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Original Research Article

Efficacy of a novel foot pan in biosecurity protocols for control of salmonellae in poultry farms

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ABSTRACT

This study aimed to replace liquid foot pan in the poultry farm, with a novel model that is used more effectively in biosecurity program convenient with the workers in Egyptian farms that avoid foot pan. This novel model dry foot pan, semiliquid (wet) foot pan and floor mat that enabled the disinfectants to be worked for a longer time. In the present study authors are looking for a durable footbath, stable, fast, easily applied and log acting in the reduction of salmonellae. The efficacy of powder disinfectant (calcium hypochlorite powder, Halamid, Staldren, Virkon S and paraformaldehyde) were tested against salmonellae in a novel form of foot pan dry, semi-liquid and floor mat models. The disinfectants were diluted by calcium carbonate or sodium chloride powder in the dry form, surfactant in the semiliquid form and use of the sponge as a mat in the third form. Daily measurement of the active principle of the tested disinfectants and the log reduction of the *Salmonellae* were done. The dry form and semi liquid form of the Calcium hypochlorite was successfully effective for 10 days in dry form and 9 days in semiliquid form. However, Halamid and Staldren were successfully effective in dry form for 14 days and 17 days respectively, semiliquid form was worked for 21 day and 3 days and floor mat was effective for 21 days and 3 days respectively. Paraformaldehyde powder was also effective for 6 days in the dry form, but in the semiliquid form was effective for 10 days, floor mat was effective for 12 days. 5% Virkon S could be effective for 3 days in the dry form and semi-liquid form but only 2 days in the floor mat form.

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1. Introduction

Nowadays, the modern poultry industry has been challenged by highly pathogenic, contagious disease organisms (bacterial, viral, even mycotic) that threatened poultry flocks resulting in serious economic consequences for the whole society. salmonellosis is an important socioeconomic problem in several counties, mainly in developing countries, where this etiological agent is reported as the main responsible for food borne disease outbreaks. It is one of the most problematic zoonosis in terms of public health all over the world because of the high endemicity, but mainly because of the difficulty in controlling them (Antunes et al., 2003), and the significant morbidity and mortality rates. As salmonellae can be introduced to the poultry house by vehicles, people, clothing, footwear, equipment, utensils, water, feed, feathers, and carcass of dead birds, litter, insects, rodents, wild birds and pets. salmonellae may survive for several years in a favorable environment (Heyndrickx et al., 2002). Thorough understanding the routes of *Salmonella* transmission is critical for successful prevention, control, and eradication

Mechanical transmission of the salmonellae throughout common behavior of farm workers, boots and personnel movement including workers and visitors. Soles of personal footwear could contaminate fomites into farm setting, resulting in contamination of the floor of the farm anteroom and the ventral surfaces of containers after contact with the contaminated anteroom floor (OIE-FAO, 2008). Egyptian stockman behavior always averts the wet foot bath in the farm calming that wet foot bath may destroy foot wear. The same says in wheals dip of the farm.

Calcium hypochlorite is an inorganic compound with formula $Ca(ClO)_2$ known as "bleaching powder," contains from 30 to 95 % of available chlorine. As a mixture of lime and calcium hypo chloride, it is marketed as chlorine powder or bleach powder for water treatment and as a bleaching agent (Vogt et al., 2010). This compound is relatively stable and has greater available chlorine than sodium hypochlorite (liquid bleach) (Connell, 2006). Hypochlorite showed higher antibacterial activity against *Salmonella Typhimurium* compared to *E. coli*. However, sodium hypochlorite was less potent against the tested Gram-negative bacteria (Kang et al., 2013).

Chloramine T. in the percentage of 24.4% of chlorine (Halamid®) is a universal, effective, readily biodegradable disinfectant with superior storage stability. It is an active chlorine compound, (but not a hypochlorite releasing compound), an effective biosecurity program may base on Halamid® brings the total bacterial and viral load in the poultry house down to a safe level. At the recommended use concentrations of 0.5 - 1 %, all harmful pathogens are killed (Axcentive Sarl, 2015). While in outbreak condition has been determined to double the use concentrations (to 1-2 %).

Staldren is a Danish product and it is 2.5 g/kg chloramine T, 21 g/kg Iron Sulphate, 25 g/kg copper, which is kind to the environment, and, moreover, has a well-documented effect on the most common bacteria and fungi spores. Among others, a very efficient product against *Salmonella* and *Campylobacter* (Jorenku, 2010).

Virkon S is a broad spectrum disinfectant containing potassium peroxymonosulfate 20.4%, sodium chloride 1.5 %, other ingredients 78.1%, total 100%. The full formulation of Virkon S is containing the triple salt of potassium monopersulphat oxidizing agent, sulphamic acid organic acid/catalyst, malic acid organic acid/catalyst, sodium hexametaphosphate buffering agent, sodium dodecyl benzene sulphonate surfactant, sodium chloride, amaranth colour & lemon peel perfume (DuPont, 2010). It is marketed as a bactericidal, virucidal, fungicidal and sporicidal agent for use in human and veterinary health settings (Dunowska et al., 2005).

Paraformaldehyde actually the powder of formalin when dissolve in water with a small amount of methanol as a stabilizer to limit the extent of polymerization (Yates, 1973). Paraformaldehyde is used as a disinfectant in commercial poultry hatcheries to reduce the number of pathogens (*Salmonella, Escherichia coli,* and *Pseudomonas*) in the hatchery environment (Sander et al., 1995).

