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Research Article
Monitoring the Effect of Disinfection Methods on *Mycoplasma gallisepticum* in Commercial Layer Farms

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Abstract

Background and Objective: It is important for poultry owners to control disinfectants resistance of *Mycoplasma gallisepticum* circulating amongst poultry farms. On a field level among poultry flocks a survey study was conducted to identify and to estimate the effects of the most common poultry pathogens (*Mycoplasma gallisepticum*) on production performance of commercial layer hens and the efficacy of the most common disinfectants against *Mycoplasma gallisepticum* was determined through traditional and new methods of application against MG strain isolated from commercial layer farms in Egypt and recorded in Gen-Bank. **Materials and Methods:** A survey study was carried out to identify and to estimate the effects of Mycoplasma gallisepticum on production performance of 15 commercial layer flocks. In addition, the efficacy of some commercially available disinfectants against *Mycoplasma gallisepticum* with different application methods was also monitored. **Results:** The results showed that: (1) The prevalence of *M. gallisepticum* (33.3%) and mortality rate (12%) was high in the flocks at 78 weeks of age and the current egg-production performance was also significantly reduced ($p<0.05$). (2) MG isolate accessed on Gen-Bank and coded as; MZ826700 , 26 bp DNA linear BCT 30-SEP-2021, DEFINITION *Mycoplasma gallisepticum* strain EGY2021 mgc2 gene, partial cds. ACCESSION MZ826700-authers: Alfeteehy, N.M., Mohamed, M. and Kaoud, H.A. (3) Fogging method showed the highest reduction in *Mycoplasma gallisepticum* populations. **Conclusion:** *Mycoplasma gallisepticum* infection leads to tremendous economic losses in poultry production as a result of decreased hatchability, egg production and mortalities; it is evident that, fogging will increase the efficacy of the used disinfectants for 15 min contact of exposure time.

Key words: *Mycoplasma gallisepticum*, egg-layer flocks, production performance, commercial poultry farm, mortality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Avian mycoplasmosis was primarily described in turkeys in 1926 and in chickens in 1936[1]. *Mycoplasma* infections are pandemic in multiage layer chicken flocks, with *Mycoplasma gallisepticum* being the species of greatest concern to commercial egg producers[2,3].

Economic losses caused by mycoplasma's infection in chicken and turkey flocks, solely or in conjunction with other organisms are strongly associated with an increase of condemnation rate, decrease in final weight and egg production and poor feed conversion ratio[4-6].

Mycoplasma gallisepticum (Mg) infection usually causes chronic respiratory disease in poultry, it is characterized by respiratory rales, coughing, nasal discharges and an acute-to-chronic infectious disease for chickens and turkeys involving primarily the synovial membranes of joints and tendons sheaths. However, during recent years, Mycoplasma synoviae (Ms) has less frequently been associated with synovitis but more frequently associated with air sacculitis in chickens and sometimes in turkeys[7]. Both egg transmitted and hatchery disseminated diseases are economically important. They lead to huge economic losses in poultry industry as a result of reduced hatchability and egg production, decreased quality of day-old chicks, restarted growth rate, increased costs of control which involve site cleaning and depopulation and increased costs of medication and vaccination[8,9].

Mycoplasma gallisepticum is transmitted vertically (trans-ovarian) from infected parents to progeny and horizontally through contamination of feed, water, infectious aerosols of the environment and by human activity on fomites (shoes, equipment, etc.). When birds are stressed latent infection may be occur through horizontal transmission via aerosols and respiratory route, after which infection and clinical disease spread through the flock latent (in some birds for days to months)[4].

Mycoplasma gallisepticum can survive in varying reservoirs within a poultry farm. Among these reservoirs, food, drinking water, feathers, droppings or dust are the most common[10]. Although *Mycoplasma* spp. has been reported to be airborne transmittable[11,12], the factors affecting *M. gallisepticum* aerosolization from its reservoirs, its dispersion and transmission remain unknown. In laying hen houses, infection by the respiratory pathogen *Mycoplasma gallisepticum* is very common[13]. This pathogen can cause a decrease in laying eggs and their quality, without showing any clinical signs[14].

