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Effect of cold fog disinfection on *Escherichia coli* affecting commercial egg layer flocks

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Abstract

On a field level among poultry flocks a study was conducted to estimate the efficacy of the most common disinfectants against E. coli (*APEC* isolates) was determined. The selected disinfectants were; Formalin, Phenol, QAC, Halamid, Virkon" S and Micro Sept M against *E. coli* isolates of commercial egg- layer flocks in Egyptian farms. The recovered results showed that: (1) the incidence of *E. coli* in the observed commercial egg-layer flocks; the isolation of E. coli (*APEC* of rfbO157 gene) from 4 commercial egg-layer flocks (26.7 %) out of 15 flocks, the mortalities rates at the end of 78 weeks of age was 20% and also, the current egg-production, average egg weight, hen housed day, hen housed egg and percent peak of egg-production were: 69.2%,58.3 gm, 70%,326,78% and 69%,respectivel. (2) Formalin, Micro Sept M and Virkon'S treatment demonstrated a significant reduction in E. coli populations especially by cold fogging method.

Keywords: *E. col;* Egg -Layer Flocks; Performance; Disinfection; Cold Fogging; Rate of Application

1. Introduction

Colibacillosis, a syndrome caused by *Escherichia coli*, is one of the most common infectious bacterial diseases of the layer industry. *E. coli* are always found in the gastrointestinal tract of birds and disseminated widely in faeces; therefore, birds are continuously exposed through contaminated faeces, water, dust and the environment [1]. *Escherichia coli* infection leads to high morbidity and mortality causing economic losses on a farm especially at the peak of egg production and at the production cycle**.**

Infectious diseases of poultry are responsible for tremendous economic losses in the poultry production worldwide. Most of these diseases are caused by bacterial pathogens. Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *Escherichia coli* [2]. It is an economic problem due to reduction of feed intake, growth retardation, uniformity reduction, and mortality as well as it causes respiratory problems and act as a welfare issue consequently, it is globally spreading infectious disease that represents a main concern in the poultry industry. [3].

Colibacillosis in egg-laying flocks is characterized by acute mortality without prior clinical signs of disease and with a some effect on egg-laying, production and quality. Normally, colibacillosis is a secondary infection that appears after a situation of immune-suppression caused by another bacterial or viral infection, although Vandekerchove et al., 2004b [4] proposed that it may act as a primary pathogen as well. Furthermore, this situation is made complicated by environmental stress such as improper ventilation, temperature, and dust [5]. The common route of infection is mostly via the respiratory tract which usually followed by septicemia.

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Using disinfectants without evaluation & adequate validation may cause the increased selective pressure on the pathogen that, leading to the gradual or slow decrease in susceptibility or resistance of the pathogens to the disinfectants used and even cross-resistance to antibiotics of public health concerns [6]. Thus, improper sanitation procedures might be ineffective in disease control lowering bird performance [6]. So, the evaluation of the efficacy the disinfectants' must be considered in priority for the selection of the suitable disinfectant that reducing or minimizing the bacterial load.

The current study was conducted to evaluate some commercially available disinfectants against *Escherichia coli*.

2. Material and methods

2.1. 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.

2.2. Properties of the house

Natural housing with Ventilation system - Floor system on litter -Capacity- 10.000 hens – Stocking density m^2 / bird - 7.5 - 8 -Lighting system included of 16 h light and 8 h dark. Birds had free access to food and drink. They received all necessary vaccinations except for *E. coli.*

2.3. Samples collection

Swab samples were collected from cloaca and tracheal of 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, and were subjected to various bacteriological and biochemical examination in the laboratory. Case history and the performance of each flock were recorded.

2.4. Isolation of *E. coli*

Samples were sent on ice to the Laboratory, stored overnight, and tested the following day. Testing for *E. coli* was performed according to the standardized methods currently used in the U.S. Food and Drug Administration. 2001. [7] (for fecal samples. Briefly, swab samples were added in 10 ml BPW, and incubated for 37°C ± 1°C for 18 h ± 2 h for pre-enrichment. Selective media like blood agar, Eosin Methylene Blue (EMB) Agar or MacConkey are used for isolating *E. coli* (Arathy et al., 2011; Khatun et al., 2015) [8,9].

2.5. Biochemical Tests

Nutrient Broth (NB) and Nutrient Agar (NA) were used to grow the organisms from the collected samples before performing biochemical test according to the procedure describe by Cheesebrough [10]. Eosin Methylene Blue (EMB) agar medium was used for observing growth of *E. coli.* Suspected isolates of *E. coli* organisms were identified according to MacFaddin [11].

2.6. Serological identification of *E. coli*

The isolates were serologically identified according to Kok *et al*. [12] by using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co., Japan**)** for diagnosis of the Enteropathogenic types.

2.7. Polymerase Chain Reaction (PCR) Using E. coli rfbO157gene (Maurer et al., 1999)

Ten sero-typed *APEC* isolates were tested for the presence of ***rfbO157* gene**, encoding *E. coli* species, by PCR technique were detected on PCR-confirmed *APEC* isolates. DNA extracts were prepared by the boiling method according to Sambrook et al. [13].