This study aimed to replace liquid foot pan in the poultry farm, with a novel model that easily used, effective in biosecurity program convenient and acceptable with the vice of workers in Egyptian farm who avoid foot pan. This novel model dry foot pan, semi-liquid (wet) foot pan and floor mat that enabled the disinfectants to be worked for a longer time. A footbath is a very simple form of biosecurity that helps prevent the potential spread of disease. Organisms have the potential to survive for several days or weeks in the dirt stuck to the bottom of shoes. Footbaths can eliminate these organisms. So this study aimed to elongate the period which requires to change the disinfectant and to overcome the bad habits of the Egyptian workers in poultry farms.

2. Materials and methods

2.1. Location of the study

This study was carried out in the Lab of Faculty of Veterinary Medicine, Cairo University.

2.2. Period of the study

The tested disinfectants were tested daily until they give not the desired results.

2.3. Tested organism

Reference strain of *Salmonella typhimurium* (ATCC@14028). The strain is inoculated according to ISO (6579:2002): Microbiology of food and animal feeding stuff-Horizontal method for the detection of *Salmonella*. ISO 6579: 2002(E) International Standards Organization, Geneva.

2.4. Preparation of bacterial suspension for biocidal activity

Cultures of the bacterium *Salmonella typhimurium* (ATCC@14028) inoculated into 9 ml of Buffered peptone water (BPW). Reconstituted cultures were incubated at 37 °C for 24 h. One ml of the overnight culture was inoculated into a test tube containing nine ml tryptic soy broth, diluted 1:10 (Tryptic soy broth TSB).

2.5. Disinfectants: The efficacy of disinfectants agents used against *Salmonella* spp.

Table 1. Diluents used in foot pan.

Diluent	Active chemical	Manufacture
Pril @	Non-ionic surfactants (5%) and anionic surfactants (5-15%).	Henkel factory production (Made in Egypt)
Calcium carbonate	Ca2Co3	Egyptian company for chemicals production (Made in Egypt)
Sodium chloride	NaCl	Egyptian company for chemicals production (Made in Egypt)

Table 2. Disinfectants used in foot pan.

Disinfectant used	Active chemical	Manufacture
Commercial calcium hypoclorite powder	Chlorine conc. 89%	Egyptian company for chemicals production(Made in Egypt)
Halmid	Chlorine conc. 24.4%	Product by Axcentive Sarl. (Made in Netherland)
Staldren	Chloramine percentage is 10 %	Product from J.N. Jorenku (Made in Germany)
Virkon S	Chlorine percentage is 5 % Potassium peroxymonosulfate 21.41% Sodium Chloride 1.50% Other ingredients 77.09% Total ingredient 100.00%.	Product by DuPont (Made in U.S.A)
Paraformald-ehyde	Paraformaldehyde 95%	Product by Loba chemie (Made in India).

Table 3. Composition of neutralizing agent.

Disinfectant agent	Neutralizer	Reference
For all the disinfectants	3% Tween 80 (polysorbate 80) (Mp Biomedicalis), 0.3 Lethcine (Fisher chemicals), 1% Histidine (Fisher chemicals), 0.5% Sodium thiosulphate(Fisher chemicals), 3% Saponine (Fisher chemicals).	Horejsha and Kampf (2010)

2.6. Chemical analysis of disinfectants used

2.6.1. Determination of available Chlorine (British pharmacopeia 2012)

By dissolving 0.28g in 100 ml of water and carries out the complex metric titration of Calcium. One mL of 0.1 M sodium edetate is equivalent to 14.7 mg of $CaCl_2$, $2H_2O$. Determination of chlorine is according to the molecular weight.

2.6.2. Determination of the formaldehyde (British pharmacopeia 2012)

Into a 100 ml volumetric flask containing 2.5 ml of water and 1 ml of dilute sodium hydroxide solution, introduce 1.000 g of solution to be examined, shake and dilute to 100 ml with water. To 10 ml of a solution add 30 ml of 0.05 M iodine. Mix and add 10 ml of dilute sodium hydroxide solution. After 15 minutes, add 25 ml of dilute sulfuric acid and 2 ml of starch solution. Titrate with 0.1 M sodium thiosulphate. 1 ml of 0.05 M iodine is equivalent to 1.501 mg of formaldehyde CH_2O.

2.7. The Novel Model foot pan:

Three model systems for foot pan were used, at the Faculty of the Veterinary Medicine respectively, 1-dry foot pan 2-Semi liquid foot pan 3-floor mat pan.

2.7.1. Dry foot pan model

The active principle of disinfectant which used in the experiment was firstly determined then mixed with lime or other diluent in a blender for five minutes at the third speed in order to obtain homogenous powder then spread in boot or foot (Fig. 1).

Fig. 1. Dry foot pan model.

2.7.2. Semi liquid foot pan model

The disinfectant used in this experiment was firstly determined their active substance then mixed with (surfactant) diluent in blender for five minutes at the third speed in order to obtain homogenous pasty material or semiliquid matter that spread in foot or boot bath as 3 liters / meter^2. The chlorine powder when mixed with surfactant it becomes like white paste while the paraformaldehyde takes about 24 hours to dissolve in the surfactant and give a good result (Fig.2).

Fig. 2. Liquid foot pan.

2.7.3. Floor Mat model

The same of the wet model was done but the disinfectant was immersed in a piece of sponge (Fig. 3).

