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RESEARCH ARTICLE

Salmonella in broiler chickens: Biofilm formation, disinfectant resistance, and contribution to microbial risk in housing environments

Hanan S. Khalefa[1], Amr Elkelish[2]*, Marwa S. Khattab[3], Amr Fouda[4], Heba M. Salem[5,6], Soha A. Alamoudi[7], Mohammed Musa Alzahrani[2], Mohammed Alghonaim[2], Shereen Fawzy[8], Mohamed T. El-Saadony[9], Asmaa M. Ali[1]

[1]Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt; [2]Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), P. O. Box: 90950, Riyadh 11623, Saudi Arabia; [3]Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt; [4]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, 11884, Egypt; [5]Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt; [6]Department of Diseases of Birds, Rabbits, Fish & their Care & Wildlife, School of Veterinary Medicine, Badr University in Cairo (BUC), Badr City, Cairo, Egypt; [7]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; [8]Department of Medical Microbiology, Faculty of Medicine, University of Tabuk, Tabuk 71491, Saudi Arabia; [9]Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
Corresponding author: aaelkelish@imamu.edu.sa (Amr Elkelish)

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ABSTRACT

Avian salmonellosis poses a significant threat to the poultry sector. The ability of *Salmonella* to form biofilms enhances its long-term persistence in poultry houses, thereby increasing the risk of zoonotic transmission. Therefore, this study aims to identify the prevalent *Salmonella* species in various broiler chicken farms and conduct a risk analysis for their occurrence. The poultry samples, which included 220 bird organs and environmental samples, had a *Salmonella* isolation rate of 4.45%. From the examined bird and environmental samples, 10 *Salmonella* isolates were recovered (seven from bird samples and three from environmental samples). The ten *Salmonella* isolates were identified in this investigation using serological analysis. The study revealed the presence of five different serotypes: *S.* Enteritidis (40%), *S.* Kentucky (30%), *S.* Typhimurium (10%), *S.* Infants (10%), and *S.* Gueuletapee (10%). On the experimental level, the tube adherence test confirmed biofilm formation on the inner surface of the test tubes, resulting in 20% of the *Salmonella* isolates exhibiting a strong positive reaction, 50% displaying a moderate response, and 30% showing a weak reaction. Additionally, our second objective is to conduct an *in vitro* assessment of the *S.* Enteritidis strain's ability to form biofilms on PVC coupons, given its zoonotic significance and detrimental effects on chickens. Additionally, the biofilm's susceptibility to various disinfectants was evaluated. The results show that Virkon S® (1% wt./vol) and H_2O_2 (1.0% vol/vol) were able to reduce the count of microbes on the PVC surface by 5.6 and 5.8 log, respectively. The effectiveness of disinfectants in eliminating biofilm on contaminated surfaces varies depending on factors such as concentration, duration of contact, active ingredients, biofilm age, and environmental variables that simulate real situations.

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INTRODUCTION

Salmonella is a common pathogen that has been found and affects an extensive variety of avian species at various ages (Elsayed *et al.*, 2024). It has a substantial economic impact on poultry production because of its severe clinicopathological profile, reduced productivity, elevated mortalities, and zoonotic significance (Cosby *et al.*, 2015; Ebeid and Al-Homidan, 2022; Elsayed *et al.*, 2024). Throughout its supply chain, numerous vertical and horizontal transmission ways complicate the epidemiology of salmonellosis (Elsayed *et al.*, 2024; Naheed *et al.,* 2025).

For example, hatcheries, cloacal infection, and the transportation of feed and equipment can all cause horizontal transmission of *Salmonella* species to chicks and vertical transmission through infected parents (Crabb *et al.*, 2018). More than 2600 serotypes in the genus *Salmonella* pose a public health concern due to their rapid mutation and emergence/reemergence rate (Ali and Alsayeqh, 2022). In addition, season and geographical distribution determine the diversity of *Salmonella* serotypes recorded from avian sources (Jassim and Limoges, 2017).

However, researchers have reported high-incidence cases for several serotypes, like *S.* Enteritidis, *S.* Typhimurium, *S.* Kentucky, *S.* Infantis, *S.* Newport, and *S.* Derby (Merino *et al.*, 2019; Khan and Chousalkar, 2020). *Salmonella* contamination and biofilm formation are at risk in zones that are hard to access, like drinking water or feeding systems, wall crevices, and areas with inadequate cleaning and disinfection (González-Rivas *et al.*, 2018; Laban *et al.,* 2025). Most of the *Salmonella* serovars that are present in the surroundings of hatcheries, feed mills, and broiler farms are relatively insignificant in terms of human health (Ogundipe, 2025). Still, they are difficult to eradicate (Davison *et al.*, 2005).

The formation of biofilms encourages their persistence (Imran-Ariff *et al.,* 2025). Poultry farms in Egypt, like those throughout the world, are dealing with the devastating issue of microbial biofilm. *Salmonella, Escherichia coli, Campylobacter* and *Staphylococcus aureus* primarily cause biofilm production in chicken farms (Abd-Elall *et al.*, 2023; Ishaq *et al*., 2022; Yaseen *et al*., 2025). *Salmonella* has the potential to generate biofilms on different types of surfaces, including contact surfaces like aluminum, stainless steel, rubber, nylon, polystyrene, plastic, or glass, as well as on poultry farm processing surfaces such as walls, floors, pipelines, and drains (Lee *et al.*, 2020). Moreover, the food production chain poses a growing risk of exposing consumers to resistant bacterial strains, including those capable of forming biofilms (Obe *et al.*, 2020; Laban *et al.*, 2024).

Bacterial biofilms are groups of many cells that grow on both inorganic and organic surfaces, surrounded by a biopolymer extracellular matrix. This biofilm is a mechanism of cellular survival that increases cell resistance to harmful environmental factors and various antibiotic intervention regimens (Hosseinidoust *et al.*, 2013). Over half of the *Salmonella* strains isolated from avian farms generated biofilms in the processing zones and contact surfaces (Merino *et al.*, 2019).

Poultry waterlines structure a significant portion of poultry water systems, and polyvinyl chloride (PVC) was the material of choice for their construction. The formation of biofilms in the water systems of poultry houses is an essential factor in disease transmission (Wingender and Flemming, 2011). Numerous investigations (Fairchild and Ritz, 2009; Marin *et al.*, 2009) have found microbes creating biofilm in poultry water systems. Over time, the progressive deposition of numerous dirt, minerals, rust, and algae in poultry house drinker lines generates microbial biofilms. According to Zimmer *et al.* (2003), biofilm continues to threaten birds, especially young ones. Researchers have also linked poor flock performance to biofilms blocking water pipelines and filters, restricting water flow (Fairchild and Ritz, 2009; Maharjan *et al.*, 2015; Ibrahim *et al.*, 2023).