Table 1 Designed primers of *rfbO157* gene, encoding *E. coli* species

Primer	Target gene	Gene product	PCR amplicon (bp)	Reference
CGTGATGATGTTGAGTTG	rfbO157	LPS O157	420	Maurer et al., 1999 [14]

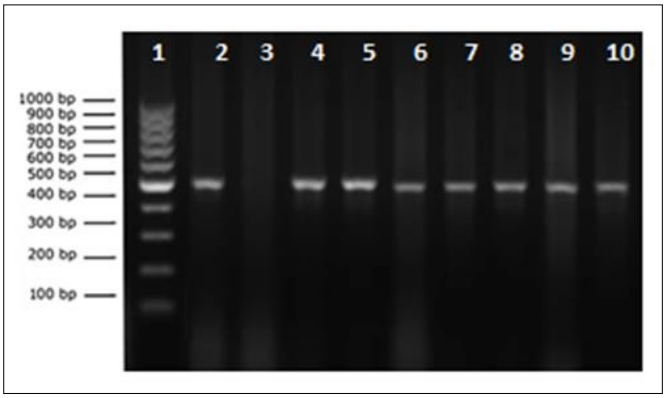

Figure 1 PCR assay for *E. coli*. *Lane* 1: *Ladder DNA 1kbp; Lane* 2: (positive control); *Lane3*: negative control and Lane 4-10 specific amplification at 420 bp of DNA of isolates

2.8. Evaluate some commercially available Disinfectants

The efficacy of some common disinfectants was tested on pathogenic strain of *Escherichia coli*. The experiment carried out as a field study by different methods of application inside poultry house (Low & high rates of spray and Cold Fogging).

2.9. Experimental test

Experimental test units were 1-ft^2 floor plots randomly blocked with a 1-ft^2 space between each experimental plot. The treatments consisted of 6 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_2 o, Micro Sept M 1: 5 (for spraying)).

2.10. Preparation of microbial culture

Escherichia coli were propagated using pour plate method, [15]. A loopful was transferred from all bacterial strains that was stored onto nutrient slopes into 10 ml nutrient broth and incubated at 37C for 20-24 h.

One ml from in-activator tubes was used for the bacterial count using pour plate method [16]. The colony count of bacteria on each plate was carried out. The calculation was applied using the following formula:

Log (average CFU/ drop vol.) (Dilution factor) (Vol. scrapped into/ surface area) [17, 18].

2.11. Bacterial Inoculation into plots

For each experimental plot the inoculums (10 $^{6.8}$ of *E. coli* 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. 40 ml/plot via pipette were applied for each plot, whereas the positive control plots received 40 ml/plot of distilled water.

2.12. Application of the disinfectants [19]

Six treated plots for bacterial pathogen and 2 as control (each group for one disinfectant).

- As a coarse spray at a low application rate of 55 ml/plot to create a good surface coverage.
- As a spray at a high application rate of 125 ml/plot (. as a common disinfectant usage level of 500 gal/16,000 ft2)

- As cold fogging: a rate of 125 ml/plot (fogging for 5 min) was chosen because it correlated to a common disinfectant usage level. Disinfectant Fog Machine of nano-atomizer adjustable fogger. Two untreated plots, receiving no disinfectant, served as the negative control group.

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 [20] that were hydrated with 20 mL of laboratory prepared Butterfield's phosphate diluents (BPD) [21] prior to sampling. Sponges were used to sample the surface of the plot. A was then placing each sponge into sterile bottles containing 180 ml of BPD (1: 10 dilution). Samples were put in ice packs and transported to the laboratory.

2.13. Counting

Briefly, BPD samples were incubated at 37°C for 24 h, and then 1 mL was transferred into 9 mL nutrient broth and incubated at 37°C for 24 h. One ml from broth was used for the bacterial count using pour plate method [15]. The numbers of survival bacteria on each plate were counted. The calculation was carried out using the following formula: Log (average CFU/ drop vol.) (Dilution factor) (Vol. scrapped into/ surface area) [17, 18].

2.14. 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 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 Reduction = $\log_{10} \frac{A}{B}$

3. Results

Table 2 The incidence of *Escherichia coli* in observed commercial egg-layer flocks

The incidence	No. of infected flocks	Percent
Escherichia coli	4	26.7 %

*Number of infected flocks = 4 (26.7 %).
* Number of studied flocks = 15

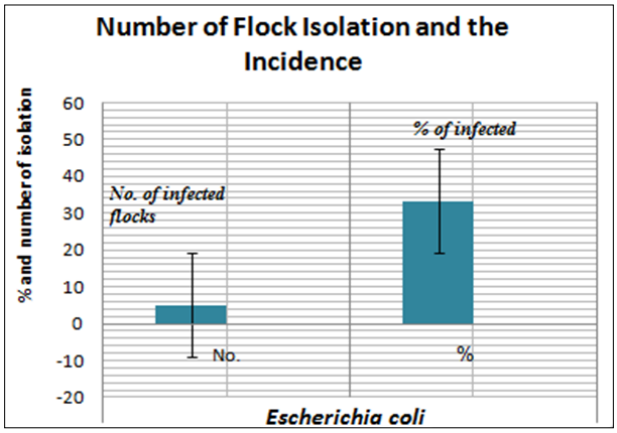

Figure 2 The incidence of *Escherichia coli* in observed commercial egg-layer flocks: The results showed that: *Escherichia coli* were isolated from 4 commercial egg-layer flocks (26.7 %) out of 15 flocks

