

Effectiveness of Some Disinfectants Commonly Used in footbaths inside Poultry Farms

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Abstract: Footbath is a very simple form of biosecurity that helps preventing the potential spread of disease. Three experimental studies were conducted to evaluate and compare the bactericidal effectiveness of five commercial disinfectants (CleanZix®, Zix-Virox®, Synergize®, GroundZero® and Halamid®) in liquid and semi-liquid form after one minute contact time, Besides, comparing the capacity of liquid and semi-liquid foot baths to withstand and remain effective along the study duration for three successive days challenged daily against 12 cm² contaminated rubber shoes with poultry deep litter. After one-minute contact time, all the disinfectants were not effective in reducing the bacterial load of the contaminated shoes. Otherwise, the Chloramine-T disinfectant (Halamid®) in its first day of use (liquid form) showed the highest log reduction 4.17. the semi-liquid bath achieved 100% bacterial reduction and showed no viable bacteria along the whole experiment days., the semiliquid foot baths need much time to act correctly as it was more condensed, but this did not lessen its powerful and efficient capacity to remain clean for 3 successive days than the liquid baths.

Key words: disinfectants, Chloramine-T, liquid and semi-solid foot bath, poultry, organic matter.

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I. Introduction

Although the main risk factor for introducing infection to a poultry flock is direct contact to infected poultry, humans are supposed to be another risk factor who may act as mechanical vectors. Subsequently, most biosecurity protocols on poultry production facilities include that employees, visitors, and veterinarians disinfect their shoes before entering facilities. Yet, few studies have evaluated the efficacy of footbaths for reducing bacterial count on footwear. Footbaths will not sterilize footwear, but they could benefit in reducing the risk for infection when used with effective disinfectants. [1].

Numerous disinfectants are commercially available, but careful consideration should be taken before selecting the suitable one for footbath. [2]. Appropriate attention should be taken while mixing disinfectants. Sub-lethal concentrations of disinfectant may keep organisms viable but in non-cultural state. [3,4] or even develop antimicrobial resistance. [5]. Poor maintenance of footbaths and its obvious contamination with organic matter are frequently observed in most facilities. People usually avoid stepping into foot baths or just step through the bath without cleaning their footwear. [6]. Many disinfectants do not function well in the presence of organic matter, such as soil or litter, as quaternary ammonium compounds [2].

Disinfectants may have a limited lifespan after their initial dilution in foot baths due to heat, sunlight, time, organic matter (OM), and other contaminants that reduce their efficacy [7].

The aim of disinfection is to decrease microbial populations. The various classes of disinfectants include, quaternary ammonium compounds (QAC), halogens, oxidizing agents, aldehydes, chlorhexidine compounds, and alcohols [8].

Hydrogen peroxide and glutaraldehyde products are most commonly used disinfectants in cleansing and disinfection steps in poultry farms and footbath [9,10]. Peroxygen disinfectant had higher decontamination properties on rubber boots, compared with the QAC disinfectant [1]. The low efficacy of peroxygen was associated to its susceptibility to organic matter [11,12]. The combination of glutaraldehyde and QAC increases their antibacterial effectiveness [13]. Iodophors have powerful bactericidal efficacy against both Gram-positive and Gram-negative bacterial, and act through their high affinity to microbial membrane lipids [14,15]. Chloramine-T produces a more stable residual in water than free chlorine providing long-lasting protection against regrowth. Moreover, chloramine could penetrate more deeply than chlorine within the biofilm [16].

From the light of this literature, the presented study was conducted to determine bactericidal efficacy of disinfectants commonly used in foot bath inside poultry farms, comparing bactericidal effectiveness of liquid and semi-liquid foot baths, moreover monitoring the survival of total viable aerobic colony count that contaminate the footbaths from sole of shoes and ability of liquid and semi-liquid foot baths to withstand and remain effective with progressive increase the microbial and organic load.

II. Materials and methods

This study aimed to judge the validity of five disinfectants commercially recommended as footbaths in poultry farms (table 1). Footbaths were evaluated in two forms; either liquid or semi-liquid dilutions, for one-minute contact time against soiled shoes. The test was repeated for three successive days.

