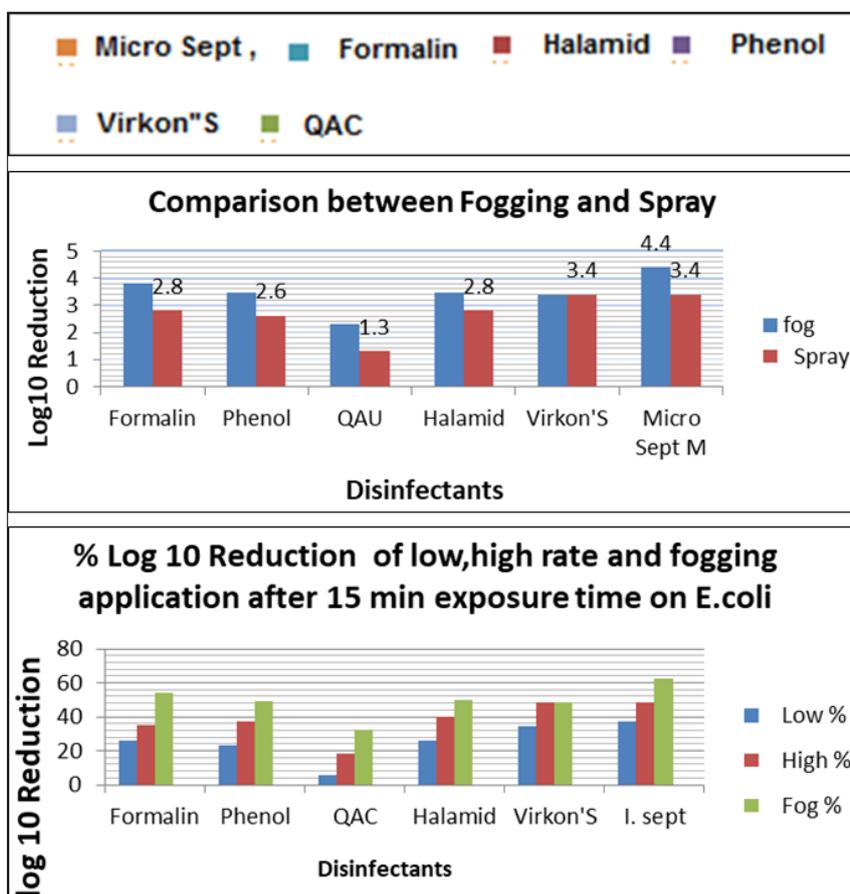
**Table 7** The effect of disinfectants exposure time (15min) when applied at low, high and fogging application rates on *S. typhimurium* populations obtained from a poultry house floor (log10 reduction)

Method of appl.	<i>S. typhimurium</i> Count		
	low	high	fog
Formalin	1.8 <sup>b</sup>	2.8 <sup>a</sup>	3.8 <sup>a</sup>
Phenol	1.6 <sup>b</sup>	2.6 <sup>b</sup>	3.46 <sup>a</sup>
QAU	0.4 <sup>b</sup>	1.3 <sup>b</sup>	2.3 <sup>b</sup>
Halamid	1.8 <sup>b</sup>	2.8 <sup>b</sup>	3.48 <sup>a</sup>
Virkon'S	2.4 <sup>b</sup>	3.4 <sup>a</sup>	3.4 <sup>a</sup>
Micro Sept M	2.6 <sup>b</sup>	3.4 <sup>a</sup>	4.4 <sup>a</sup>

A–b Column values with different superscripts differ significantly ( $P < 0.05$ ). 2n = 8 plots per disinfectant. Control: *S. typhimurium* =7



**Figure 7** The effect of disinfectants exposure time (15min) when applied at low, high and fogging application rates on *S. typhimurium* populations (log10 reduction)



**Figure 8** While, using Formalin, Phenols, QAC, Halamid, Virkon'S and Micro-Sept by fogging other than spraying the efficacy of the disinfectants were increased on the tested pathogen *S. typhimurium* after 15 min contact time

## 4. Discussion

### 4.1. Identification of *S. typhimurium* by PCR.

*Salmonella typhimurium* isolates of the studied commercial egg-layers in Egypt were contained 309 bp (*hfq* gene of *Salmonella typhimurium*) (Table 1 and Fig.1)

### 4.2. Incidence of *S. typhimurium*

*Salmonella* spp. are utmost enteric pathogens, they have high morbid manifestation. *Salmonella* in poultry acts as an important reservoir for other animal and humans. *Salmonella* can cause clinical or sub-clinical disease or asymptomatic infection in animals. Infected poultry showed reduction in eggs of layer flocks and marked gross lesions in infected birds.