In poultry houses, the most prevalent approach to preventing microbial adhesion and biofilm formation is to employ chemical attacks through cleansing and disinfection. Nevertheless, these methods are not entirely successful in removing biofilm (Garcia *et al.*, 2017). Only a deeper understanding of the devastating impact of bacterial biofilm can lead to the development of better control measures, like the use of effective disinfectants. In poultry farms, the most widely used disinfectants are hydrogen peroxide (H_2O_2) (Marques *et al.*, 2007), sodium hypochlorite (NaOCl) (Ismail *et al.*, 2019), Virkon S (Elsayed *et al.*, 2020), glutaraldehyde (Günther *et al.*, 2017), and copper sulfate (Sallami *et al.*, 2022).

These disinfectants should be safe, efficient, environmentally friendly, and free of toxic residues (Arnold and Silvers, 2000). Furthermore, it is imperative to implement the appropriate concentration of disinfectants. The current study is designed to determine the following objectives: first, investigate the possible risk factors that may have caused different types of *Salmonella* to get into eleven broiler farms in Egypt; next, test the potential of the different types of *Salmonella* that can form biofilms *in vitro* and see how fast biofilms form over 7 days on PVC sections used as test coupons in warm, static microbial water (>4.5 log10 CFU/mL) and finally, the capability of two disinfectants (Virkon S® and H_2O_2-based product) to work at different concentrations and contact times to eliminate of biofilms that would normally be killed by the rate of the drinking water for birds is tested.

MATERIALS AND METHODS

Ethical approval: Each farm's identity and location were coded, and its informed consent and ethical approval were documented. Before collecting environmental samples, it was necessary to obtain permission from the owner of each poultry farm. The Institutional Animal Use and Care Committee approved the research (Vet CU 25122023872), founded on 25.12.2023.

Sampling and postmortem examination

Poultry farms and sampling: The farms were selected based on their varying hygiene levels, housing systems, and production varieties. We collected a total of 165 environmental samples (15 samples from each farm). Litter samples were obtained from 5 places, with four in the corners and one in the middle. There were also 22 samples from feed, 33 from the floor, and 33 from fan dust. Water and ration samples were obtained directly from each farm's drinking water and chicken ration. Immediately after being collected aseptically, the environmental samples were transported to a portable container filled with ice and then taken to the laboratory. Upon their arrival, they were either processed or stored at 4°C overnight. In addition to environmental samples, organ samples were obtained from 55 birds (diseased and healthy) between the ages of 3 and 30 days (five birds were sampled per farm). The diseased birds experienced diarrhea, loss of appetite, ruffled feathers, and elevated mortality rates. The postmortem (PM) examination included the sterile collection of organ

samples for subsequent bacteriological testing, including the liver, cecum, spleen, and heart.

Histopathological examination: Tissue specimens were collected from the lungs of birds and then fixed in 10% buffered neutral formalin. Tissues were processed by ascending concentrations of ethanol & xylene, embedded in paraffin wax, and then sectioned by rotary microtome into 4 µm-thick sections. Hematoxylin and eosin stain were applied for staining, and a light microscope equipped with a digital camera was used for examination (Spencer *et al.*, 2012).

***Salmonella* isolation, identification, and serotyping**

Isolation of *Salmonella* species: *Salmonella* was identified using protocols suggested by the International Organization for Standardization (ISO, 2002). In summary, the samples were pre-enriched in a non-selective solution called buffered peptone water (BPW; Oxoid) at a ratio of 1:9 ml. The pre-enrichment process was done at 37 °C for 18–24 hours. To selectively enrich *Salmonella*, transfer 0.1 ml of the pre-enriched culture to 10 ml of Rappaport Vassiliadis (RV) broth. Next, kept aerobically at 41–42 °C for 24 hours. *Salmonella* was selectively isolated using Xylose Lysine Deoxycholate (XLD) agar plates (Oxoid). After being moved from RV broth to XLD agar, the inocula were cultured for 24 hours at 37 °C. On XLD agar, *Salmonella* colonies are translucent, colorless, and extremely light. The dark center of the colonies is generated by hydrogen sulfide (+). For confirmation, representative *Salmonella* colonies were taken up and sub-cultured on nutrient agar (Oxoid) for 18 to 24 hours at 37 °C.

Biochemical identification: Bergey's Manual of Determinative Bacteriology provides specific guidelines for identifying biochemicals. We biochemically confirmed each identified colony for *Salmonella* morphology using triple sugar iron agar (TSI), urease, Simmons' citrate agar, indole, lysine iron agar (LIA), methyl red (MR), and Voges-Proskauer (VP). By ISO (2002), *Salmonella* was identified in colonies exhibiting red slant (alkaline), yellow butt (acidic), bubbles/cracking at the butt (gas production), negative urea utilization (yellow), positive citrate utilization (deep blue slant), a positive MR test (positive), & a negative VP test. Utilizing LIA, hydrogen sulfide and lysine decarboxylation, or deamination, were synthesized simultaneously. Samples that tested positive for *Salmonella* may have an alkaline slant or butt.

Serotyping: *Salmonella* strains were serotyped applying the Kauffman-Whitney typing system for the identification of somatic (O) as well as flagellar (H) antigens using standard antisera from Bio-Rad Laboratories, Hercules, CA, USA.

Detection of the biofilm-forming ability of *Salmonella* Enteritidis

Tube method: *Salmonella* isolates were assessed for their capacity to establish biofilms using test tubes, as previously stated by Stepanović *et al.* (2000). In summary, 2 mL of 1/10 dilutions prepared from the overnight culture were aseptically poured into sterile tubes. Each tube was maintained at 37 °C for 24 hours. Following the incubation period, we rinsed the tubes to remove any planktonic cells, dried them, and then stained them for 20 minutes using 1% crystal violet. The extra stain was rinsed with sterile distilled water three separate times. After being inverted overnight, the tubes were allowed to dry at ambient temperatures. The test tube walls exhibited blue and had rings, indicative of biofilm formation. The experiment was conducted three times.