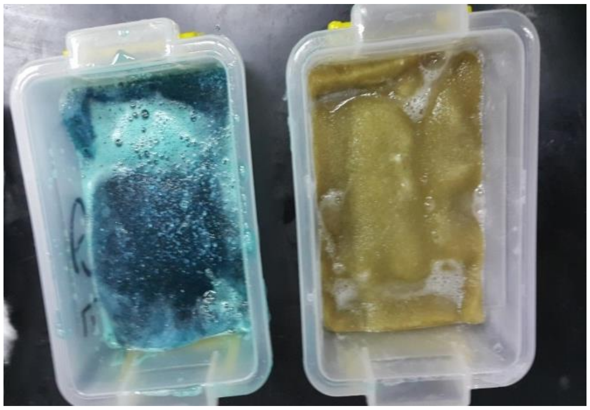

Fig. 3. Floor mat model.

2.8. Building substrate

Coupon of the same material that used in the sole of the shoes of the stockmen in poultry farm which was made of rubber. Diameter of Coupon is cm×3cm. All coupons were sterilized in 15-cm glass petri dishes by autoclaving them at 121° C for 15 minutes. A template was used in sampling procedure of square area one cc.

2.9. Surface test

The surface test is based on EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Hard Surfaces-Efficacy Data Recommendations (2012) on daily basis each model is tested against salmonellocidal activity by count after one minute and determination of the concentration of the active principle of the disinfectant as mentioned before.

3. Results

Table 4. Results of effectiveness of the used diluents in the foot pan for one minute.

Diluent	Log reduction
Slaked lime (calcium carbonate)	0.1
Sodium chloride	Zero
Surfactant	4.9

Table 5. Longevity of calcium hypochlorite in different model of foot pan as 5% chlorine is a starting point.

Days	Dry foot pan[1]		Semi liquid pan[2]	
	Log reduction	Chlorine concentration	Log reduction	Chlorine concentration
Zero	10.1	5 %	10.1	5 %
1st	10.1	3.5%	10.1	3.5%
2nd	10.1	3.5%	10.1	3.8%
3rd	10.1	3%	10.1	3.8%
4th	10.1	2.8%	10.1	3.5%
5th	10.1	2.8%	10.1	2.5%
6th	10.1	2.5%	10.1	2.5%
7th	9.1	2.5%	10.1	2.5%
8th	8.8	2%	10.1	2.5%
9th	8.6	2%	10.1	2.5%
10th	4.5	1.5%	4.5	1.8%

1-5% chlorine powder in diluted slaked lime (calcium carbonate).
2-5%chlorine powder diluted in surfactant.

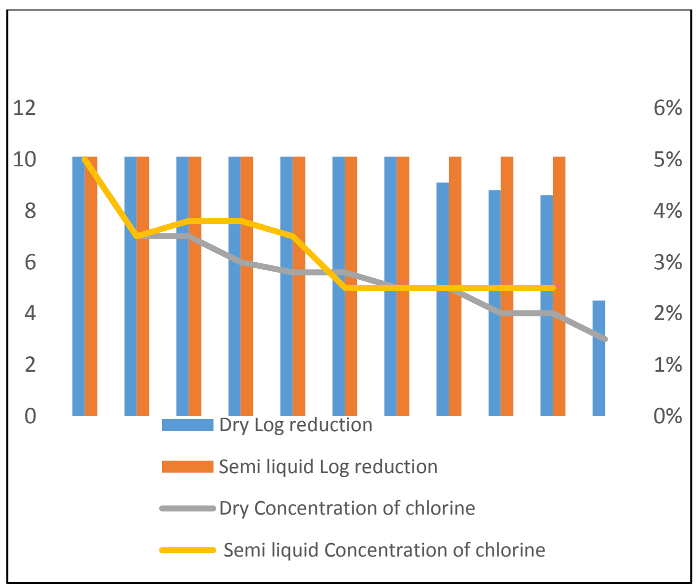

Fig. 4. Longevity of calcium hypochlorite in different models of foot pan as 5% chlorine starting point.

Table 6. Longevity of Halamid powder in different models of foot pan as 5 % chlorine starting point.

days	Dry Foot pan[1]		Semiliquid pan[2]		Floor mat[3]	
	Log reduction	Chlorine concentration	Log reduction	Chlorine concentration	Log reduction	Chlorine concentration
Zero	10.1	4.9 %	10.1	4.9%	10.1	4.9 %
1st	10.1	4.9%	10.1	5%	10.1	5%
2nd	10.1	4.5%	10.1	6.5%	10.1	6.5%
3rd	10.1	4.5%	10.1	6.8%	10.1	6.8%
4th	10.1	4.5%	10.1	7%	10.1	7%
5th	10.1	4%	10.1	7.3%	10.1	7.3%
6th	10.1	4%	10.1	7.5%	10.1	7.5%
7th	10.1	4%	10.1	7.7%	10.1	7.7%
8th	10.1	4%	10.1	8%	10.1	8%
9th	10.1	4%	10.1	8%	10.1	8%
10th	10.1	3.5%	10.1	8.5%	10.1	8.5%
11th	10.1	3.5	10.1	8.5%	10.1	8.5%
12th	10.1	3.5%	10.1	11%	10.1	11%
13th	8.1	3.5%	10.1	10%	10.1	10%
14th	7.1	2%	10.1	10%	10.1	10%
15th	4.3	2%	10.1	10%	10.1	***
16th	***	***	10.1	10%	10.1	***
17th	***	***	10.1	10%	10.1	***
18th	***	***	10.1	11%	10.1	***
19th	***	***	10.1	11.7%	9.1	***
20th	***	***	10.1	11%	9.1	***
21th	***	***	10.1	11%	9.1	***

1-5%chlorine powder in slaked lime (calcium carbonate) ; 2-5%chlorine powder in surfactant;
3-5%chlorine powder in surfactant using mat.; ***It means these values couldn't be estimated.