The incidence of *S. typhimurium* in studied commercial egg-layer flocks; the isolation of *E. coli* was from 5 commercial egg-layers flocks (33.3 %) out of 15 flocks, (Table 2 and Fig.2). This indicated that, the pathogen's horizontal transmissibility characteristics among birds of a same flock.

*S. typhimurium* was isolated from the lesions of the infected layer-birds. Serotype strains that belonged to somatic groups' of no previous clinical manifestations, they were characterized severe lesions of septicemia and fibrinous polyserositis and sudden mortality which may reach to 4.0% or more [13].

### 4.3. Effect of *S. typhimurium* infection on egg production and mortality

The results revealed that, there were a significant difference ( $P < 0.05$ ) among the control flocks and the infected ones by *S. typhimurium*. in mortality rate (4.6, 16%), current percent egg-production (81, 68), average egg weight (62.8,

59.2, gm), hen housed day (80 %, 68.4%), hen housed egg (351.7 –362.4, 318,) and percent peak of egg-production (95–97 %, 78%). (Table 3 and Fig.3).

Salmonellosis is one of the most prevalent food borne illnesses. The outbreak of this disease is often associated with eggs, the prevalence of *Salmonella* surveyed layer farms in Korea, were; Of 32 farms and 67 flocks examined, 19 farms (59.3%) and 34 flocks (50.7%) were observed to be positive for *Salmonella* contamination [43].

Twenty-three different serotypes were identified, with *S. Kentucky* and *S. Isangi* as the most prevalent (32.9% and 11%). Serotypes showed some geographic variation. *Salmonella* detection was strongly associated with disposal of poultry waste and with presence of other livestock on the farm. *Salmonella* was commonly detected on commercial poultry farms in North West Nigeria [44].

The majority of the outbreaks occur around the period of peak egg production, which is believed to be an important stress factor contributing to the disease but continues as the flock ages. Cumulative mortality is normally between 5 and 10% during a single outbreak [45, 46]. However, the proportion of hens that suffer from the disease often reaches more than 50% [47].

Airborne *Salmonella* infections between houses or farms are uncommon [5, 42], thus they are mainly introduced with any vehicle that comes into the poultry house. These vehicles either have to enter the poultry house (feed, the poultry itself, water, litter and people who attend to the poultry), or their introduction can be avoided (e.g., wild animals or equipment) by bio-security measures. Though it is difficult to document the infection source in every single case, it is generally agreed that many *Salmonella* infections are introduced either by contaminated feed or the poultry itself [3, 5, 48]. Several studies have described *Salmonella* contamination of water, mainly due to contamination from sewage or sludge [15, 16, 13, 48, 6]. but this infection source is not common in poultry, although it has been reported [17].

In addition, *Salmonella* can occur in very low numbers in feed and still infect chicks [2, 3], and the distribution in a solid is often uneven, so when only a minor fraction of the feed is sampled, *Salmonella* will often be undetected [4].

#### 4.4. Identification of the isolates

##### 4.4.1. Evaluation the efficacy to reduce *Salmonella typhimurium*

###### Low rate of application

Concerning *Salmonella typhimurium*, it was observed that it affected significantly only by Micro Sept M and Virkon'S, the Log 10 of populations after 15 mint exposure were 4.2, 4.4, respectively as shown in (Table 4 and Fig. 4).

The lack of response for the disinfectant treatment is in agreement with literature, which states that most disinfectants do not perform well when applied at low rate of application or in the presence of organic material [33, 32, 49]. Not all products work the same on different species of microbes; therefore, the disinfectant should be tested in the field for the specified application to ensure its effectiveness [50].

###### High rate of application

Disinfectants impacted and affect significantly on *Salmonella Typhimurium* populations at the high application rates, respectively ( $P < 0.05$ ) (Table 5 and Fig. 5).

*Salmonella typhimurium* populations were affected significantly ( $P < 0.05$ ) with Formalin, Phenol, Halamid, Virkon'S and Micro Sept M where their logs 10 were 4.2, 4.4, 4.2, 4.6 & 3.6 respectively. Micro Sept M and Virkon'S treatment demonstrated a significant reduction in *Salmonella typhimurium* populations.