***In vitro* biofilm formation on PVC coupons by *Salmonella* Enteritidis**

Polyvinyl chloride (PVC) coupons: coupons have an internal diameter of 1.90 cm and a length of 2.54 cm, which is equivalent to the dimensions of commercial poultry water lines. The coupons were rinsed with detergent and then immersed in 8% sodium hypochlorite for five hours. After being cleaned five times with water and air-dried, the object was subsequently treated with 100% ethanol at room temperature for one hour. The coupons were then sterilized via autoclaving for 15 minutes at 121°C. The sterilized coupons were allowed to cool to room temperature before use.

Biofilm formation: A pre-sterilized PVC coupon is utilized as a surface to assess the biofilm-forming capability of the *S.* Enteritidis strain. Following Maharjan (2016) description, we used eight PVC coupons, for biofilm growth and some as control negatives. In three containers, each with a volume of 250 mL. One container was designated as the control negative, while the other two were used for testing. Each container was filled with 150 mL of tryptic soy broth (TSB) that was enriched with 1% glucose. A fresh culture of *Salmonella*, which had been incubated overnight, was distributed into cubes, except the negative control, at a concentration of roughly 8 log CFU/ml. The sample was placed in an incubator and allowed to grow as a biofilm for seven days at 25 °C. After the incubation period, we rinsed each coupon with 10 mL of phosphate-buffered saline (PBS) to get rid of any free-floating cells. Cells that adhered to the surface were removed by using a damp cotton swab soaked in 0.85% saline solution to wipe the PVC coupons inside the marked region. A swab with a biofilm layer was then placed in a tube containing 10 ml of saline solution. The tube is then subjected to vortex-generated vibration at a speed of 2,800 rpm for 1 minute to disperse the biofilm in the saline solution. Preparations for dilutions were made and then submitted to a total bacterial count. This count was utilized to determine the magnitude of reduction generated by the disinfectants, expressed as decimal reductions.

Evaluation of disinfectant efficacy against seven-day-old biofilm: The disinfectants used were disinfectant A (Aqua plus® 1%, which composed of Stabilized Hydrogen peroxide 50%), disinfectant B (Virkon S® 1%, which composed of Potassium peroxy mono sulphate 50%) and a general neutralizer (3% polysorbate 80, 0.3% lecithin, 0.1% Histidine, 0.5% sodium thiosulphate, 3% Saponin, and 1% sodium Laureth Sulphate) used for evaluation according to ASTM E1054-02 (2002). Fresh stock solutions for each disinfectant were set according to the manufacturer's conditions. After incubation, the tested

coupons with 7-day biofilm growth were removed from TSB and immediately rinsed with PBS to remove the culture broth and planktonic cells. For each tested disinfectant, a total of two PVC coupons were soaked in 10 ml of diluted disinfectant solution for 10 min at room temperature. After contact time, they transfer to 10 ml neutralizing agent for 5 min to inactivate the disinfectant's killing effect. After this step, they were swabbed with a sterile cotton swab and placed in a tube containing 10 mL of saline. The tube was then vortexed at 2,800 rpm for 2 min. Next, ten-fold serial dilutions were prepared, followed by plating and incubation at 35°C for 24 hours. The control positive coupon is the one that has biofilm growth but is not treated with disinfectant. The anti-biofilm effect or log reduction (R) of tested disinfectants was assessed by subtracting the log of viable count (CFU) post the effect of disinfectants from the log of bacterial count after 7-day growth (before exposure to disinfectants).

Scanning Electron Microscope: With some modifications to the protocol explained by Brown *et al.* (2015). The authors estimated the biofilm development of the *S.* Enteritidis strain on PVC coupons using scanning electron microscopy (SEM). A microscope examination was also conducted on the treated PVC coupons with disinfectants A and B after 7 days of biofilm formation. A scanning electron microscope (FEI Quanta 3D 200i) was used for the inspection at the Grand Egyptian Museum. The instrument was operated under a low vacuum with an acceleration voltage ranging from 20.0 to 30.0 kV, and a large field detector with a working distance of 15 to 17 mm was employed.

Statistical analysis: We used SPSS Statistics version 16 to examine the data that was entered into Microsoft Excel 2010. Pearson's Chi-square (X^2) test was applied to examine the risk of *Salmonella* occurrence from various samples from birds and the surroundings of poultry houses. For the variables examined using X^2, a difference was judged significant if the P-value was less than 0.05 at a 95% confidence level. Before analysis, we logarithmically transformed the bacterial counts to standardize the data distribution.

RESULTS

During the field investigation, the investigated broiler farms suffered from mortalities ranging from 6% to 15.5%. General clinical signs included ruffled feathers, unthriftness, reduced appetite, poor feed conversion rate (FCR), diarrhea with varying colors from white to brown, and a pasty vent in most cases. As mentioned in Fig. 1, the PM examination of freshly dead birds revealed that the liver was enlarged (hepatomegaly) with the existence of