Table (1). Selected disinfectants with its manufacture recommended use dilution

Brand name	Active ingredients	Use dilution	Company
CleanZix®	Alkali combination with sequestering agents, emulsifying, saponification, humectants.	1%	Biocidas Biodegradables ZIX, España
Zix-Virox®	Hydrogen Peroxide 25% peracetic acid synergized 5%, organic acids, stabilizers and surfactants	0.5%	Biocidas Biodegradables ZIX, España
Synergize®	Alkyl (C12 67%, C14 25%, C16 7%, C18 1%) dimethyl benzyl ammonium chloride 26.00% Glutaraldehyde 7.00% Inert Ingredients 67.00%	0.4%	Preserve International. Reno, NV, USA
GroundZero®	Iodophore 3% Glutaraldehyde 3.5% Acid base	0.4%	Electrostatics, Bradenton, FL, USA
Halamid®	sodium N-chloro-para-toluenesulfonamide	1%	Axcentive, France
Pril®	Aqua. Sodium laureth sulfate. Cocamidopropylbetaine Sodium chloride Parfum Limonene 2-Bromo-2-nitropropane-1,3-diol colorant methylchloroisothiazolinone methylisothiazolinone	-	Henkel, Egypt

Foot baths vessel:

Each of liquid and semi-liquid dilutions were placed in clean plastic containers of 75 cc length, 50 cc width, and 20 cc depth. were used for each disinfectant bath. Solution of at least 10 cc depths., from the bottom. The same foot baths were tested for the 3 days of the study. Foot bathes were kept at room temperature (approx. 25° C) for 3 successive days of experiment duration, for calculating the bacterial log reduction, a daily sampling before and after immersion of contaminated shoes was conducted.

Foot wears:

Sterile Rubber Shoes with an average tested surface area of 12 cm² were soiled with deep-litter obtained from a broiler farm aging 32 days' litter was packed in flat plastic vessel of length 40x40width depth of the litter ranging from 10 to 15 cc. Then, the soiled shoes were done by standing over the box for 10-minute minimum time for rooming inside poultry folk. Soles of shoes were swabbed and tested for the total viable aerobic colony counts (TVCC) before and after contact to foot baths. The same steps were followed for three successive days.

Disinfectants:

Five commercially available disinfectants (table 1), were assessed for their bactericidal efficacy as liquid and semi-liquid foot baths (**1% CleanZix®**, **0.5% Zix-Virox®**, **0.4% Synergize®**, **0.4% GroundZero®**, and **1% Halamid®**). Liquid foot baths, were diluted with tape water, according to the manufacturer recommended working solution. Semi liquid foot baths were prepared by mixing two parts of the diluted liquid disinfectant to three parts of viscous detergent **Pril®**.

Foot bathes were kept uncovered at room temperature (approx. 25° C) for the 3 successive days of the study duration.

Survival of total viable aerobic colony in footbaths

A daily 1 ml samples from the foot baths were counted for their TVCC (before and after immersion of the soiled shoes into the foot baths); to evaluate their contamination load.

Contact time

The bactericidal effectiveness of the tested disinfectants foot bath was assessed after only 1 min contact time (usual personnel time in foot bath in poultry farms).

Neutralization of the disinfectants:

Neutralization broth was prepared by using a mixture solution of different neutralizers that have the ability to deactivate the bacteriostatic effect of the active compounds of the tested disinfectants (table 2).

Table (2). The composition of the neutralizing broth

Neutralizer	Conc.	Company
Tween 80 (polysorbate 80)	3%	MpBiomedicals
Lecithin	0.3%	Fisher chemicals
Histidine	1%	Fisher chemicals
Sodium thiosulphate	0.5%	Fisher chemicals
Saponine	3%	Fisher chemicals

Disinfectant evaluation method

Sampling process

Shoes' swabs after contact with litter for 10 minute and contact with foot baths for one minute subjected to 10-fold serial dilution in sterile saline solution. before and after foot bath immersion for 1 min contact time with disinfectant, the immersed contaminated rubber shoes were swabbed followed by neutralization. Both the original and diluted samples were plated on Nutrient agar (Oxoid). Plates were incubated aerobically for 24 hours at 37°C. Bacterial colonies were enumerated on the plates that yielded 20 to 200 colonies forming units (CFU).