This study was conducted to isolate and identify mycoplasmas as well as to estimate the effects of *Mycoplasma gallisepticum* on production performance of commercial layer hens and the efficacy of the most common disinfectants against *Mycoplasma gallisepticum* was also determined through traditional and new methods of application.

MATERIALS AND METHODS

This survey was conducted from October 2019 to January 2021. Individual cloacal and tracheal swabs were collected from 300-layer hens. A total of 10,000 birds were kept for eggs production.

Nonstructural properties of the house: A total of 10000 hens were housed on floor system with litter having density 7.5-8 m^2 $bird^{-1}$ and ventilation system. Lighting schedule was 16 h light and 8 h dark. Measurements were conducted during spring-summer season. Birds had free access to food and water. They received all necessary vaccinations except for *M. gallisepticum.*

Flock management:

- **Vaccination program:** Birds were vaccinated against different diseases like Merck, New Castle, Gambro, Infectious Bronchitis, Infectious Laryngotracheitis, Avian Influenza and Pox
- **Feeding program:** The flocks were fed standard commercial diet for layers having balance and necessary nutrients according to the recommendations of Hyaline company
- Cleaning and Disinfection program was implemented

Samples collection: A total of 600 cloacal and tracheal swab samples were collected from the commercial layer flocks (Triple swabs). Samples were collected aseptically and transferred immediately into sterile Petri-dishes. The samples were then brought to the laboratory in the Department of Veterinary Hygiene and Management, Faculty of Veterinary, Cairo University. These samples were subjected to various bacteriological and biochemical examination in the laboratory. Case history and the production performance of each flock was recorded.

Isolation and Identification of *Mycoplasma gallisepticum*: Swabs were streaked on PPLO (pleuro-pneumonia-like organism) agar plates, incubated for 7 days at 37°C. When the

growth of the colonies was obtained, digitonin test was performed to differentiate the colonies of *Mycoplasma* from *Acholeplasma*[15].

Broth culture: Identification of Mg and Ms was made by the growth inhibition test using specific anti-sera (BioChek) as described by Khalifa *et al*.[16] and the rapid serum agglutination tests for the two species. Then, positive cultures were lyophilized and kept in -20°C. Colonies of fried egg appearance on solid media were observed in all cultures. Colonies found sensitive to digitonin, ensuring that they were mycoplasmas. Serological tests include the rapid slide agglutination test, the haemagglutination inhibition test and ELISA for Mg[3] were performed.

***Mycoplasma* isolates:** The examined isolates of this study were *M. gallisepticum* (MG) recovered from layers having respiratory problems, mortalities and reduction of egg production. DNA detection methods based on the polymerase chain reaction (PCR) were used to detect mgc2 gene of MG[17-19]. MG isolate accessed on Gen-Bank and coded as; MZ826700, 26 bp DNA linear BCT 30-SEP-2021, DEFINITION *Mycoplasma gallisepticum* strain EGY2021 mgc2 gene, partial cds. ACCESSION MZ826700-authers: Alfetehy, N.M., Mohamed, M. and Kaoud, H.A.

Identification of *M. gallisepticum* by PCR: The isolates were subjected to identification through species specific PCR consisting of oligonucleotide paired primers sequences source through Midland Certified Company (USA). Genomic DNA was extracted by TIANamp Bacteria DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. PCR reaction was performed in a thermo-cycler PCR machine (BIO RAD T100) in a total of 25 µL. The PCR reaction comprised of the first step of an initial denaturation at 95°C for 3 min followed by 34 cycles with denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec and extension at 72°C for 90 sec and a final extension step of 5 min at 72°C. The expected amplified product was analyzed through agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio Rad, USA). Materials for isolation and bacterial growth were purchased from Oxoid (Oxoid, UK). Primers were synthesized through Invitrogen. PCR master mix was purchased from Bioline (Bioline, London, UK).

- **Monitoring the effectiveness of selected disinfectants on *Mycoplasma gallisepticum***

Experimental tests: The floor of the poultry house was cleaned between feed and water lines. Duplicate rows (Each raw consisted of 8 plots, each plot equal to $1ft^2$) were sterilized by thermal tractor. Mounted flaming devices were used for each disinfectant (One for each application).