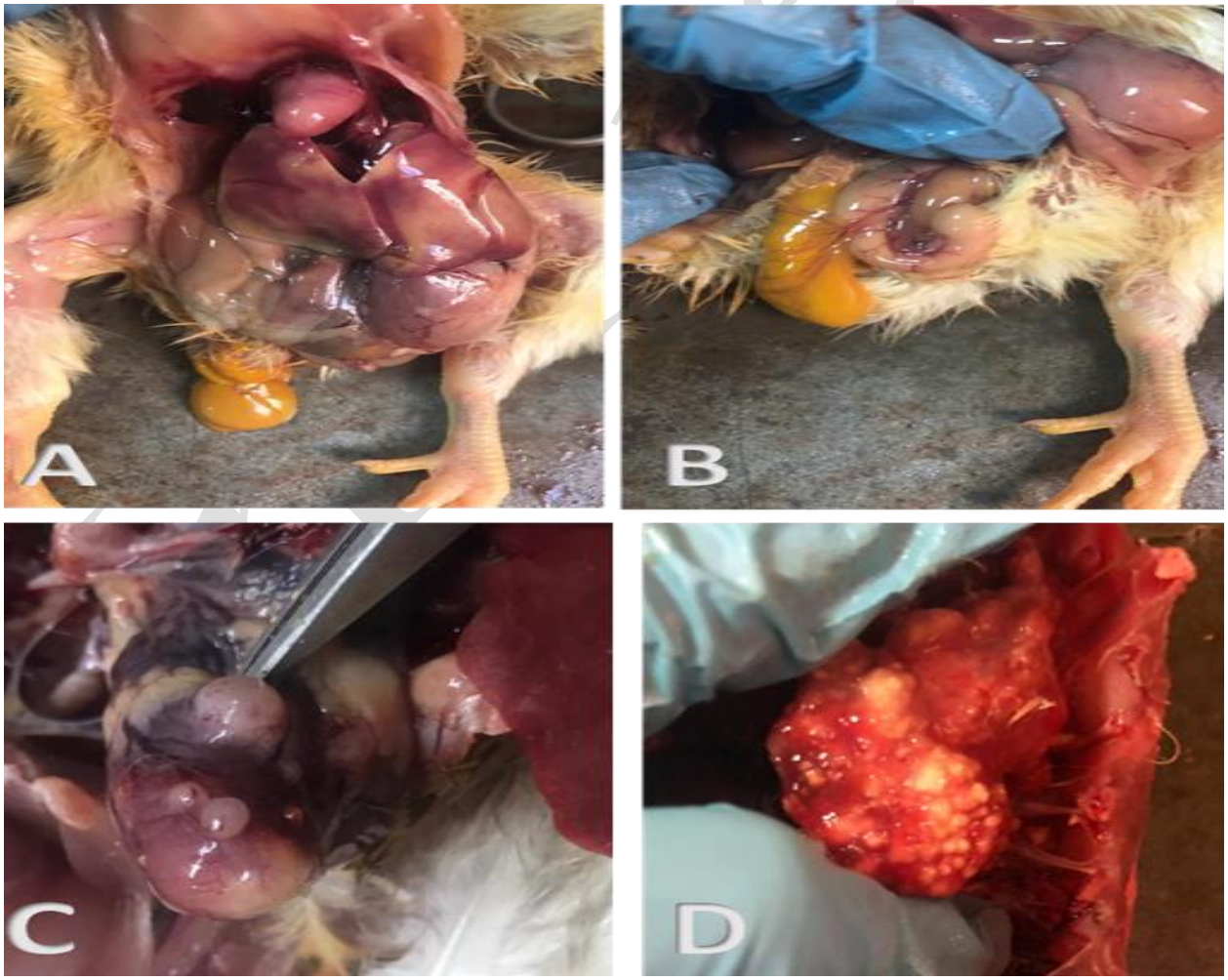

Fig. 1: Freshly dead chicks, A: PM exam showing enlarged liver with subcapsular hemorrhages, B: *S.* Enteritis with unabsorbed yolk sac, C: nodules on the heart, D: nodules on the lung tissue.

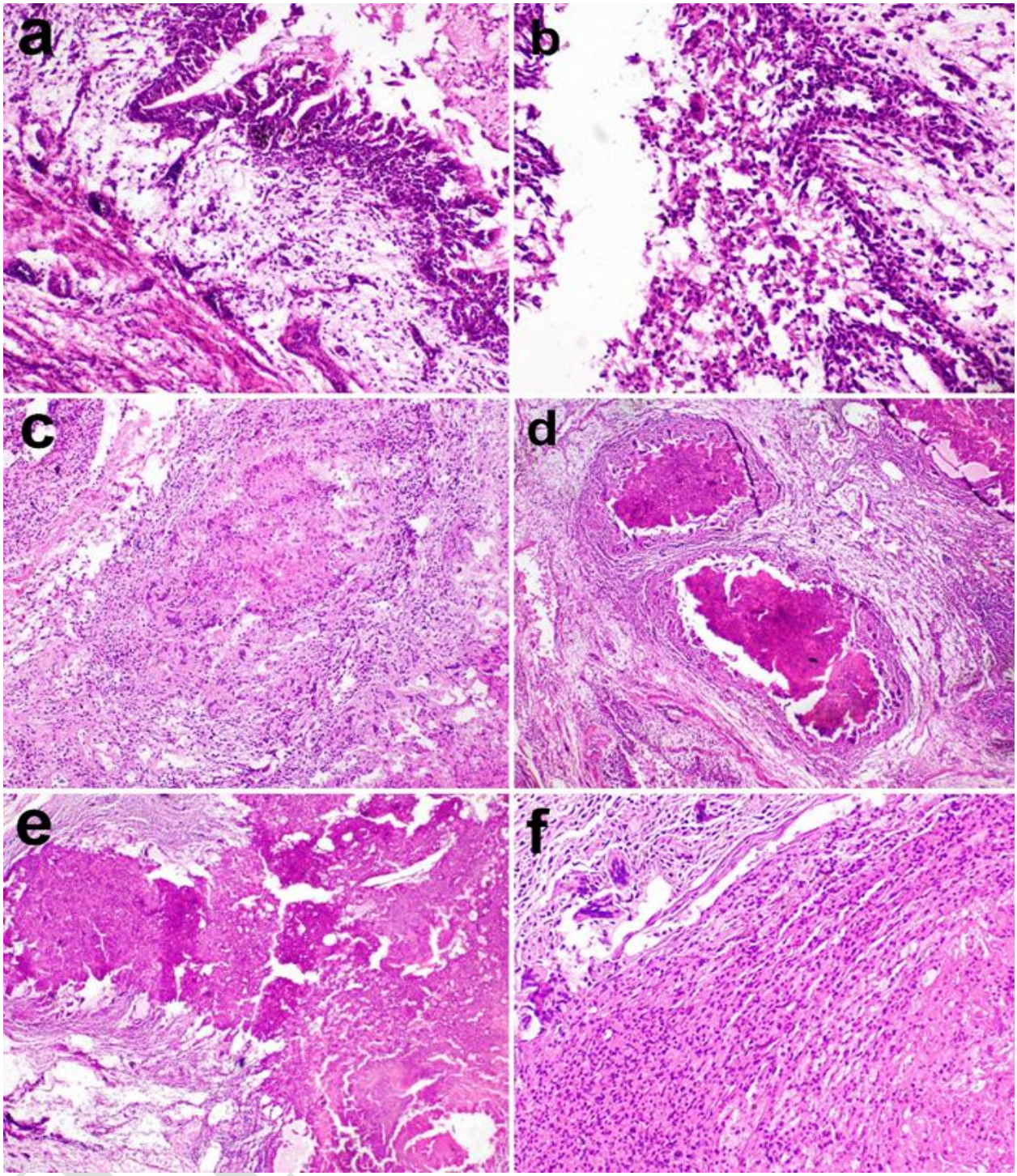

Fig. 2: histopathology of pulmonary tissue of affected birds. (a) severe diffuse edema and leukocytic cell infiltration in the submucosa of bronchi (X100). (b) hyperplastic bronchial epithelium with erosions (X200). (c) necrotic areas surrounded by leukocytes (X40). (d) multifocal, demarcated, distinct necrotic areas surrounded by leukocytes and fibrous connective tissue (X40). (e) coalescing large granulomas (X40). (f) Multinucleated giant cells among the leukocytes demarcating the necrotic areas (X100). Hematoxylin and eosin stain.

subcutaneous hemorrhage and necrosis, unabsorbed yolk sac, enteritis, nephrosis, and ureters distended with creates, congested spleen and some cases revealed septicemic pictures (congestion in subcutaneous tissues, congestion in all parenchymatous organs with petechial hemorrhages on heart muscle), in some cases, nodules were observed in the lung and live tissues.