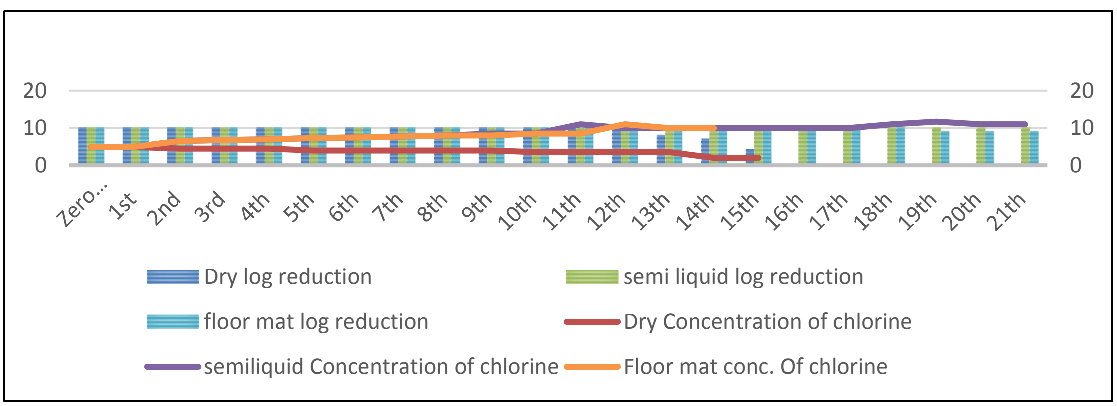

Fig. 5. Longevity of Halamid powder in different models of foot pan as 5% chlorine a starting point.

Table 7. Longevity of Staldren in different model of foot pan as 5%chlorine is a starting point.

Days	Dry foot pan[1]		Semi liquid pan[2]		Floor mat[3]	
	Log reduction	**Chlorine concentration**	**Log reduction**	**Chlorine concentration**	**Log reduction**	**Chlorine concentration**
Zero	10.1	5 %	6.1	5 %	6.1	5 %
1st	8.7	5%	5.5	10%	5.7	10%
2nd	8.1	5 %	5.1	7%	5.1	8%
3rd	8.1	5 %	4.8	7%	4.7	7%
4th	7.8	5%	***	***	***	***
5th	7.7	5 %	***	***	***	***
6th	6.8	5 %	***	***	***	***
7th	6.7	4.5 %	***	***	***	***
8th	6.7	4 %	***	***	***	***
9th	6.7	4%	***	***	***	***
10th	6.5	3%	***	***	***	***
11th	6.3	3 %	***	***	***	***
12th	6.3	3 %	***	***	***	***
13th	6.2	3 %	***	***	***	***
14th	6.1	3 %	***	***	***	***
15th	5.6	3%	***	***	***	***
16th	5.2	3%	***	***	***	***
17th	4.1	2.5%	***	***	***	***

-5%chlorine powder in slaked lime (calcium carbonate); 2-5%chlorine powder in surfactant
3-5%chlorine powder in surfactant using mat; ***It means these values couldn't be estimated.

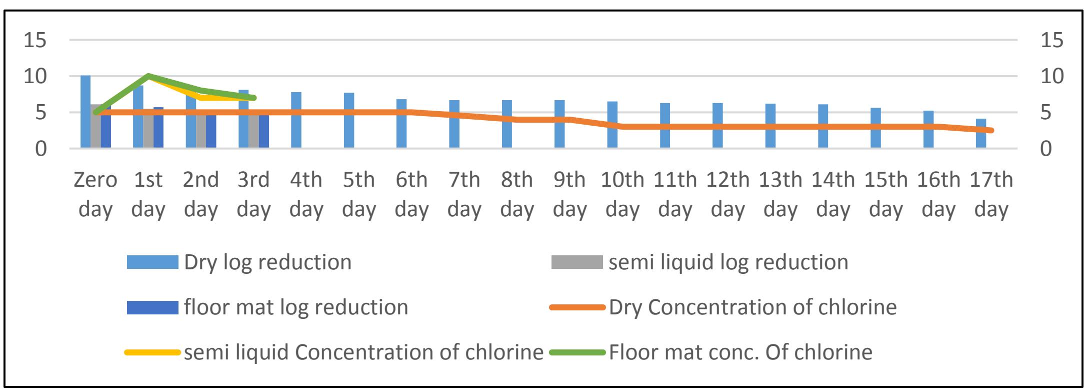

Fig. 6. Longevity of Staldren in different models of foot pan as 5% chlorine a starting point.

Table 8. Longevity 2% Virkon and 5%Virkon in slaked lime (calcium carbonate) and sodium chloride as a dry foot model.