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
E. coli	69.2	58.3	70%	326	78%	69 %	20 %

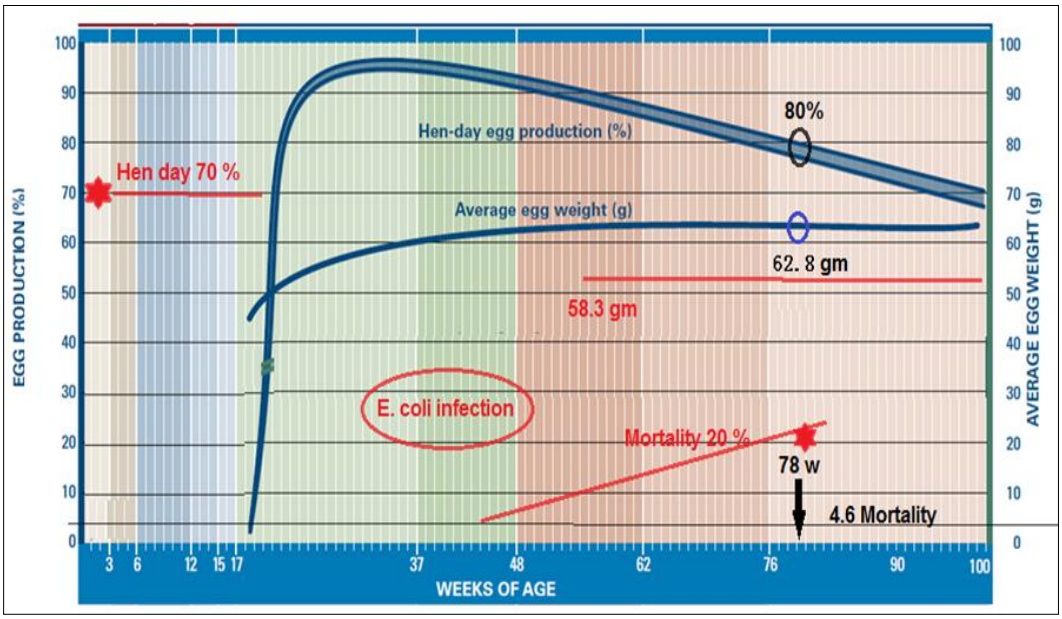

Figure 3 The results revealed that, there were a significant difference (P< 0.05) among the control flocks and the infected ones infected by *E. coli*. In mortality rate (4.6, 20%), current percent egg-production (78 – 81, 69.2), average egg weight (62.8, 58.3 gm), hen housed day (80 %, 70%), hen housed egg (351.7 –362.4, 316) and percent peak of egg-production (95–97 %, 78%)

3.1. Effect of rate of application and exposure time of the disinfectant on *E. coli* of poultry floor.

Table 4 The effect of low rate of application and exposure time on *E. coli* of poultry floor

Time	*E. coli* Count		
	15 min	6 hr.	24hr
Control	7.2	7.2	6.8
Formalin	5.8^{b}	5.75^{b}	5.72^{b}
Phenol	6.1^{b}	6^{b}	5.9^{b}
QAC	6.1^{b}	6.2^{b}	6.1^{b}
Halamid	5.9^{b}	5.8^{b}	5.8^{b}
Virkon'S	4.7^{a}	4.6^{a}	4.55^{a}

A–b Column values with different superscripts differ significantly ($P < 0.05$). A 10-ml application rate per plot (surface coverage). 2n = 6 plots per disinfectant in the floor

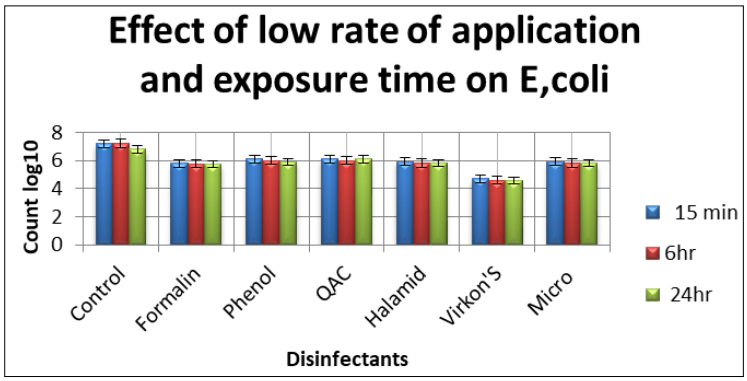

Figure 4 The effect of low rate of application and exposure time on *E. coli* of poultry floor

Table 5 The effect of high rate of application and exposure time on *E. coli* of poultry floor