In a 5×2 factorial design, one-half of the plots for each disinfectant was sampled 15 min and 6 h post-application. The treatments consisted of 6 different disinfectants, which included: Formalin, Phenol, QAC, Halamid, Virkon'S, 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), QAC Diluted 1:3, Halamid Diluted 1:18, Virkon" S 1% (w/v) potassium peroxymonosulfate and sodium chloride in H_2O, Micro Sept M 1:5 (for spraying and conc. for cold fogging)}.

Preparation of *Mycoplasma gallisepticum*: MG was cultivated for 7-10 days at 37°C +5% CO_2 on mycoplasma agar plates prepared with supplement G (Oxoid, Dardilly, France). Colonies from three to four agar plates were harvested by swabbing into Dulbecco's modi ed Eagle's medium (Gibco) +10% foetal calf serum. The stock culture was diluted in fresh mycoplasma broth medium to give inoculums of $10^{6.2}$ colony forming unit (CFU) per milliliter[20].

Experiment I: Three hours prior to disinfectants treatment, all plots were inoculated with 40 mL $plot^{-1}$ of $10^{6.2}$ CFU mL^{-1}. After 3 h, each disinfectant was applied to the plots as a spray at a high application rate of 125 mL $plot^{-1}$.

Application of the disinfectants[21]: Six treated plots inoculated with the isolated *M. gallisepticum*, received each tested disinfectant alone as a spray at a high application rate of 125 mL $plot^{-1}$. The rate of 125 mL was chosen because it correlated to a common disinfectant usage level of 500 gal/16,000 $feet^2$. Two untreated plots, receiving no disinfectant, served as the negative control group (There were 2 replicate trials per treatment).

Experiment II: As experiment I, six treated plots inoculated with the isolated *M. gallisepticum*, received each tested disinfectant alone as a fog for 5 min. Two untreated plots, receiving no disinfectant, served as the negative control group (There were 2 replicate trials per treatment).

Sampling: Cellulose drag sponges contained in sterile whirl pack bags with 20 mL of Butter field's phosphate diluents (BPD) were used prior to sampling. Placing each sponge into sterile bottles containing 180 mL of BPD (1:10 dilution). Samples were immediately stored in a cooler with ice packs and transported to the laboratory.

Counting: Swabs were stricken into Dulbecco's modi ed Eagle's medium (Gibco) +10% foetal calf serum. The titer of the inoculums was estimated by making appropriate 10-fold dilutions of culture in Frey's broth[22] and then plating six discrete 25 µL drops of each dilution onto surface dried mycoplasma agar plates[23,24]. Plates were incubated at 37°C for up to seven days under reduced oxygen tension and visible colonies were counted.

Statistical analysis: Data were converted to $\log_{10}$ 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 one-way ANOVA. Variables were compared and were considered to be significant at $p<0.05$.

$$\text{Reduction (\%)} = \frac{\text{A- B}}{\text{A}} \times 100$$

Where

A : No. of microorganism before treatment

B : No. of microorganism after treatment

$$\text{Log reduction} = \frac{\log_{10}(\text{A})}{\text{B}}$$

RESULTS AND DISCUSSION

Identification of the isolates: MG isolates was accessed on Gen-Bank and coded as; MZ826700, 26 bp DNA linear BCT 30-SEP-2021, DEFINITION *Mycoplasma gallisepticum* strain EGY2021 mgc2 gene, partial cds. ACCESSION MZ826700 (Table 1 and Fig. 1).

Incidence of *M. gallisepticum*: The results indicated a high prevalence of mycoplasma in the evaluated flocks (33.3%), with *Mycoplasma gallisepticum* (MG), mainly in layer hens with respiratory problems (Table 2 and Fig. 2). The high prevalence of mycoplasma indicated the horizontal transmissibility characteristics of pathogens among birds of a same flock. Consequently, mycoplasmosis does not display high horizontal transmissibility, when compared with diseases (Influenza or Newcastle), which may infect even up to 100% of birds of a same flock within a few days. Considering the already acknowledged vertical transmission of this disease[23], the prevalence of the mycoplasmas in the layers (33.3%) indicates continuous dissemination in the commercial farms increasing the occurrence of disease and economic losses.