Days	Calcium carbonate as a base of dry foot				Sodium chloride as a base of dry foot			
	Log reduction		Chlorine concentration		Log reduction		Chlorine concentration	
	2% Virkon	5% Virkon	2% Virkon	5% Virkon	2% Virkon	5% Virkon	2% Virkon	5% Virkon
Zero day	4.1	6.1	0.9	4.1	4.4	7.1	0.7%	3.8%
1st	***	5.8	***	2	negative	5.8	***	2.5%
2nd	***	5.1	***	1.7	negative	5.7	***	1.3%
3rd	***	4.1	***	0.8	negative	5	***	1.2%

*** It means that values could not be detected.

Table 9. Longevity 2% Virkon and 5%Virkon in surfactant as a semi liquid foot model.

Days	Log reduction		Chlorine concentration	
	2% Virkon	5% Virkon	2% Virkon	5% Virkon
Zero day	4.1	8.1	1	2
1st	***	6.4	***	3.5
2nd	***	5.1	***	1.8
3rd	***	3.1	***	1

***It means these values couldn't be estimated.

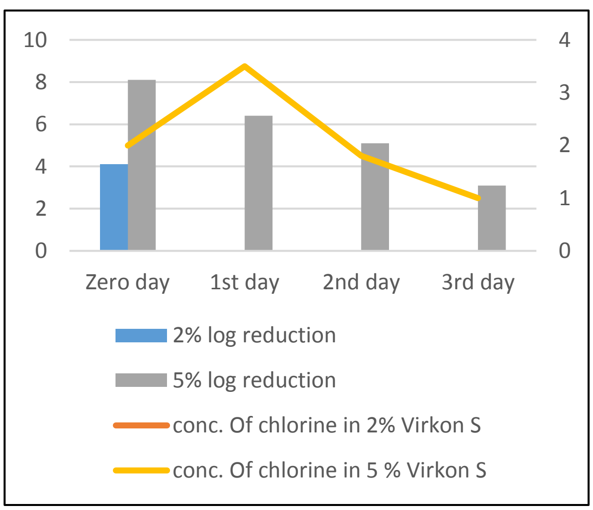

Fig. 7. Longevity 2% and 5% Virkon as a semiliquid foot model.

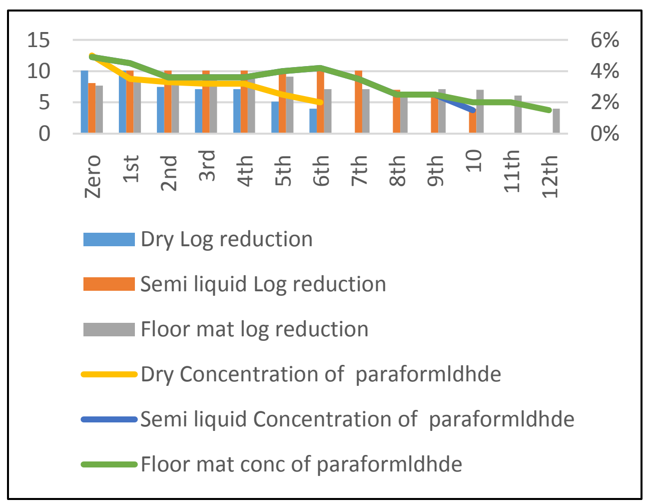

Fig. 2. Longevity of paraformldhyde in different model of foot pan as 5% formaldehyde is a starting point

Table 10. Longevity 2% Virkon S and 5%Virkon S in surfactant as a floor mat.

Days	Log reduction		Chlorine concentration	
	2%Virkon	5%Virkon	2%Virkon	5%Virkon
Zero	4	8	1	2
1st	***	7	***	3.5
2nd	***	4	***	2

***It means these values couldn't be estimated.

Table 11. Longevity of paraformaldehyde in different model of foot pan as 5% formaldehyde is a starting point.

Days	Dry foot pan[1]		Semi liquid pan[2]		Floor mat[3]	
	Log reduction	Formaldehyde concentration	Log reduction	Formaldehyde concentration	Log reduction	Formaldehyde concentration
Zero	10.1	5 %	8.1	4.9 %	7.7	4.9 %
1st	10.1	3.5%	10.1	4.5%	8.8	4.5%
2nd	7.5	3.3%	10.1	3.6%	9.1	3.6%
3rd	7.1	3.2%	10.1	3.6%	9.1	3.6%
4th	7.1	3.2%	10.1	3.6%	9.1	3.6%
5th	5.1	2.5%	10.1	4%	9.1	4%
6th	4	2 %	10.1	4.2%	7.1	4.2%
7th	***	***	10.1	3.5%	7.1	3.5%
8th	***	***	7	2.5%	6.4	2.5%
9th	***	***	6.1	2.5%	7.1	2.5%
10th	***	***	4	1.5%	7	2%
11th	***	***	***	***	6.1	2 %
12th	***	***	***	***	4	1.5%

1-5% formaldehyde powder in slaked lime (calcium carbonate); 2-5% formaldehyde powder in surfactant; 3-5% formaldehyde powder in surfactant using mat; ***It means these values couldn't be estimated

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4. Discussion

The present study looking for footbath durable, stable, easily applied fast and log acting in reduction of salmonellae, from the available powder disinfectants (calcium hypochlorite powder, Halamid, Staldren, Virkon S and paraformaldehyde) which tested against *Salmonella* in a novel form of foot pan dry, semi liquid and floor mat models. As main purpose of our different type of foot pans is to reduce the number of pathogens in the environment. By reducing pathogen numbers and the potential for disease occurrence in the poultry farm is reduced (Block, 2001).