Time	*E. coli* Count		
	15 min	**6 hr.**	**24hr**
Control	7.2	7.2	6.8
Formalin	4.82[a]	4.65 [a]	4.7 [a]
Phenol	4.1 [a]	4 [a]	3.9 [a]
QAC	5.14[b]	5.2 [b]	5.12 [b]
Halamid	4.9 [a]	4.8 [a]	4.8 [a]
Virkon'S	4.7 [a]	4.6	4.55 [a]
I. sept	3.9 [a]	3.8 [a]	3.8 [a]

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 ft²). 2n = 12 plots per disinfectant in the floor

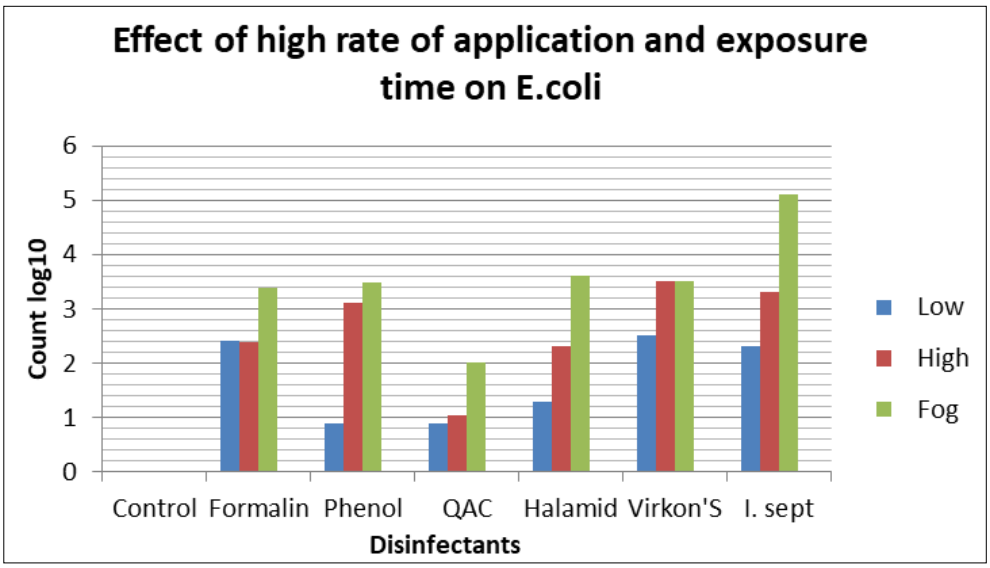

Figure 5 The effect of high rate of application and exposure time on *E. coli* of poultry floor

Table 6 The effect of fogging application and exposure time on *E. coli* of poultry floor

Time	*E. coli* Count		
	15 min	**6 hr.**	**24hr**
Control	7.2	7.2	6.8
Formalin	4.82 [a]	3.75 [a]	3.77 [a]
Phenol	4. 1 [a]	3.7 [a]	3.9
QAU	5.2 [a]	5.2 [a]	5.12 [a]
Halamid	4.6 [a]	4.6 [a]	4.8 [a]
Virkon'S	4.7 [a]	4.6 [a]	4.55 [a]
I. sept	3.9 [a]	3.8 [a]	3.8 [a]

A–b Column values with different superscripts differ significantly ($P < 0.05$). 1A 55-mL application rate per pan (common usage level of 500 gal/16,000 ft²). 2n = 6 pans per disinfectant. Control: *E. coli* =7.2

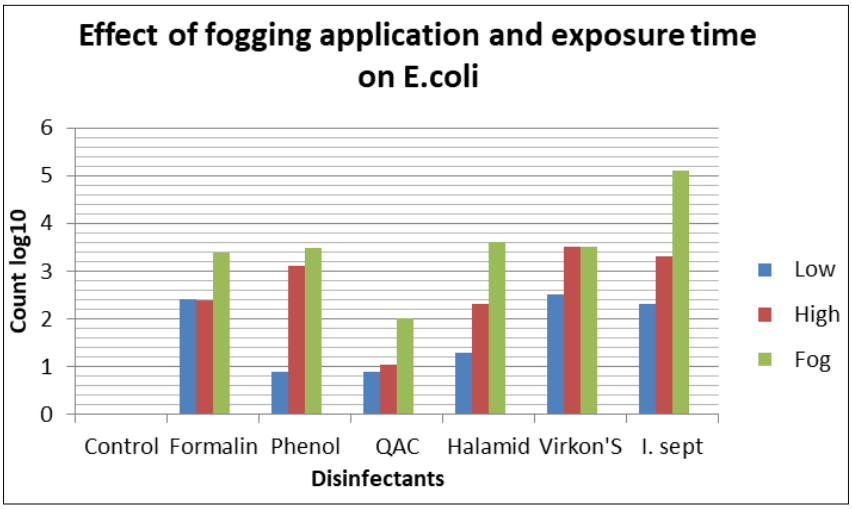

Figure 6 The effect of fogging application and exposure time on *E. coli* of poultry floor