Microscopy of the lungs in affected birds revealed severe diffuse edema and leukocytic cell infiltration in the submucosa of bronchi (Fig. 2a). The lining epithelium of the bronchi was hyperplastic and sometimes eroded (Fig. 2b). The bronchial lumen was filled with exudates, leukocytes, and desquamated epithelium. Severe chronic multifocal granulomas were observed replacing the pulmonary tissue. The lesions varied from necrotic areas surrounded by leukocytes to well-demarcated, distinct necrotic areas surrounded by leukocytes and fibrous connective tissue (Fig. 2c, d). These necrotic areas may coalesce, forming large granulomas (Fig. 2e). Multinucleated giant cells were sometimes observed among the leukocytes, demarcating the necrotic areas (Fig. 2f).

Salmonella ferments dextrose, mannitol, and maltose to produce gas and acid. Based on the cultural and biochemical characteristics listed in OIE (2004), *Salmonella* shows positive results for the methyl red, triple sugar iron, citrate utilization, & catalase but negative results for the VP, urease, & indole tests. Table 1 shows that 5 of the 11 farms in Egypt that underwent examination had positive results for *Salmonella* isolation and identification from internal organs and environmental samples (45.4%). The incidence rate of *Salmonella* isolation from different samples. The incidence rate of *Salmonella* isolation was 4.45% for the poultry samples, including 220 bird organs and environmental samples, for 10 isolates (seven from birds, and 3 from the environment). Serological analysis was used to identify the ten *Salmonella* isolates in this study, and five serotypes, *S.* Enteritidis (40%), *S.* Kentucky (30%), *S.* Typhimurium (10%), *S.* Infants (10%), and *S.* Gueuletapee (10%), were reported (Fig. 3).

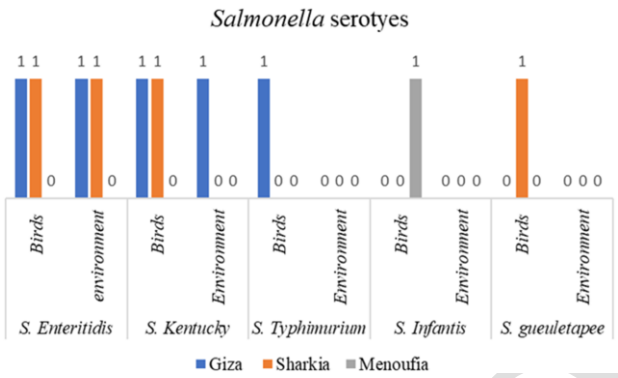

Fig. 3: Number of *Salmonella* serotypes isolated from broiler farms in different governments.

Table 1: Isolation of *Salmonella* from the eleven broiler poultry farms collected from diseased birds and environmental samples

Measurments	Governorate			Total
	Giza	Sharkia	Menoufia	
No. of examined farms	4	3	4	11
No. of Positive farms	2	2	1	5
%	50	66.6	25	45.45
Total no. of isolated *Salmonella* strains	5	4	1	10
Positive (bird's organs)	3	3	1	7
Positive (Environmental samples)	2	1	0	3

Our risk analysis survey at the farm level (Table 2) revealed that the highest isolation rate was observed in individuals under the age of two weeks (6 isolates out of a total of 10 isolates), with a significant difference in isolation rates among individual ages ($P<0.05$). A statistically significant difference was observed based on health status, frequency of cleaning, and sampling source ($P<0.05$). Conversely, there is no discernible difference ($P>0.05$) between the other factors, which include flock size, farm location, housing type, water supply, and foot bath usage.

Table 3 indicates that all *Salmonella* isolates in this study were positive, as evidenced by a visible film lining the bottom & wall of the test tubes, as determined by the tube adherence test. Fig. 4 illustrates that 20% of the *Salmonella* isolates were strongly positive, 50% were moderately positive, and 30% were weakly positive.

As seen in Table 4, the results of antibiofilm efficacy of tested disinfectants in our study showed that Virkon S® (1% wt./vol) & H_2O_2 (1.0% vol/vol) were effective in reducing the microbial load on the PVC surface after 10 min contact time by 5.6 and 5.8 log, respectively, without causing the removal of all of the bacteria as the viable bacterial count (after 7-day biofilm growth) was 8.6 x 10^8 CFU/mL.

Table 2: The risk analysis of *Salmonella* occurrence from different sampling points: Association between *Salmonella* prevalence and variables of chickens from our studied broiler poultry farms

Characteristic	Variables	Positive	Negative	Total	% Positive	Chi-square (X^2)	P-value
Age distribution	<2 wks.	6	54	60	10	6.189	.045*
	2-3 wks.	4	126	130	3.07		
	>21 days	0	30	30	0		
	Total	10	210	220			
Flock size	<1200	5	115	120	4.16	0.087	.767
	>1200	5	95	100	5		
	Total	10	210	220			
Health status	Sick	9	120	129	6.97	4.248	.039*
	Healthy	1	90	91	1.09		
	Total	10	210	220			
Location	Government 1	5	95	100	5	1.815	.403
	2	4	56	60	6.66		
	3	1	59	60	1.66		
	Total	10	210	220			
House type	Open	7	143	150	4.66	0.016.	.899
	Closed	3	67	70	4.28		
	Total	10	210	220			
Source of Water	Tap	5	145	150	3.33	1.596	.206
	Well	5	65	70	7.14		
	Total	10	210	220			
Frequency of cleaning	Frequent/spot cleaning	3	162	165	1.8	11.3143	.0008*
	Infrequent/between flocks	7	48	55	12.7		
	Total	10	210	220			
Use of footbath	No	9	171	180	5	0.471	.492
	Yes	1	39	40	2.5		
	Total	10	210	220			
Sample type	birds' organs	7	48	55	12.72	11.3143	.0008*
	Environmental samples	3	162	165	1.81		
		10	210	220			

The result is significant at $P<.05$.