In the current study, calcium hypochlorite powder was applied in two forms only dry powder and semi-liquid form. Meanwhile, floor mat form couldn't be applied due to the calcium hypochlorite powder destructs the sponge or the mat. Two models of the foot bath the first one is in the dry model using of 5% calcium hypochlorite powder in diluted slaked, the logarithmic reduction of average bacterial count of *S. typhimurium* (10.1 log) with a contact time one minute was 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 9.1, 8.8, 8.6, 4.5 in zero day till the 10th day respectively, also the concentration of chlorine was 5%, 3.5%, 3.5%, 3%, 2.8%, 2.5%,2.5%, 2%, 2% and 1.5% in zero day to 10th day respectively (Table 5). While semiliquid model by using 5% calcium hypochlorite powder diluted in surfactant, the logarithmic reduction of average bacterial count of *S. typhimurium* (10.1 log) with a contact time one minute was 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1 and 10.1, 4.5 in zero day to 10th day respectively, also the concentration of chlorine was 5%, 3.5%, 3.8%, 3.8%, 3.8%, 3.5%,2.5%, 2.5% , 2.5% and 1.8% in zero day till the 10th day respectively. As a mixture with lime and calcium chloride, it is marketed as chlorine powder or bleach powder for water treatment and as a bleaching agent (Vogt et al., 2010). These compounds are relatively stable and have greater available chlorine than (liquid bleach) (Connell, 2006). This results are in according to (Kang et al., 2013) who approved that hypochlorite showed higher antibacterial activity against *Salmonella typhimurium* but not against *E. coli*. Its strong inhibitory effect appeared to be due to a higher pH. However, sodium hypochlorite was less potent against the tested Gram-negative bacteria. Free-chlorine concentration (FC) of 0.25 mg/liter and exposure time of 120 s reduced populations of *S. newport* and *S. typhimurium* by only 2.1 to 3.7 log CFU/liter (Shen et al., 2012).

Days which were negative indicate to stopping effectiveness of the disinfectant. So it will be a must to change the disinfectant in the foot pan. This case repeated in all the forms of this experiment.

The effectiveness of Halamid powder was shown in (Table 6) in different three forms. The dry form revealed an average log reduction 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1 8.1,7.1 and 4.3 in zero-day till the 15th day respectively, also the concentration of chlorine was 4.9%, 4.9%,4.5%, 4.5%, 4%, 4%, 4%, 4%,4%, 3.5%, 3.5%, 3.5%, 3.5, 2% and 2% in zero day till the 10th day respectively. The semi liquid form showed an average log reduction 10.1 in zero day till the 21th day respectively, also the concentration of chlorine was 4.9%, 5%, 6.5%, 6.8%, 7%, 7.3%, 7.5%, 7.7%,8%,8%, 8.5%, 8.5%, 11%, 10%, 10%, 10%,10%,10%, 11%, 11.7%, 11% and 11% on zero day till the 21th day respectively. The floor mat form showed an average log reduction 10.1 in zero day till the 18th day and 9.1 in 19th day to 21th day, also the concentration of chlorine was 4.9%, 5%, 6.5%, 6.8%, 7%, 7.3%, 7.5%, 7.7, 8%, 8%, 8.5%, 8.5%, 11%, 10% and 10% in zero day to 14th day respectively, the rest of days we couldn’t have an amount to be estimated but it was still work in a good performance. Halamid® is a unique professional disinfectant as it is a large activity spectrum, noncorrosive in solution for materials, easy to use and versatile, stable, readily biodegradable, no risk of building up resistant microorganisms. The present results are slightly similar to that found by Parkar et al. (2004) who approved that Halamid has a bactericidal effect against thermophiles bacilli.

Concerning Staldren powder, using dry foot pan gave an average log reduction was 10.1, 8.7, 8.1, 8.1, 7.8, 7.7, 6.8, 6.7, 6.7, 6.7, 6.5, 6.3, 6.3, 6.2, 6.1, 5.6, 5.2 and 4.1 Form zero day to 17th day respectively, while the concentration of chlorine was 5%, 5%, 5%, 5%,5%,5%,5%, 4.5%, 4%,4%, 3%, 3%, 3%,3%,3%,3%, 3% and 2.5% in zero day to 17th day respectively. The semi liquid form showed the log reduction 6.1, 5.5, 5.1 and 4.8 in zero day to 3rd day respectively. Moreover, the concentration of chlorine was 5%, 10%, 7% and 7% in zero day to the 3rd day respectively. The floor mat of Staldren showed the average log reduction 6.1, 5.7, 5.1 and

4.7 and the concentration of chlorine was 5%, 10%, 8% and 7% in zero day to 3rd day respectively. On the other hand, both the wet or pasty pan and the Floor mat gave a nearly results with a log reduction of 5.1 for 2 days (Table7). Results were found in agreement with those revealed by Shetty and Gowda (2004), Ura and Sakata (2007) saying that chloramine-T is capable of inhibiting with bacterial growth.

The study noticed that the semi liquid pans especially which contain chloramine T that the concentration of the active principle decreased then increased then decreased again, it may be due to the evaporation of the water led to this phenomenon. We have no explanation about that, it needs further investigation. On the other hand Virkon S in this study showed that the log reduction of dry form of 2% Virkon S with calcium carbonate was 4.1 in the zero day, also the concentration of chlorine was 0.9%. The result of 2% with sodium chloride that the log reduction was 4.4 and the concentration of chlorine was 0.7%.