Effect of *M. gallisepticum* on egg production and mortality: The mortality rate (4.6 and 12%), current percent egg-production (81, 63.5), average egg weight (62.8, 58.2 g), hen housed day (80 and 71%), hen housed egg (362.4, 320) and peak of egg-production (97 and 80%) was significantly different between control and infected flocks (Table 3, Fig. 3a-b).

Table 2 and Fig. 2 shows the mortality rates, the current egg-production, average egg weight, hen housed day, hen housed egg and percent peak of egg-production at the end of 78 weeks of age.

Table 1: Identification of *Mycoplasma gallisepticum* by PCR. The isolates were subjected to identification through specie specific PCR. Primer pair. Oligonucleotide primers Sequences, Source: Midland Certified Company (USA)

Agent	Gene	Sequence	Amplified product	References
MG	Mgc2	CGCAATTTGGTCCTAATCCCCAACA TAAACCCACCTCCAGCTTTATTTCC	300 bp	Lysnyansky *et al.*,[18]
MS	!6SRNA	GAGAAGCAAAATAGTGATATCA CAGTCGTCTCCGAAGTTAACAA	210pb	OIE,[19]

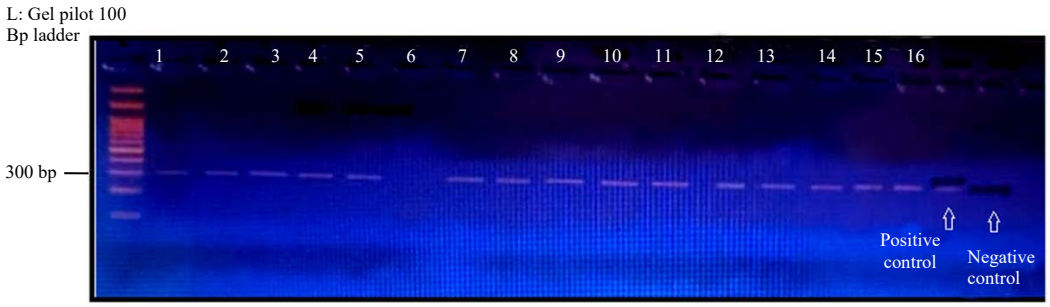

Fig.1: Amplified PCR product of molecular size of 300 bp using primer of Mgc2 Gene of *M. gallisepticum*
L: Gel pilot 100 bp ladder. !-16: positive Mgc2 gene of *M. gallisepticum* except 6 was negative. Pos: Control positive *M. gallisepticum*

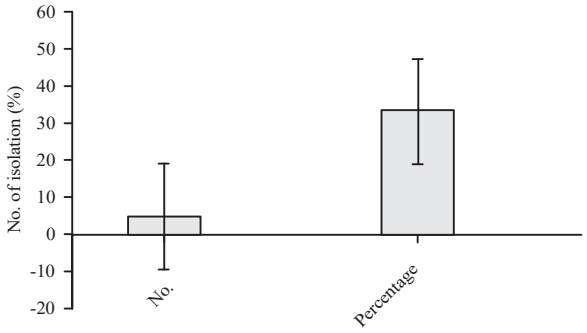

Fig. 2: The incidence of *M. galisepticum* in observed commercial egg-layer flocks

The results showed that: *Mycoplasma gallisepticum* was isolated from 5 commercial egg-layer flocks (33.3%) out of 15 flocks

Mycoplasma gallisepticum is commonly involved in the polymicrobial "chronic respiratory disease" in chickens, leading to increased condemnations in the processing plant. In layers and breeders, it is usually subclinical but causes a reduction in the number of eggs laid per hen over the production cycle. In laying hen houses, infection by the respiratory pathogen *Mycoplasma gallisepticum* is very common[13,24]. The most important pathogens associated with avian mycoplasmosis were *M. gallisepticum* and *M. synoviae*. *M. gallisepticum* producing an infectious contagious avian respiratory disease with a large range of clinical lesions as increase of mortality%, decrease eggs and meat production, decrease of fertility and hatchability%, combined with high cost of treatment and control[25].

Mycoplasma gallisepticum can survive in varying reservoirs within a poultry environment. Among these reservoirs, food, drinking water, feathers, droppings or dust are the most common[10].