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

Time	*E. coli* Count		
	low	***high***	***fog***
Formalin	2.4 [a]	2.38 [a]	3.38 [a]
Phenol	0.9 [b]	3.1 [a]	3.49 [a]
QAC	0.9 [b]	1.04 [b]	2 [b]
Halamid	1.3 [b]	2.3 [a]	3.6 [a]
Virkon'S	2.5	3.5	3.5
Micro Sept M	2.3 [a]	3.3 [a]	5.1 [a]

A–b Column values with different superscripts differ significantly ($P < 0.05$). 1A 55-mL application rate per pan (common usage level of 500 gal/16,000 ft²). 2n = 6 pans per disinfectant. Control: *E. coli* =7.2

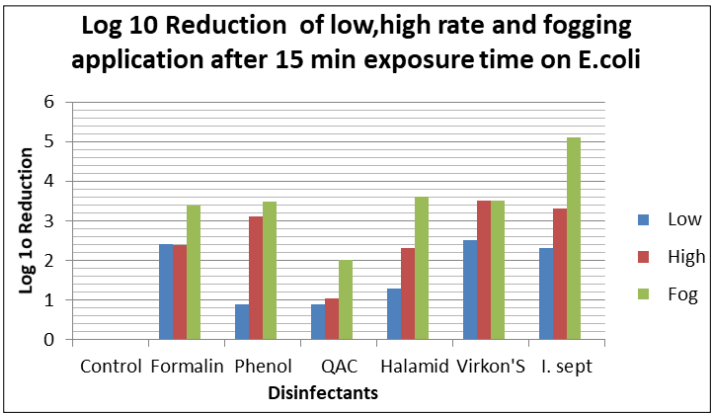

Figure 7a The effect of disinfectants exposure time (15min) when applied at low, high and fogging application rates on *E. coli* populations obtained from a poultry house floor (log10 reduction)

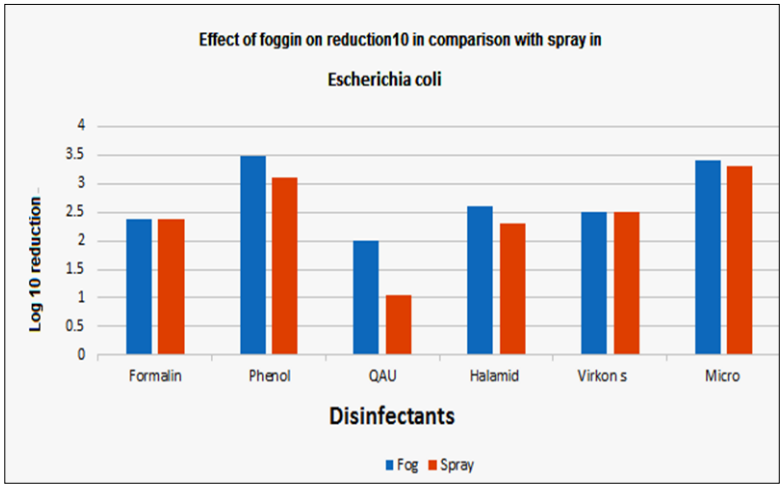

Figure 7b Using phenols, QAC and Halamid at concentration of5%, 5 % and 18 % by fogging other than spraying had increased action on the tested pathogens *E. Coli* after 15 min contact time (the increased percent were; 12%, 96 % and 13 %, respectively).

4. Discussion

4.1. Incidence of *Escherichia coli*

The etiology of colibacillosis can be either due to primary infection with poultry pathogenic *Escherichia coli (APEC)* or due to secondary and/or (opportunistic) infection after a primary insult has occurred. *E. coli* are gram-negative, rod-shaped bacteria considered normal inhabitants of the avian digestive tract. While most strains are considered to be non-pathogenic, certain strains have the ability to cause clinical disease

Polymerase chain reaction (PCR) analysis of *E. coli* O157 virulence markers revealed that all O157: H7/ isolates were positive ***rfb*$_{O157}$** (Table 1 and Fig.1)

The incidence of *E. coli* in studied commercial egg-layer flocks; the isolation of *E. coli* was from 4 commercial egg-layers flocks (26.7 %) out of 15 flocks, and 18 isolates (Table 2 and Fig.2). This indicated that, the pathogen's horizontal transmissibility characteristics among birds of a same flock.

The global poultry industry loses millions of dollars every year because of Colibacillosis. Caused by infection with Avian Pathogenic *Escherichia Coli* (*APEC*), Colibacillosis is among the most morbid and mortal of poultry bacterial infections (Kabir et al.,2010)[22], which in turn leads to significant reduction in the production of poultry meat and eggs (Kabir et al.,2017) [23]. The disease appears in different forms from acute (septicemia) to sub-acute including pericarditis, perihepatitis, arthritis, airsacculitis and cellulitis (Calnek et al., 1997) [24].

Escherichia coli were 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 whish may reach to 4.0% or more [25].

4.2. Effect of Escherichia coli infection on egg production and mortality

The results revealed that, there were a significant difference (P< 0.05) among the control flocks (Standard) and the infected ones by ***Escherichia coli*.** The mortality rate at the end of 78 weeks of age was 20% and also, the current egg-production, average egg weight, hen housed day, hen housed egg and percent peak of egg-production were: 69.2%, 58.3 gm, 70%, 326, 78% and 69%, respectively. (Table 3 and Fig.3).