Experiment I. Disinfectant testing:

Bactericidal effectiveness of tested disinfectants foot bath was determined in liquid and semi-liquid form, by calculation of log reduction on the contaminated shoes surfaces after immersion in the foot bath for 1 min contact time.

Experiment II. Comparing effectiveness of Liquid versus semi-liquid foot bath:

Bactericidal effectiveness of liquid and semi-liquid foot baths were compared after immersion of the contaminated shoes for 1 min contact time.

Experiment III. Survival of bacterial contamination in footbaths (Bacterial load of the footbath):

TVCC in foot bathes fluid before and after immersion of contaminated shoes were estimated by one ml samples from the foot baths were received on test tubes containing neutralizing broth prior to the serial dilution were monitored and compared for 3 successive days for each tested disinfectant type foot bath, where the shoes and the foot bath were sampled before and after the contact time, and TVCC was determined.

Data analysis

Log reduction was calculated from the bacterial counts of shoes, before and after exposure to disinfectants. The following formula was used according to **The Australian Guidelines for Water Recycling [17]**.

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

Where,

A = Mean viable colony count before disinfection

B = Mean viable colony count after disinfection

The microbicidal effect (ME) was calculated so, by subtracting the log number of CFU ml⁻¹ after action of the disinfectant (B), from the log number of CFU ml⁻¹ of contaminated rubber shoes before disinfection (A), to pass the test, disinfectants must achieve a five log reduction in viable bacterial counts.

III. Results

In Experiment I and II, the greatest reduction of total aerobic bacterial populations of shoes was reported for Halamid® 1% liquid foot bath (4.17, 2.53, and 2.00 log reduction) for the three successive days of experiment duration, respectively. Other types of disinfectants varied in their log reduction value, with reduced effectiveness after each day (tables 3 and 4).

In experiment III., Both types of Halamid® 1% foot bathes; (liquid and semi-liquid) recorded the cleanest condition along the experiment duration, and the least affected by progressive organic load of deep litter contaminated shoes where, Halamid® 1% semi-liquid foot bath recorded totally clean foot bath media with no bacterial count, while Halamid® 1% liquid foot bath recorded only 1 log 10 bacterial count in the 2nd and 3rd days. Other disinfectants baths showed increased microbial load count each day after repeated contamination with deep litter. It was noticed that semi-liquid foot baths showed lower progress of microbial count in the second day of use of foot baths, and some degree in the third day than the liquid foot baths (tables 5 and 6).

Table (3). The mean viable colony count of shoes after 1 min contact time with the tested disinfectants in liquid foot baths; expressed as log 10

Days	First day			Second day			Third day		
	Before	After	Log reduction	Before	After	Log reduction	Before	After	Log reduction
CleanZix® 1%	6.65	5.86	0.79	6.30	4.00	2.30	8.20	7.00	1.20
Zix-Verox® 0.5%	7.39	6.00	1.39	6.65	4.14	2.51	8.38	6.89	1.49
Synerges® 0.4%	7.46	5.50	1.96	7.60	5.68	1.92	7.90	6.40	1.50
GroundZero® 0.4%	8.47	6.40	2.07	6.47	5.84	0.63	6.47	5.80	0.67
Halamid® 1%	8.17	4.00	4.17	6.00	3.47	2.53	6.69	4.69	2.00

Table (4). The mean viable colony count of shoes after 1 min contact time with the tested disinfectants in semi-liquid foot baths; expressed as log 10

Days	First			Second			Third		
	Before	After	Log reduction	Before	After	Log reduction	Before	After	Log reduction
CleanZix® 1%	6.69	4.00	2.69	6.10	3.89	2.21	7.00	6.00	1.00
Zix-Verox® 0.5%	6.30	5.00	1.30	6.00	3.70	2.30	7.65	6.47	1.18
Synerges® 0.4%	7.30	4.80	2.50	6.40	4.07	2.33	6.07	5.90	0.17
GroundZero® 0.4%	7.00	5.77	1.23	6.40	5.50	0.90	8.25	7.30	0.90
Halamid® 1%	6.69	5.69	1.00	6.50	5.77	0.73	6.07	4.88	1.19