- **Efficacy of some commercially available disinfectants on *Mycoplasma gallisepticum***

Effect of high rate of application:

- **Fifteen min exposure:** Disinfectants (Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M) decreased the count of MG where; the log_{10} count values were 3.2, 4.2, 5.2, 4, 3.75 and 3.52, respectively).

Formalin, Phenol, Virkon'S and Micro Sept M significantly decreased ($p<0.05$) the count of *M. gallisepticum* populations after 15 mint exposure as compared to the control (Table 4 and Fig. 4). The Reduction percent of the bacterial population for Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M were; 48.49, 32.26, 16.13, 35.48, 38.83 and 40.32%, respectively. Formalin, Micro Sept M and Virkon'S treatment demonstrated a significant reduction in *M. gallisepticum* populations.

Table 2: The incidence of *Mycoplasma galisepticum* in observed commercial egg-layer flocks

The incidence	No. of infected flocks	Percentage
M. galisepticum	5	33.3

*No. of infected flocks: 5 (33.3 %). *No. of studied flocks: 15

- **Six h exposures:** Disinfectants (Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M) decreased the count of MG where; the log_{10} count values were 3.1, 2, 1.1, 2.2, 2.46 and 2.52log10, respectively). The Reduction percent for Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M were; 50, 32.26, 17.74, 35.48, 39.68 and 44%, respectively. These results indicated that, there was no difference between 15 min and 6hrs of exposure time

Effect of cold-fogging application: Fogging by Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M resulted in the greatest reduction in *M. gallisepticum* count where; the log_{10} reduction in count were 3.38, 3.49, 2,3.6, 3.5 and 5.1) (Table 5 and Fig. 5).The Micro Sept M, Formalin, Virkon'S, Halamid and Phenol, treatment demonstrated a significant reduction in *M. gallisepticum* populations. The Reduction percent were 59.39, 48.38, 39.51, 35.48 and 32.25%, respectively as shown in Fig. 6. Fogging procedures in swine confinements are practical approach to reduce air contamination. It is evident that as compared to spraying, fogging by Micro Sept M (PHMB), Formalin, Virkon'S, Halamid and Phenol, at the same concentration had increased action on the tested pathogens *M. gallisepticum* after 15 min contact time[26,27].

The recent reports showed that most of the poultry farms do not practice the benchmark guidelines of biosecurity[28]. Spraying disinfectants in sheds and removing feces were the only sanitation schemes adopted in the farms[29,30]. Even these Disinfectants are used without regular evaluation and adequate validation where the efficacy of the disinfectants is influenced by formulation, level of organic load, humidity, temperature, dilution rate, pH and hardness of water and many other factors[31-33]. So, the evaluation of the disinfectants' efficacy should be in priority to select the suitable disinfectant