Escherichia coli 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 poultry the infection is by the respiratory route. It can existed viable and survive in varying reservoirs within a poultry environment. Among these reservoirs, food, drinking water, feathers, droppings or dust are the most common.

.The impact of colibacillosis on laying hens of the Hy-Line lineage (at 32 weeks old) characterized by approximately 40% laying, per day and large number of birds affected with diarrhea and then apathy followed by death [26]. Pathogenic strains are commonly of the O1, O2 and O78 serotypes. Since *E. coli* is a common inhabitant of the intestine, it is widely disseminated in faecal material and litter. Sources of *E. coli* infection for a flock may be contaminated drinking water, ration & feed, ingredients and rodent droppings [27].

Da Rosa et al., 2020, [28] proved that colibacillosis was the cause of oxidative stress in poultry breeder flocks that, negatively affecting their weight gain and egg production. Outbreaks of acute mortality in layer flocks were occurred in Europe, due to colisepticaemia have frequently been observed since the mid-1990s. Disease was usually acute without clinical symptoms [29].

4.3. Evaluation the efficacy of the selected disinfectants to reduce *Escherichia coli*

The empty houses of poultry flocks after birds are eliminated or transferred from the house are left contaminated with different microbial groups. Cleaning and disinfection of empty houses are a significant or utmost step in prevention and controlling diseases in large-scale poultry farms. Disinfection can reduce or kill potential pathogenic microorganisms in the house and prevent the transmission of pathogenic microorganisms between batches. To prevent or control the occurrence of infectious diseases in poultry houses effectively, several disinfection procedures and steps have applied.

Selecting appropriate cleanizers and disinfectants as well as disinfection methods such as cleaning, soaking, fumigating, spraying, and UV irradiating can minimizing microbial loads in the farm.

The current observations and reports showed that most of the poultry farms do not practice the benchmark guidelines of bio-security [30]. Spraying disinfectants in sheds and removing feces were the only sanitation schemes adopted in the farms [31, 32]. In addition, disinfectants are used without regular validation and adequate evaluation of efficacy. The efficacy of the disinfectants is affected by formulation & concentration, level of organic matter, humidity & moisture content, temperature, pH and hardness of water, and other factors [33, 34].

4.3.1. Effect of low rate of application

E. coli was affected significantly with Virkon'S, compared with the control group and other disinfectants when applied at the low application rate ($P < 0.05$) (Table 4 and Fig. 4). The lack of response for the disinfectant treatment is in agreement with literature, as not all products work the same on varying types of pathogens; therefore, the disinfectant should be evaluated in the field for the specified application to ensure its effectiveness [34]. Also most disinfectants do not perform well when applied at low rate of application or in the presence of organic material [35-37]. It has been recorded that ability of the Enterobacteriaceae groups to certain disinfectants, including QAC and substituted phenols, may increase or decrease depending on cell density, growth rate and the limiting nutrient [37-39]

4.3.2. Effect of high rate of application

Disinfectants impacted and effect on ***Escherichia coli*** populations at the high application rates (The log 10 values their log 10 were 4.82, 4.1, 5.14, 4.9, 4.7 and 3.9 for Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M where, respectively) as shown in Table 4 and Fig. 4). Formalin, Phenol, Halamid, Virkon'S and Micro Sept M resulted significantly ($P < 0.05$) in reduction of *E. coli* populations (log10 reduction were; 3.2, 4.2, 4, 3.75 and 2.52, respectively) compared with the control.(Table 5 and Fig. 5).

4.3.3. Effect of fogging application

Fogging the disinfectants were decreased *Escherichia coli* populations (The log 10 values of their log 10 were; 4.82, 4.1, 5.14, 4.9, 4.7 and 3.9 for Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M where, respectively) as shown in Table 6 and Fig. 6a).

From the results we observed that fogging by Formalin, Phenol, QAC, Halamid, Virkon'S and Micro Sept M resulted in greatest reduction in *E. coli* populations where log10 reduction were: 3.3, 3.8, 3.49, 2, 3.6, 3.5 and 5.1, respectively). The Micro Sept M, Halamid and Virkon'S treatment demonstrated a significant reduction in *E. coli* populations. Using Phenols, QAC and Halamid at concentration of 5, 33.3 % and 5.5 % by fogging other than spraying had increased action on the tested pathogens *E. Coli* after 15 min contact time (12%, 96 % and 13 %, respectively) (Table 7 and Fig. 7a and 7b).

Fogging machines to transform liquid into droplets that are dispersed into the atmosphere use large volumes of air at low pressures. That type of fog- machine can give extremely small sized droplets with diameters ranging from 1–150 μm.

Thus, the small *sized droplets* are less carrier for the applied disinfectants, although the 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 the small sized droplets composed of 10^5 molecules or more, will form a much of dielectrons which resulted during the splitting process lead to the liberation of molecular hydrogen and formation of two solvated hydroxide anions. All disinfectants need a minimum time of 5 – 10 minutes to destroy various types of microorganisms in the absence of organic matter. [40].