Table 3: Different isolated *Salmonella* serotypes and their biofilm formation potency (adherence level) by the Test Tube Method

Strain	Serotype	Origin	Adherence level
1	S. Enteritidis	Birds	++
2	S. Enteritidis	Birds	+++
3	S. Enteritidis	Enviro. (fan swabs)	++
4	S. Enteritidis	Enviro. (water)	+++
5	S. Kentucky	Birds	++
6	S. Kentucky	Birds	++
7	S. Kentucky	Enviro. (water)	++
8	S. Typhimurium	Birds	+
9	S. Infantis	Birds	+
10	S. *gueuletapee*	Birds	+

Table 4: Antibiofilm Efficacy of tested disinfectants

Disinfectants	Aqua plus® H_2O_2	VIRKON S
Average initial count (bacterial growth in TSB) CFU/ml	5×10^8	
Viable bacterial count (after 7-day biofilm growth) CFU/ml	8.6×10^8	
Contact time	10 min	10 min
Viable bacterial count (After disinfection) CFU/ml	13×10^2	19×10^2
Log reduction	5.8	5.6

SEM results showed that contaminated PVC with *Salmonella* had matrix formations in very similar *Salmonella*-shaped biofilms (Fig. 5a, b, and c). When treated PVC was exposed to disinfectants after biofilm formation, the microbes exhibited different morphologies from those observed in the positive biofilm (Fig. 5D, E, and F). Meanwhile, the effect of disinfectant H2O2 was analyzed at different magnifications: 5.000, 6.000, and 10.000 (Fig. 5G, H, and I).

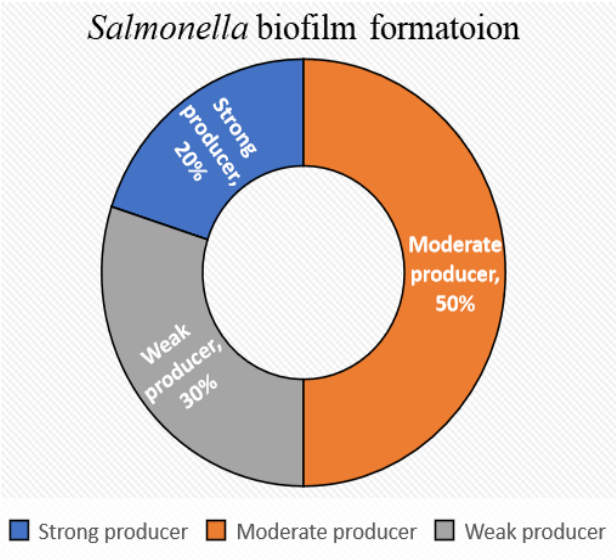

Fig. 4: Degree of biofilm production.

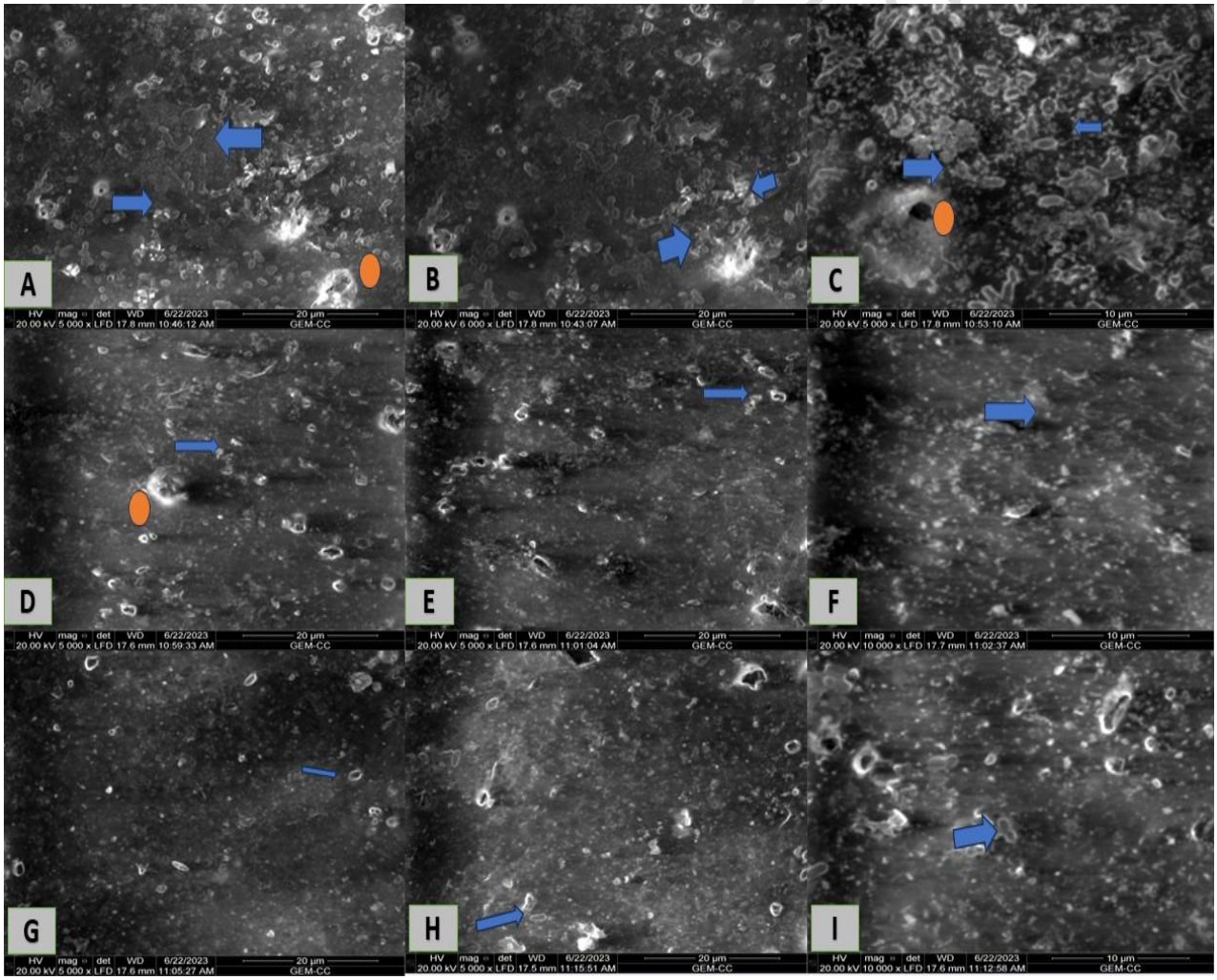

Fig. 5: PVC analysis by scanning electron microscopy. Biofilm formation at different magnifications 5.000-6.000-10.000 (A, B, & C). The effect of disinfectant Virkon S was analyzed at different magnifications 5.000, 6.000 and10.000 (D, E, and F), while the effect of disinfectant H_2O_2 was analyzed at different magnifications 5.000-6.000-10.000 (G, H, and I). Blue arrow: for bacteria accumulation, red circle: PVC surfaces.