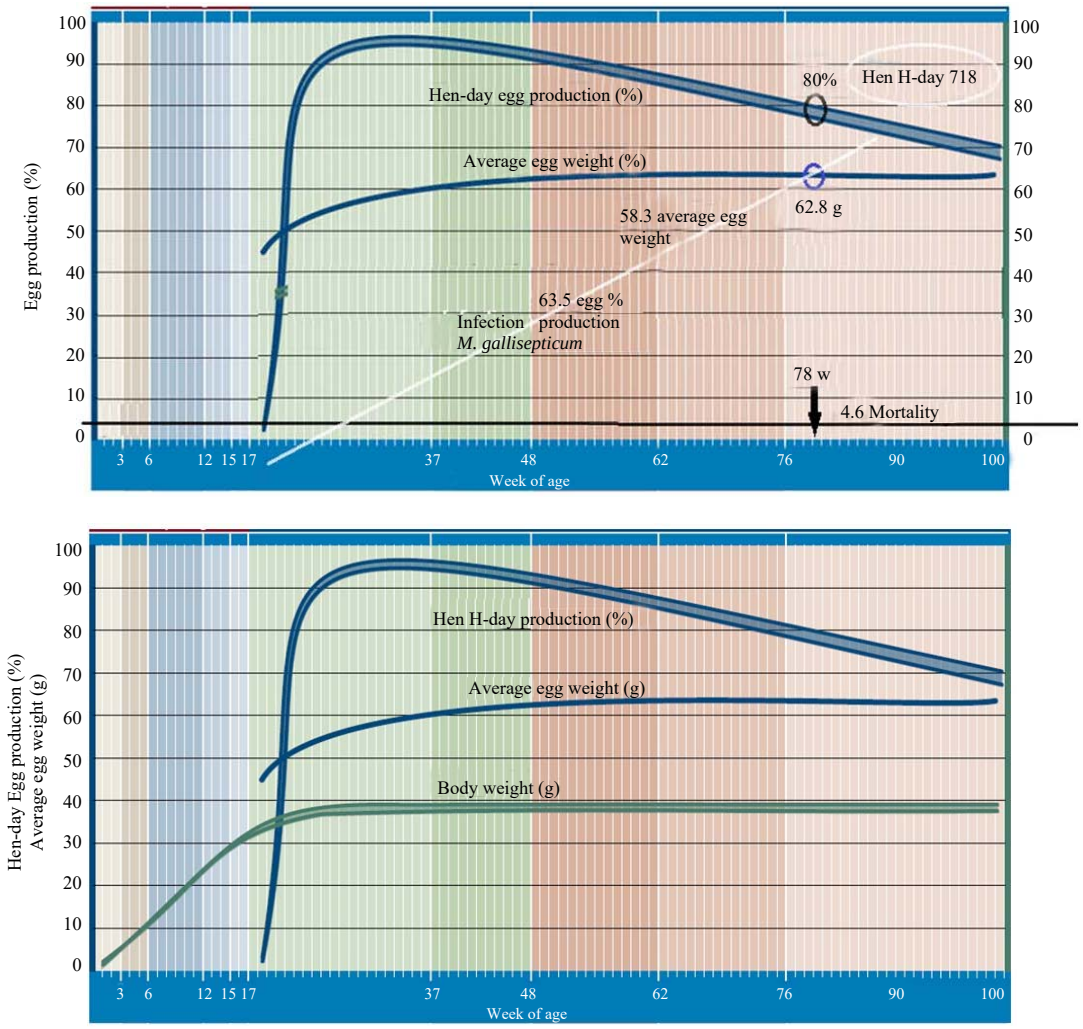

Fig. 3(a-b): (a) Standard egg production performance and mortality on commercial egg-layer flocks, (b) Effect of *M. galisepticum* on egg production performance and mortality in commercial layer flocks

Table 3: Effect of *M. galisepticum* on egg production performance and mortality in commercial layer flocks

	Average egg production at 78 weeks					Average egg production	Mortality
Parameters	Current (%)	Average egg weight	Hen housed day (%)	Hen housed egg	Peak (%)	Cycle of production (%)	At 78 w Average (%)
Control	78-81	62.8	80	351.7-362.4	95-97	86	4.6
M. galisepticum	63.5	58.2	71	320	80	72	12

to minimize the microbial load before slaughtering and processing of the carcasses. Disinfectant efficacy was increased when high-volume directed mist application of accelerated hydrogen peroxide and peroxymonosulfate disinfectants were used in a large animal hospital[34-37]. So, it is important to select the suitable disinfectant that has the ability to reduce the pathogens load before raising the birds. The efficacy of the disinfectants was accelerated and increased through the application of the disinfectants huge directed mist in animal houses and hospitals, where, hydrogen peroxide and peroxymonosulfate disinfectants were used[34-37].