5. Conclusion

The incidence of *E. coli (APEC)* in observed commercial egg-layer flocks was 26.7%. 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 *Escherichia coli* infection.

Disinfectants were affecting on *Escherichia coli* survival at high rate of application. It is evident that, fogging will increase the efficacy of the used disinfectants for 15 min contact of exposure time. Proper care should be taken for the application rates of the disinfectants, and to take into consideration factors, such as water pH, temperature, and surfaces on which application will occur. To minimize persistent infections in the flock a highly effective disinfectant should be used. It has been suggested that increased *Escherichia coli* susceptibility may occur from best management practices that reduce exposure to protective gut flora, which can be found in litter. The intention of disinfectant programs in poultry facilities is to reduce the populations of disease associated bacteria. However, if disinfectants are used without properly cleaning the facility prior to application, then the effectiveness of the disinfectant may be compromised.

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.

References

[1] Charlton BR. Avian Disease Manual. 6th edition. Athens: American Association of Avian Pathologists (AAAP), 2006. Print. Edited by: BR, Carlton. 2006.

[2] Barnes HJ, AM Fadly JR, Glisson LR, McDougald DE, Swayne YM. Diseases of Poultry (11th ed.), Iowa State University Press, Ames. 2003; 631-656.

[3] Ask B, EH van der, Waaij JHH, van Eck JAM, van Arendonk JA, Stegeman. Defining susceptibility of broiler chicks to colibacillosis. Avian Pathol. 2006; 35: 147-153.

[4] Vandekerchove D, PDe Herdt, H Laevens, F Pasmans. Colibacillosis in caged layer hens: Characteristics of the disease and aetiological agent. Avian Pathol. 2004; 33: 117-125.

[5] Shane SM. *E. coli* continues to influence poultry health. World Poult. 2007; 23: 44-45.

[6] McDonnell G, AD Russell."Antiseptics and disinfectants: activity, action, and resistance," *Clinical Microbiology Reviews*. 2011; 14(1): 227.

[7] US, Food and Drug Administration. Center for Food Safety and Applied Nutrition, Bacteriological Analytical Manual online, Rockville, MD. 2001. www.cfsan.fda.gov/~ebam/bam-ri.html. Accessed Nov. 2004.

[8] Antimicrobial Drug Resistance in Escherichia coli Isolated from Commercial Chicken Eggs in Grenada, West Indies DS Arathy1, G Vanpee2, G Belot 2, V Mathew1, C DeAllie1, R Sha West Indian Med J. 2011; 60(1): 53.

[9] Most. Nazmunnaher Khatun , ATM, Mahbub-E-Elahi , Sultan Ahmed, Md. Shafiullah Parvej , Sharmin Akhter , Wahedul Karim Ansari , Mohammad Shaokat Ali. Frequency of drug resistant Escherichia coli isolated from commercial broiler chicken in Bangladesh. International Journal of Natural and Social Sciences. 2015; 2(4): 01-05.

[10] Cheesbrough, M, Culture Media. In: Cheesbrough, M, ed. Medical Laboratory Manual for Tropical Countries. Tropical Health Technology and Butterworth-Heineman, Cambridge. 1984; 60-69.

[11] MacFaddin JF. Biochemical Tests for Identification of Medical Bacteria, 3rd ed. Williams and Wilkins, Philadelphia, P A. 2000.

[12] Kok T, Worswich D, Gowans E. Some serological techniques for microbial and viral infections. In: Practical Medical Microbiology, Collee J, Fraser A, Marmion B, Simmons A, eds. 14th ed., Edinburgh, Churchill Livingstone, UK. 1996.

[13] Sambrook, J, Fritsch, EF,Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA. 1989.

[14] Maurer JJ, Schmidt D , Petrosko P, Sanchez S , Lance Bolton L , Lee, MD. Development of Primers to O-Antigen Biosynthesis Genes for Specific Detection of *Escherichia coli* O157 by PCR. Applied and Environmental Microbiology. 1999; 65(7): 2954-2960.

[15] Cruickshank, RJP, Duguid BP, Marimion RH. Swain. Medical microbiology. ELBC, 12th Ed., vol. 11, reprinted Churchill Livingstone and Robert Stevenso. Edinburgh, EHI, 3AF. 1980.

[16] Zelver N, Hamilton M, Pitts B, et al., Measuring antimicrobial effects on biolm bacteria: from laboratory to eld. Methods Enzymol .1999; 310: 608-628.

[17] Herigstad B, MartinHamilton, JoannaHeersink . How to optimize the drop plate method for enumerating bacteria. Journal of Microbiological Methods. 2001; 44 (2, 1): 121-129.

[18] Payne, JB, Kroger, E C, Watkins, SE. Evaluation of Disinfectant Efficacy When Applied to the Floor of Poultry Grow-Out Facilities. J. Appl. Poult. Res. 2005; 14: 322–329.

[19] US; Food and Drug Administration. Center for Food Safety and Applied Nutrition, Bacteriological Analytical Manual online, Rockville, MD. 2001.