DISCUSSION

Infectious disease outbreaks and dissemination can have a catastrophic impact on the commercial chicken industry, particularly if the illness is foodborne or zoonotic, and can significantly affect public health (Salem *et al.*, 2023). Biofilm-producing bacteria cause severe economic losses to the livestock and food industries by causing food deterioration, disease outbreaks, and even mortality (Yousef *et al.*, 2023; Laban *et al.*, 2025). In poultry farms, the main zoonotic pathogens that form biofilms and provide a genuine risk to animal and human health include *Salmonella, Staphylococcus*, *Campylobacter*, *Pseudomonas*, *Clostridium*, *E. coli*, *Klebsiella*, and *Aeromonas* species (Ibrahim *et al.*, 2023). The primary risk factors for the biofilm-producing pathogenic bacteria that might contaminate a poultry farming environment are contact with chicken ration, plants, dust, pipes, utensils, excrement, contact surfaces, and equipment (Butucel *et al.*, 2022).

Salmonella biofilm formation in poultry houses represents a significant hazard, as biofilms allow the bacteria to persist on surfaces such as drinkers, feeders, litter, and equipment despite cleaning and disinfection (Marin *et al.,* 2009; Krüger *et al.,* 2025). Within the biofilm matrix, *Salmonella* cells are protected from environmental stress, antimicrobials, and host immune responses, enabling long-term survival and acting as a continuous source of flock infection. This persistence increases the risk of vertical and horizontal transmission, contamination of poultry products, and subsequent public health threats through the food chain (Biyashev *et al.,* 2025).

The current study's observations of the mortality rate, clinical manifestations, PM lesions, and histopathological alterations in lung tissue from various *Salmonella* paratyphoid infections were consistent with those made earlier by El-Saadony *et al.* (2022), Marouf *et al.* (2022), and Elsayed *et al.* (2024). The nodule found in the lung and heart tissues and the lung histological changes matched (Nazir *et al.*, 2012), who documented similar PM findings in hens with paratyphoid illness. Desmidt *et al.* (1997) also noted that *Salmonella* Enteritidis tended to produce granulomatous nodules in chickens during the experimental infection. Furthermore, during a parallel study, Pecoraro *et al.* (2017) observed the establishment of granulomatous lesions in many organs during a paratyphoid infection with *Salmonella* Dublin in cattle.

Numerous risk factors influence *Salmonella* contamination in avian production, with the condition of the prior flock and the appropriate management practices implemented before introducing a new flock to a chicken farm being the most significant ones (Butucel *et al.*, 2022). It is indeed difficult to effectively control and eradicate *Salmonella* from housing facilities. Marin *et al.* (2009) thoroughly studied the frequency of *Salmonella* on 44 broiler farms & 51-layer farms. *Salmonella* was identified in 27.2% (n = 2678) samples from broiler farms & 22.4% (n = 1409) samples from laying hen farms. The frequency of droppings, dust, machine surfaces, delivery box liners, water dispensers, water tanks, litter, and vectors (rodents, beetles, and flies) was studied, and it was found that surfaces, dust, and droppings had the highest rates of contamination.

Salmonella isolation from farms was 45.4% in our study, as demonstrated in Table 1. The highest prevalence of isolation was from poultry, followed by their environment. Several theories link the high maintenance of *Salmonella* in chicken farms to the lack of established cleaning & disinfection protocols. Table 2 displays the *Salmonella* serotypes isolated (n=10) from the poultry samples, which included 220 bird organs and environmental samples, by 4.45%. Maharjan (2016) identified the possible origins and vectors of contamination on poultry farms, reporting nearly comparable results. These vectors and sources included infected livestock, free-living animals, flies, rodents, polluted surface water, personnel, and farm equipment. Fig. 3 illustrates the five *Salmonella* serotypes identified in chickens: S. Enteritidis (40%), *S.* Kentucky (30%), *S.* Typhimurium (10%), *S.* Infants (10%), and *S.* Gueuletapee (10%). *Salmonella* Enteritidis often persists in small areas of waste and fan dust outside chicken houses, which remain after the location has been cleaned and disinfected. It can also survive on artificially contaminated poultry feed for at least 26 months (Davies and Wray, 1996). In another research, Mir *et al.* (2010) identified the predominant serotypes of *S.* Typhimurium and *S.* Enteritidis in 6.88% and 6.31% of poultry, respectively. The incidence of *Salmonella* serotypes in chickens was 3.35%. The most common serotypes found were *S. Enteritidis*, accounting for 68.1% of the isolates, and *S.* Typhimurium, accounting for 31.8% (Shivaning Karabasanavar *et al.*, 2020). Table 3 demonstrates that the isolation rate was significantly higher in the younger age group (less than two weeks) than in the older age group (two to three weeks), with rates of 60% and 40%, respectively. In agreement with Fagbamila *et al*. (2018), Salmonellosis in day-old chicks constituted a significant risk factor. Our results in Table 3 indicated that, based on sample type, environmental samples from water and fan swabs are the avian samples with the highest risk of *Salmonella* pollution. Rose *et al.* (2000) state that the subsequent flock may become infected due to insufficient cleaning and disinfection practices. When the new flock becomes contaminated, *Salmonella* quickly spread throughout the house and irrigation system. To prevent the spread of *salmonella,* it is critical to shield samples from environmental cross-contamination, specifically from water tanks, final water lines, and feed sources.