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

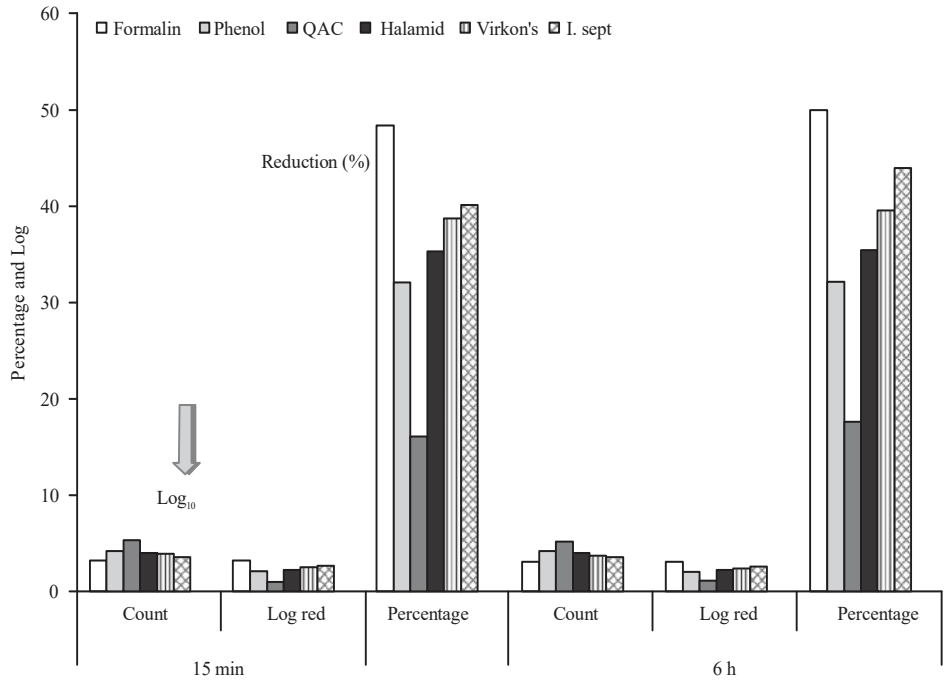

Fig. 4: The effect of high rate of application and exposure time on *Mycoplasma gallisepticum* of poultry floor

Table 4: The effect of high rate of application and exposure time on *Mycoplasma gallisepticum* in poultry floor

	15 min			6 h		
		Reduction			Reduction	
Disinfectants	Count: log_{10}	Log	Percentage	Count: log_{10}	Log	Percentage
Formalin	3.2[a]	3	48.49	3.1[a]	3.1	50.00
Phenol	4.2[ab]	2	32.26	4.2[ab]	2	32.26
QAC	5.2[c]	1	16.13	5.1[c]	1.1	17.74
Halamid	4.0[ab]	2.2	35.48	4.0[ab]	2.2	35.48
Virkon'S	3.75[a]	2.45	38.83	3.74[a]	2.46	39.68
I. sept	3.52[a]	2.5	40.32	3.51[a]	2.52	44.00
Control	6.2					

[a-c]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^2). 2n = 16 plots per disinfectant in the floor

Table 5: The effect of fogging application and exposure time on *Mycoplasma gallisepticum* of poultry floor

	15 min			6 h		
		Reduction			Reduction	
Disinfectants	Count: log_{10}	Log	Percentage	Count: log_{10}	Log	Percentage
Formalin	3.20[a]	3.00	48.38	3.91[a]	2.29	36.94
Phenol	4.20[ab]	2.00	32.25	3.32[a]	2.90	46.77
QAC	5.72[c]	0.48	7.74	5.71[c]	0.49	7.90
Halamid	4.00[ab]	2.20	35.48	4.00[ab]	2.20	35.48
Virkon'S	3.75[a]	2.45	39.51	3.84[a]	2.36	38.00
I. sept	2.52[a]	3.68	59.39	2.51[a]	3.69	59.52
Control	6.20					

[a-c]Column values with different superscripts differ significantly ($p<0.05$). 1A 55-mL application rate per plot (common usage level of 500 gal/16,000 ft^2). 2n = 16plots per disinfectant. Control: *M. gallisepticum* = 6.2

produce extremely small droplets with diameters ranging from 1-150 μm. Thus, the small sized droplets are less carrier for the applies 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 containing 10^5 molecules or more, dielectrons are formed in excess during