[20] Oh JY, MS Kang, JM Kim, BK An, EA Song, JY Kim, EG Shin, MJ Kim, JH Kwon, YK Kwon. Characterization of *Escherichia coli* isolates from laying hens with colibacillosis on 2 commercial egg-producing farms in Korea. Poultry Science. 2011; 90(9): 1948-1954.

[21] Rosa G, ,Aleksandro S, Da Silva Carine, F Souza, Matheus D, Baldissera Ricardo E, Mendes Denise, N Araujo Davi, F Alba Marcel, M Boiago , Lenita, MouraStefani . Impact of colibacillosis on production in laying hens associated with interference of the phosphotransfer network and oxidative stress. Microbial Pathogenesi. 2019; 130: 131-136.

[22] Kabir SM. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *International journal of environmental research and public health*. 2010; 7(1): 89–114.

[23] Kabir SML, Sikder MH, Alam J, Neogi SB, Yamasaki S. Colibacillosis and Its Impact on Egg Production In: Hester PY, editor. *Egg Innovations and Strategies for Improvements*: Academic Press. 2017; 523–35.

[24] Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM. *Diseases of poultry* 10th ed. Iowa State University Press, Ames, IA. 1997.

[25] Kahn, CM, Ed. The Merck Veterinary Manual. 10th edition. Whitehouse Station: Merck & Co., Inc. 2010.

[26] da Rosa G, Alba DF, Silva AD, Gris A, Mendes RE, Mostardeiro VB, Lopes TF, Schetinger MRC, Stefani LM, Lopes MT, Boiago MM, da Silva AS. da Rosa G, et al.,2020. Impact of Escherichia coli infection in broiler breeder chicks: Microb Pathog. 2020.

[27] Vandekerchove D, PDe Herdt, H Laevens, F Pasmans. Colibacillosis in caged layer hens: characteristics of the disease and the aetiological agent. Journal of the Hellenic Veterinary Medical Society. 2021; 71(4): 2425.

[28] Barua HPK Biswas KE, Olsen SK, Shil JP. Christensen. "Molecular characterization of motile serovars of *Salmonella enterica* from breeder and commercial broiler poultry farms in Bangladesh," *PloS One*. 2013; 8(3): e57811, 2013.

[29] Rimi NA, R Sultana, M Muhsina et al., "Biosecurity conditions in small commercial chicken farms, Bangladesh 2011-2012," *EcoHealth*. 2017; 14(2): 244–258.

[30] Stringfellow KP, Anderson D, Caldwell et al. "Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions," *Poultry Science*. 2009; 88(6): 1151–1155.

[31] Islam KN, KN Monira, R Sultana, IM Azharul. "The effect of timsen and ambicide as disinfectant on hatchability traits of kasila broiler parents eggs in Bangladesh," *Livestock Research for Rural Development*. 2007; 19(44).

[32] Singh S, RK Agarwal, SC Tiwari, H Singh. Antibiotic resistance pattern among the *Salmonella* isolated from human, animal and meat in India. *Tropical Animal Health and Production*. 2012; 44: 665–674.

[33] Suwa M, Oie, S, Furukawa, H. Efficacy of disinfectants against naturally occurring and artificially cultivated bacteria. *Biol. Pharm. Bull.* 2013; 36: 360–363.

[34] Chidambaranathan AS, Balasubramanium M. Comprehensive review and comparison of the disinfection techniques currently available in the literature. *Journal of Prosthodontics*. 2019; *28*(2): e849-e856.

[35] Maertens H, De Reu K, Van Weyenberg S, Van Coillie E, Meyer E, Van Meirhaeghe H, Van Immerseel F, Vandenbroucke V, Vanrobaeys M, Dewulf J. Evaluation of the hygienogram scores and related data obtained after cleaning and disinfection of poultry houses in Flanders during the period 2007 to 2014. *Poultry science*. 2018; *97*(2): 620-627.

[36] Cozens RM, Brown MRW. Effect of nutrient depletion on the sensitivity of *Pseudomonas cepacia* to antimicrobial agents. *Journal of Pharmaceutical Sciences.* 1983; *72*: 1363-1365.

[37] Brown MR, Collier PJ, Gilbert P. Influence of growth rate on susceptibility to antimicrobial agents: modification of the cell envelope and batch and continuous culture studies. *Antimicrobial Agents and Chemotherapy.* 1990; *34*: 1623-1628.

[38] Berchieri Jr, Barrow. Found that phenolic compounds and glutaraldehyde are efficient against *Salmonella s*, and also reported that quaternary ammonium compounds were inefficient. Braz. J. Poult. Sci. 1996; 10(2).

[39] Si-EunByeon, JinwookLee. Differential responses of fruit quality and major targeted metabolites in three different cultivars of cold-stored figs (*Ficus carica* L.) Scientia Horticulturae. 2020; 260: 108877.

[40] Linton Y, Hugo WB, Russel AD. Disinfection: In Veterinary and Farm Animal Practice. 2nd Ed. Oxford, London, Edinburgh, Blackwell Scientific Publication, UK. 1987.