The results of log reduction of 5% Virkon S with calcium carbonate was 6.1, 5.8, 5.1 and 4.1, Moreover the concentration of chlorine was 4.1%, 2%, 1.7% and 0.8% on zero day to the 3rd day. However, the results of 5% Virkon S with sodium chloride was 7.1, 5.8, 5.7 and 5 and the concentration of chlorine was 3.8%, 2.5%, 1.3% and 1.2% on zero day to the 3rd day respectively. Two diluents were used in the dry form of Virkon S calcium carbonate and sodium chloride, the choice of sodium chloride because it is one of the components of the Virkon S.

The semi liquid form was done with two concentrations, the log reduction of 2% Virkon S was 4.1 and the concentration of chlorine was 1%, beside that the log reduction of 5% Virkon S was 8.1, 6.4, 5.1 and 3.1 and the concentration of chlorine was 2%, 3.5%, 1.8% and 1%.

The floor mat was done with two concentrations, the log reduction the log reduction of 2% Virkon S was 4 and the concentration of chlorine was 1%, beside that the log reduction of 5% Virkon S was 8, 7, and 4 and the concentration of chlorine was 2%, 3.5%, and 2%.

Virkon S is a broad spectrum disinfectant containing peroxygen compounds (peroxymonosulfate). It is marketed as a bactericidal virucidal, fungicidal and sporicidal agent for use in human and veterinary health settings (Dunowska, 2005). Furthermore, disinfectant preparations and concentrations need to be carefully scrutinized (Ruano et al., 2001). For example, peroxides preparations (Virkon-s) commercially available for the poultry industry are more frequently recommended and used at a concentration of 1%. Unfortunately, this concentration had led to unsatisfactory results (Gehan et al., 2009) and when this product was used as a disinfectant under field spectrum activity against bacteria and their spores, fungi conditions as reported by Spielholz (1998), Gasparini et al. (1995) who found that Virkon-s is effective against *Pseudomonas* and *E. coli*.

Concerning to the paraformaldehyde powder, using the dry foot pan gave the average log reduction 10.1, 10.1,7.5, 7.1, 7.1,5.1 and 4 with a concentration of paraformaldehyde was 5%, 3.5%, 3.3%, 3.2%, 3.2%, 2.5% and 2%. The semiliquid form showed log reduction 8.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 10.1, 7, 6.1 and 4 the concentration of paraformaldehyde was 4.9%, 4.5%, 3.6%, 3.6%, 3.6%, 4%, 4.2%, 3.5%, 2.5%, 2.5%and 1.5%. The log reduction of the floor mat form was 7.7, 8.8, 9.1, 9.1,9.1, 9.1, 7.1, 7.1, 6.4, 7.1, 7, 6.1 and 4 and the concentration of the paraformaldehyde was 4.9%, 4.5%, 3.6%, 3.6%, 3.6%, 4%, 4.2%, 3.5%, 2.5%, 2.5%, 2.5%, 2%, 2% and 1.5%. Regarding paraformaldehyde powder, addition 5% paraformaldehyde powder in surfactant using mat (floor mat) gave excellent results with log reduction of 6.1 for 11 days, followed by addition 5% paraformaldehyde powder in surfactant (pasty pan model) with log reduction of 6.1 for 9 days, then using 5% paraformaldehyde powder in slaked lime (dry feet pan) with a log reduction of 5.1 for 5 days even after one minutes contact (Table 11).

Paraformaldehyde is very effective against a wide spectrum of organisms and it is well suited for the reduction of the bacterial population and the storage of non-wrapped sterilized instruments (Schilling et al., 1982). Formaldehyde is effective against most viruses and bacteria, including the acid-fast Mycobacteria. Solution of 1-5% formalin is sometimes used to disinfect buildings or as a prophylactic and therapeutic foot bath for foot rot (Kahrs, 1995). Martelli et al. (2016) mentioned that formaldehyde acts effectively against *Salmonella* even in the presence of some residual organic matter.

Campos et al. (2012) mentioned that 10% formaldehyde were effective against *S. aureus* strains. These results agreed with those given Bamabace et al. (2003) and Kuich et al. (2004).

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References

Antunes P, Reu C, Sousa JC, Peixe L, Pestana N. (2003). Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. Int. J. Food Microbiol., (82), 97–103.

Axcentive Sarl (2015). The professional disinfectant with over 60 years proven efficacy. http://www.halamid.com/

Bambace AMJ, Barros EJA, dos Santos SSF, Jorge AOC (2003). Eficácia de soluções aquosas de clo- rexidina para desinfeção de superfícies. Rev. Biociênc. 9, 73–81.

Block SS (2001). Disinfection, sterilization and preservation, 5th ed. Lippincott, Williams, and William, Philadelphia, PA.

British Pharmacopoeia (2012). Volume 1. Determination of chlorine. 483–484, determination of formaldehyde. 695–696.

Campos GB, Souza SG, Lob O TN, da Silva DC, Sousa DS, Oliveira PS, Santos VM, Amorim AT, Farias SV, Cruz MP, Yatsuda R, Marques LM. (2012). Isolation, molecular characteristics and disinfection of methicillin-resistant *Staphylococcus aureus* from ICU units in Brazil. New Microbiol., 35: 183–190.