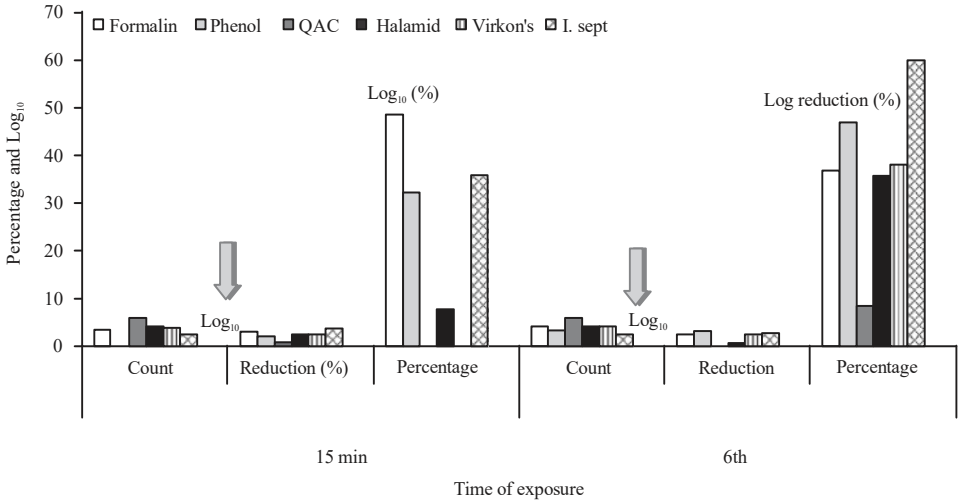

Fig. 5: The effect of fogging application and exposure time on *Mycoplasma galisepticum* of poultry floor

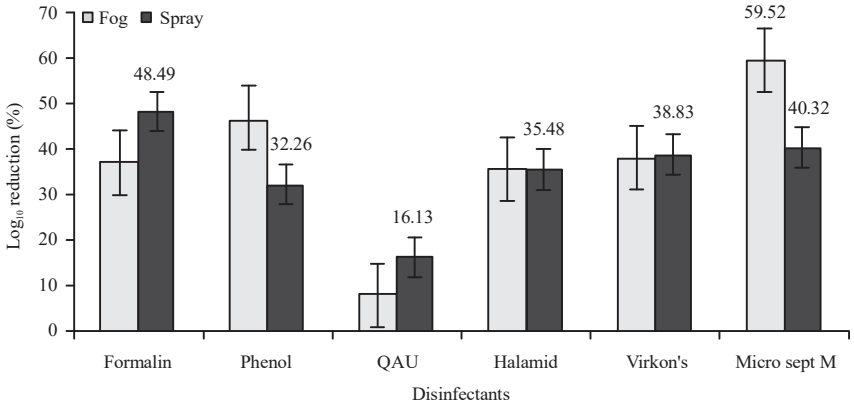

Fig. 6: Comparison between fogging and high rate spray and their effects on *Mycoplasma galisepticum* of poultry floor

the splitting process that lead to the liberation of molecular hydrogen and formation of two solvated hydroxide anions. All disinfectants need a minimum time of 5-10 min to destroy various types of microorganisms in the absence of organic matter[38].

Classes of commercially available disinfectants include aldehydes, halogens, peroxides, quaternary ammoniums, phenols and oxidizers. It was problematic to compare the efficacy of different disinfectants used on the farms due to inadequate application of the products[39] Disinfectants are efficacious against microorganisms at the manufacturer's recommended formulations within the first 10 min of contact time without organic matter[40], a successful biosecurity program, which is one of the best methods used to reduce the level of pathogens in animal facilities. 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[41].

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[41,42]. Formaldehyde and phenolic compound were effective in the presence of organic matter. The poultry houses and equipment should be fogged with formaldehyde solution which might be repeated after placing the litter[43]. 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[28].

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 in 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.

CONCLUSION

Mycoplasma gallisepticum can survive in different reservoirs within a poultry farm. Among these reservoirs, food, drinking water, feathers, droppings or dust are the most common. *M. gallisepticum* infection leads to tremendous economic losses in poultry production as a result of decreased hatchability and egg production, mortalities, reduced quality of day-old chicks, reduced growth rate, increased costs of control which involve site cleaning and depopulation and increased costs of medication and vaccination. Good management and biosecurity practices are necessary to ensure that *M gallisepticum* infections are not transmitted to commercial poultry from these and other sources. So, the evaluation of the disinfectants' efficacy should be in priority to select the suitable disinfectant by minimizing the microbial load. Improper sanitation procedures might be ineffective in disease control and further lowering the bird production performance. Cold-fogging resulted in the greatest reduction in *M. gallisepticum* count.

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