Forming biofilms may enable *Salmonella* species to endure on surfaces and remain in food processing surroundings for extended periods (Laban *et al.*, 2025). Also, because the way the bacteria are arranged in the polymer matrix makes it harder for the biocide agent to get through, biofilms are linked to higher resistance to biocides. Fig. 4 presents the results, indicating that all the examined isolates, representing ten distinct serovars, could attach to a surface and create a biofilm. However, the quantity of biofilm produced varied under different conditions. As a means of surviving and proliferating in the processing environment, *Salmonella* and other pathogens frequently develop biofilms; this enables bacteria that are lodged in the biofilm matrix to be shielded from the antibiotics required to control bacteria during processing (Morasi *et al.*, 2022). Numerous variables, like temperature, pH, and serovar identity, might affect the production of bacterial biofilms in the environment

(Borges *et al.*, 2018). Our findings demonstrated that approximately 50% of the *Salmonella* strains isolated from each hazard factor could form a moderate biofilm, regardless of where they originated. There is a direct link between serovars and the development of biofilms. They have proposed that serovar Enteritidis, isolated from different phases of chicken rearing, is a better biofilm producer than serovars Livingstone, Infantis, Saintpaul, and Virchow (Borges *et al.*, 2018). Diverse levels of biofilm production were seen in 69 distinct *Salmonella* serotypes investigated by Agarwal *et al.* (2011) (strong 19.21%, moderate 57.61%, weak 22.52%). Stepanović *et al.* (2000) also revealed that 72.9% of *Salmonella* isolates produced biofilm, with 66.3% being strong producers. According to Laban *et al.* (2025), *Salmonella* can form biofilms upon adhering to various abiotic and biotic surfaces, such as those in chicken processing environments.

Pathogens, including *Campylobacter*, *Salmonella,* and *Escherichia coli*, including avian pathogenic *Escherichia coli* strains, *Pseudomonas*, viruses, and protozoa, can be found in water system biofilm. When these pathogens enter the water system and integrate into established biofilm, it increases the hazard of flock exposure to these infections (Maharjan, 2016). Furthermore, biofilm clogs filters and water pipes, reducing water flow and potentially impairing flock performance (Fairchild and Ritz, 2009). Biofilm development is common on PVC surfaces in chicken housing; studies in a brooding environment show that if the water supply is suboptimal (APC > 4 log10 CFU/mL), bacterial growth can surpass 4 log10 CFU/cm^2 (Maharjan, 2016). The major goal of our investigation was to understand whether and how quickly biofilm would continue to grow in farm waterlines provided with clean, drinkable water with low bacterial load (3 log10 CFU/mL) and treated circumstances, particularly when the barn house is warm. Numerous investigations have demonstrated that in poultry water systems, bacteria can build biofilms; despite the cleanliness of water supplies, biofilm production can still occur (Maharjan *et al.*, 2015).

Our investigation focused on the biofilm-cide ability of widely marketed poultry drinking water disinfectants, as closed water line systems are more likely to produce biofilm due to their hidden, wet, and highly nutritious surface. We applied disinfectants based on hydrogen peroxide or chlorine to PVC coupons and waterline surfaces containing biofilm that were seven days old. Our findings indicated that Virkon S® (1% wt./vol.) and hydrogen peroxide (1.0% vol/vol) could achieve a 5.6 and 5.8 log decrease in the microbial load on the PVC surface, respectively, without causing destruction or elimination of the bacteria. This implies that the remaining bacteria may be able to recolonize and form a biofilm layer. The efficacy of chlorine compounds was evident over time; however, the current strains exhibit gene modification and can tolerate the disinfectant's effects. The disinfectant concentration, residual concentration, and contact duration significantly influence the rate of biofilm community accumulation and the control of biofilm-producing bacteria (Khalefa *et al.,* 2025). When used at the recommended dilution and contact duration, Fraise (2008) highlighted that the potent disinfectant could achieve complete biofilm elimination by reducing it by five logs (99.999%). This degree of reduction guarantees the successful eradication of biofilm. Based on this, we observed that the disinfectant alone was highly effective but did not eliminate biofilm. We have gathered data demonstrating the enhanced effectiveness of a 1% H_2O_2 disinfectant against the *Salmonella* strain, even though we couldn't achieve a complete elimination.

According to De Carvalho (2007), the high efficacy of H_2O_2 is due to the production of free radicals, which significantly influence the biofilm matrix. This result matched what Abd-Elall *et al.* (2023) found about how effective H_2O_2 is and how it can get rid of biofilm at concentrations between 2 and 5%. Nevertheless, Marin *et al.* (2009) demonstrated that hydroxide peroxide with a level of 1% had negligible efficacy against *Salmonella* biofilm, as it removed only 1.2% of the biofilm. We found that Virkon S® effectively eliminates the *Salmonella* strain's biofilm layer, thereby reducing the microbial load. The obtained result aligns with the findings of Abd-Elall *et al.* (2023), who found Virkon S® to be effective against *S.* Enteritidis biofilm. However, even after using the product at a 5% concentration for 120 minutes, the biofilm did not wholly disappear. Møretrø *et al*. (2009) evaluated two concentrations of Virkon S®, 0.1 and 4% and discovered a direct correlation between concentration and activity.

Studies on biofilms began with the development of scanning electron microscopy (SEM), which has been in use due to its excellent resolution. Conditioned surfaces encourage the excretion of exopolysaccharides that aid in attaching bacterial cells to surfaces such as metal, plastic, and organic or inorganic matrices (Laban *et al.*, 2025). SEM results showed that *Salmonella*-contaminated PVC had matrix formations in the same *salmonella*-shaped biofilms (Figure 5a, b, and c). While treating PVC with disinfectants after biofilm formation, microbes exhibited various forms, including those observed in the positive biofilm (Fig. 5D, E, and F). We also analyzed the impact of disinfectant H_2O_2 on biofilm. We expected a small amount of matrix and some other microorganisms in the treated group. However, when assessing both the biofilm and disinfectant-treated groups, there is an excellent difference in matrix formation, shape, and bacterial load.

Conclusions: *Salmonella* biofilm formation in poultry houses enables the bacteria to persist on surfaces despite cleaning, acting as a continuous infection source for flocks. These biofilms increase the risk of contamination in poultry products and pose a serious zoonotic hazard to humans through the food chain. All the tested *Salmonella* strains were able to form biofilms using the tube test, albeit with slightly different densities. For seven days, the *S.* Enteritidis strain isolated from environmental samples was chosen for *in vitro* biofilm formation on PVC coupons in BHI broth. Subsequently, we measured the potency of two different disinfectants regarding biofilm-cide. The results indicated that hydrogen peroxide (1.0% vol/vol) and Virkon S® (1% wt./vol) could lower the microbial load on the PVC surface by 5.8 & 5.6 logs, respectively, without eliminating the bacteria. However, this did not prevent the remaining bacteria from recolonizing and forming biofilm layers. The chlorine compound's effectiveness demonstrated its potency over time, but at this point.

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