Connell GF (2006). Key operating strategies for chlorine disinfection operating systems. Proceedings of the Water Environment Federation, WEFTEC, pp. 6329–6334.

Dunowska M, Morley PS, and Hyatt DR (2005). The effect of Virkon1S fogging on survival of *Salmonella enterica* and *Staphylococcus aureus* on surfaces in a veterinary teaching hospital. Vet. Microbiol., 105, 281–289.

DuPont Animal Health Solution (2010). http//www.2dupont.com/DAHS_EMEA/en_GB/products/disinfectants/virkon

Gasparini R, Pozzi T, Magnelli R, Fatighenti D, Giotti E, Polise Pratelli G, Severini R, Bonanni P, De Feo L (1995). Evaluation of *in vitro* efficacy of the disinfectant virkon. Eur. J. Epidemiol., 11:193–197.

Gehan ZM, Anwer W, Amer HM, EL-Sabagh IM, Rezk A, Badawy EM (2009). *In vitro* efficacy comparisons of disinfectants used in the commercial poultry farms. Int. J. Poul. Sci., 8 (3), 237–241.

Gregory KD (2010). Biosecurity reconsidered: calibrating biological threats and responses. Int. Secur., 34(4): 96–132.

Heyndrickx M, Vandekerchove D, Herman L, Rollier I, Grijspeerdt K, De Zutter L (2002). Routes for *Salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. Epidemiol. Infect., 129(2):253–65.

Horejsha D, Kampf G (2010). Efficacy of three surface disinfectants against spores of *Clostridium difficile* ribotype 027. Int. J. Hyg. Environ. Health, 214: 172–174.

ISO 6579 (2002). Microbiology - General guidance on methods for the detection of *Salmonella*. 4th ed. International Organization for Standardization, Geneve, Switzerland.

Jorenku JN (2010). A safe disinfectant for all livestock. http://www.jorenku.dk/en/

Kahrs RF (1995). General disinfection guidelines. Rev. sci. tech. Off. int. Epiz. 14(1), 105-122.

Kang S-N, Kim K-J, Park J-H, Kim K-T, Lee O-H (2013). Effect of antimicrobial activity of sodium hypochlorite and organic acids on various foodborne pathogens in Korean ginseng root. Afr. J. Microbiol. Res., 7(22), 2724–2729.

Koblentz GD (2010). Biosecurity reconsidered: calibrating biological threats and responses. Int. Secur., 34 (4): 96–132.

Kuich JD, Borowsky LM, Silva VS, Ramenzoni M, Triques N, Kooler FN, Cardoso MRI (2004). Evaluation of the antibacterial activity of six commercial disinfectants against *Salmonella typhimurium* strains isolated from swine. Acta Sci. Vet., 32, 33–39.

Martelli F, Gosling RJ, Callaby R, Davies R. (2016). Observation on *Salmonella* contamination of commercial duck farms before and after cleaning and disinfection. Avian Pathol., 5: 1–7.

Parkar SG, Flint SH, Brooks JD (2004). Evaluation of the effect of cleaning regimes on biofilms of thermophilic bacilli on stainless steel. J. Appl. Microbiol., 96, 110–116.

PrEN 1276, (2012). CEN. European Committee for Standardization Chemical disinfectants and Antiseptics-Quantitative suspension test for evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industerial, domestics, and institutional areas- Test method and requirements (Phase 2, Step 1). PrEN 1276: 2004. CNE, Central Secretariat: rue de Stassart 36, 1050 Brussels, Belgium.

Ruano M, El-Attrache J, Villegas PA (2001). Efficacy comparisons of disinfectants used by the commercial poultry industry. Avian Dis., 45:972–977.

Sander JE, Wilson JL, Van Wicklen GL (1995). Effect of formaldehyde exposure in the hatcher and of ventilation in confinement facilities on broiler performance. Avian Dis., 39: 420–424.

Schlling B, Wigert H, Weuffen W, Dobberkau HJ (1982). Use of paraformaldehyde tablets for bacterial count reduction, disinfection, cold sterilization, and sterile preservation of medical instruments. 3: On the use of paraformaldehyde tablets in medical institutions. Pharmazie., 37(7): 518–521.

Shen C, Luo Y, Nou X, Wang Q, Millner P (2012). Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157:H7, and non-O157 Shiga toxin–producing *E. coli*. J. Food Prot., 76 (3): 386–393.

Shetty M, Gowda TB (2004). A Study of Substituent Effect on the Oxidative Strengths of N-Chloroarenesulphonamides: Kinetics of Oxidation of Leucine and Isoleucine in Aqueous Acid Medium, in: Zeitschrift für Naturforschung. 59: 63–72.

Spielholz B (1998). Properties of hatchery disinfectants. World Poult. 14:50–51.

Ura Y, Sakata G (2007). "Chloroamines", Ullmann's Encyclopedia of Industrial Chemistry (7th ed.), Wiley, p. 5

Vogt H, Balej J, Bennett J E, Wintzer P, Sheikh SA, Gallone, P, Vasudevan, S, Pelin K (2010). Chlorine Oxides and Chlorine Oxygen Acids. Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH.

World Organization for Animal Health (2008): OIE-FAO Guide to good farming practices for animal production food safety. Bulletin de 1' OIE, 3:5–12

Yates J (1973). Adsorption and decomposition of formaldehyde on tungsten (100) and (111) crystal planes. J. Catal., 30 (2): 260–275.