###### Fogging application

Fogging by Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M resulted in the greatest reduction (log10 reduction) in *Salmonella typhimurium* populations (3.38, 3.49, 2.3.6, 3.5 and 5.1) (Table 6 and Fig. 6).

The Micro-Sept M and Virkon'S treatment demonstrated a significant reduction in *Salmonella typhimurium* populations (0.17 log reduction) [51, 52]. Fogging procedures in swine confinements are practical approaches to reduce air contamination. Dust reduction in stables can be achieved by oil fogging [51-52]. In a study, fogging with a combinatory

product with peroxide and anionic detergent in a farrowing and rearing unit resulted in a reduction of ammonia, dust particles and fungal spores [53].

#### 4.4.2. Application Methods and Rate

The lack of response to the disinfectants by the bacterial populations in field trial and 2 is most likely the result of a low disinfectant application rate.

It is evident from the results recorded in (Table 7 and Fig.7 &8) that, using formalin, phenols and QAC at concentration of 4%, 5% and 33.3 % by fogging other than spraying had increased action on the tested pathogens *S. typhimurium* after 15 min contact time (35.7%, 42.8 % and 76.8%, respectively) (Disinfectant efficacy was increased when using high-volume directed mist application of accelerated hydrogen peroxide and peroxymonosulfate disinfectants in a large animal hospital, in addition, surface disinfectant spray followed by hydrogen peroxide decontamination has potential for as the colony-forming units have been reduced further compared to spray alone and even just fog alone for all the various areas that was assessed [54-55].

Fogging machines to transform liquid into droplets that are dispersed into the atmosphere use large volumes of air at low pressures. This type of fogging machine can produce extremely small droplets with diameters ranging from 1–150 µm. Thus, the small sized droplets are less carrier for the applied disinfectants, although they cover the required surfaces

If the droplet diameter is reduced to 10 percent of its original size, then the number of droplets that can be formed will increase a thousand-fold. In droplets consisted of  $10^5$  molecules or more, formed an excess of dielectrons which resulted during the splitting process lead to the liberation of molecular hydrogen and formation of two solvated hydroxide anions.

Aldehydes have a broad spectrum of activity against bacteria, fungi, and viruses) that acts on the outer layer of bacterial cells, causing an inhibitory action on the transport of ions across the cell wall [56, 57] Formaldehyde and phenolic compound were effective in presence of organic matter. The poultry houses and equipment should be fogged with formaldehyde solution which might be repeated after placing the litter <sup>46</sup>. Cold fogging with Virkon S in animal houses and veterinary hospital would include its wide-range anti-bacterial action and reducing working-men power required to disinfect large areas. Also, fogging would potentially minimize microbial contamination in the hard to reach areas [58].

Polyhexamethylene biguanide (PHMB) is a polymeric cationic antimicrobial agent, the active ingredients bind rapidly to the bilayer membrane and, in doing so, displaces the otherwise stabilizing presence of  $Ca^{2+}$ . The hexamethylene groups of the polymer are hydrophobic so sufficiently inflexible and cannot enter into the hydrophobic core of the cell membrane. Therefore, a bridging of adjacent acidic phospholipids is brought about by the interaction of the active ingredients with the cell membrane. One additional feature of this interaction is that it will tend to become concentrated around any points of maximum charge density within the membrane normally carrier or integrated proteins. The result is the loss of their function and cellular leakage.

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## 5. Conclusion

The incidence of *Salmonella typhimurium* in observed commercial egg-layer flocks was 33.3%. This indicated that, the pathogen's horizontal transmissibility characteristics amongst birds of a same flock. The mortality rate, the current egg-production, average egg weight, hen housed day, hen housed egg and percent peak of egg-production were severely affected by *Salmonella typhimurium* infection.

It is important to follow recommended procedures, application rates, and to take into consideration factors, such as water pH, temperature, and surfaces on which application will occur. Generally, the efficiency of disinfectants depends on the concentration, method of application and exposure time. All disinfectants need a minimum time of 5 – 10 minutes to destroy various types of microorganisms. Not all products work the same on different species of pathogens; therefore, the disinfectant should be tested in the field for the specified application to ensure its effectiveness. It is evident that, using Micro Sept M, virkon'S, formalin, phenols and QAC at concentration by fogging other than spraying had increased action on the tested pathogens *S. typhimurium* after 15 min contact time. A successful bio-security program, which regularly is one of the best methods used to reduce the level of pathogens in animal facilities.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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