Table (5). The mean viable colony count of liquid foot baths along the experiment duration for 3 successive days; expressed as log 10

Days of use	First day		Second day		Third day	
	Before use	After use	Before use	After use	Before use	After use
CleanZix® 1%	0	3.00	4.00	5.97	7.10	8.40
Zix-Verox® 0.5%	0	2.90	4.47	4.20	6.70	7.30
Synerges® 0.4%	0	1.90	4.60	5.60	6.96	7.50
GroundZero® 0.4%	0	1.30	2.65	4.47	4.47	4.47
Halamid® 1%	0	1.47	1.00	1.30	1.00	2.07

Table (6). The mean viable colony count of semi-liquid foot baths along the experiment duration for 3 successive days; expressed as log 10

Days of use	First day		Second day		Third day	
	Before use	After use	Before use	After use	Before use	After use
CleanZix® 1%	0	3.00	3.69	5.40	7.00	5.90
Zix-Verox® 0.5%	0	2.36	2.39	5.47	7.07	7.60
Synerges® 0.4%	0	1.90	1.90	6.30	5.30	7.77
GroundZero® 0.4%	0	1.30	3.40	3.39	3.69	3.60
Halamid® 1%	0	0	0	0	0	0

IV. Discussion

A footbath is a very simple procedure of biosecurity that benefits in preventing the potential mechanical transmission and spread of pathogens within the farm. Footbaths should be able to eliminate hazardous organisms that could survive for several days or weeks in the dirt trapped to the bottom of personnel foot wear [18]. In a field resembling conditions, the bactericidal effectiveness of 5 commercial disinfectants were evaluated and compared after 1 min contact time (usual personnel time in foot bath in poultry farms) in liquid and semi-liquid foot bath form, For assessing the bactericidal efficacy of the tested disinfectants as a foot bath, Experiments (1) and (2) were conducted; all the five disinfectants were tested by comparing mean bacterial

count on the contaminated shoes before and after immersion then, log reduction was calculated. table (3) showed that, Only Halamid® was near to achieve 5 log reduction of total bacterial count on contaminated examined shoes surface after immersion for 1 min in diluted 1% Halamid® solution; it achieved 4.17 log reduction in the 1st day but failed on later days due to the frequent use of the foot bath and the gross contamination of organic matter that impairs disinfectant action. Halamid® [19].

Results of this study demonstrated that all footbaths either liquid or semi-liquid, except Halamide 1% (chloramine) liquid bath, failed to achieve rapid satisfactory log reduction within one minute. The minimum required bacterial log reduction should be at least 5 log¹⁰ to assess surface disinfectants as effective [20]. Organic matter on shoes' soles acted as a physical barrier that protected bacteria from contact with the disinfectants [7, 21]. Recommendations were previously set to scrub and rinse foot wears with a detergent, before contact with a proper disinfectant, provided that contact time should not be less than 15 to 30 minutes [2]. But expectations to follow these recommendations in commercial poultry husbandry environments were practically hard, similar study model and results were reported by **Morley et al.**, [1]. On the other hand, table (4) showed that, in the semi-liquid foot bath, all tested disinfectants failed to achieve efficient bactericidal action on the contaminated foot surface after immersion for 1 min contact time including Halamid®. Although the short contact time and high organic load on shoe's sole, freshly prepared liquid footbath of Halamide 1% (chloramine) disinfectant exerted acceptable bactericidal efficacy. Similar results of efficacy of Halamide® disinfectant was reported by [19, 22]. Surface viscosity of footbaths adds the advantage of the stability of the foams obtained by striking the solution [23]. Viscous semi-liquid footbaths could not reduce bacterial counts efficiently under the effect of short immersion time (1 min.). However, in contrast to the liquid solutions, semi-liquid formulations remained stuck to footwear surface for longer periods, which may extend their bactericidal action beyond the tested contact time, as proved by **EhsanBashandy et al.** [22]. These results of Halamid® foot baths could be attributed to that it is a Chloramine product which known to produce a more stable residual than free chlorine, so providing long-lasting protection against regrowth. Moreover, chloramine was reported to have deeper penetration power within the biofilm than chlorine [24].

The results of the experiments demonstrated that boot baths, as they are currently used in many poultry farms production facilities, are not efficacious for disinfecting boots due to possible time constraints that make the proper use of boot baths within production units is difficult. Although going through the motions of stepping in a boot bath can help increase employee awareness of biosecurity and maintain a clean workplace, this study indicates that this is an insufficient biosecurity measure that potentially increase the risk for infection spread through contaminated boots by farm personnel.

In experiment (3), for assessing how many days could the foot bath be clean and effective; numbers of total viable aerobic bacteria recovered from foot bathes before and after immersion of shoes were monitored for 3 successive days. In liquid foot baths, surviving of bacteria in disinfectant solution highlights the opposite role of the liquid foot bath in preventing spread of disease in poultry farms. As revealed in table (5) there was a potential surviving of bacteria in the tested disinfectants foot baths, except for Halamid®, that gave nearly constant count around 1-2 log¹⁰ for the three successive days. Approximately, 1 log¹⁰ increase in the total bacterial count was noticed every day for the other disinfectants dilutions. On the other hand, in Semi-solid foot bath, as shown in table (6) only Halamid® could achieve 100% bacterial reduction after placing of the contaminated shoes for 1 min for 3 successive days.

Continued re-use of footbaths for successive days lead to build up of organic matter, beside survival and propagation of bacteria. Residual action of disinfectants decreases by time due to oxidant demanding reactions, permitting the growth of bacteria and, moreover, the recovery of injured ones [24]. Survival and propagation of bacteria in disinfectant solution highlights the possible risky role of the used of soiled footbaths in spreading of infection in poultry farms. However, bacterial propagation rate was, to some extent, slower in semi-liquid baths than in liquid solutions (except Zix-verox® and Synerges®), and that kept semi-liquid baths relatively clean. Semi-liquid Halamid® footbath showed the highest potency to remain clean with no bacterial propagation for 3 successive days of the experiment, withstanding the bacterial and organic daily load. Chloramine (Halamid®) was described as a potent broadspectrum disinfectant, with a more stable residual action than free chlorine, sustained long-lasting protection against bacterial regrowth, and exerted deeper penetration power within the biofilm than chlorine [6,19]. Applying footbath measures on a farm without using them properly is a waste of resources and even a risk factor. Footbaths should be maintained clean through changing and cleaning them periodically in a rate depending on the intense of foot traffic on the farm. On average, footbaths require weekly cleaning [24, 25]. Disinfectants should be used after cleaning and removal of organic matter (blood, fecal matter, litter, fat, and hatchery fluff); which affords a physical barrier that protects microorganisms from contact with the disinfectant [21]. Recently prepared disinfectant foot baths achieved better in the existence of organic matter than old disinfectants [7]. These results emphasize the need to use freshly prepared disinfectant with regular cleaning of the foot baths. Furthermore, the shoes should be strongly scrapped before foot bath immersion to reduce the organic load; otherwise, prolonged foot bath contact time should be applied.

Conclusively,. Footbaths should be used after cleaning and removal of organic matter from foot wears. Re-used soiled footbaths (especially liquid solutions) may act as a reservoir container for bacterial poultry pathogens. Potent long acting disinfectants should be properly selected for footbaths, kept clean as possible, and changed frequently. Adulteration should be kept in mind, as the labeled concentrations of the active ingredients may not be real [26].

In Conclusion, Surviving of bacteria in disinfectant solutions highlights the opposite role of the liquid foot bath in preventing spread of disease in poultry farms, on the other side, the semisolid foot baths need much time to act correctly as it was more condensed, but this did not lessen its powerful and efficient capacity to remain clean for 3 successive days than the liquid baths.

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