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Application of Foam Mixture in Disinfecting *Clostridium perfringens* Isolated Form Broiler Poultry Litter

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Abstract: The use of foams in disinfection is universally advantageous and means a great advance in disinfection technology, This study was undertaken to assess the efficacy of foam mixtures with a base of calcium hypochlorite 5% or glutraldehyde 1% and with addition of 1% urea against *C. perfringens* after 5, 15 and 30 minutes of contact using cement coupons resemble poultry house floor under dirty condition, the log reduction of *Clostridium perfringens* count after addition of 1% foaming agent (nonionic and an ionic surfactants) reached 1.48 after 30 minutes and upon addition of 1% urea the reduction reached 3.38 after 30 minutes while the log reduction of *Clostridium perfringens* count after addition of 1% foaming agent and 5% calcium hypochlorite reached 5.76 after 30 minutes and upon addition of 1% urea the reduction reached 5.94 after 30 minutes and log reduction of *Clostridium perfringens* count after addition of 1% foaming agent and 1% glutraldehyde reached 6.38 after 30 minutes and upon addition of 1% urea the reduction improved and reached 7.38 after 30 minutes. In conclusion the use of foam mixture in disinfection is great advantageous and provide excellent result in reduction of *Clostridium perfringens* count attached to cement coupons resemble poultry house floor.

Key words: Disinfection · Foam · *Clostridium perfringens* · Broiler

INTRODUCTION

Foam cleaning is widely used for open plant cleaning and refers to the cleaning process where the main detergent is applied as foam. The benefits include the ease and safety of application, extended contact time between detergent and soil and overall reduced labor and time for cleaning. Alkaline based foams are the most common used for cleaning in the poultry industry. They will range from approximately pH8 to pH12 (In use) and are effective on most types of soil encountered including poultry, red meat, fish and vegetable. Some alkaline foam are inhibited and may be safe to use on soft metal such as aluminum, tin, brass etc. Uninhibited alkaline foams may cause some corrosion to soft metals. Therefore when selecting the type of foam to be used, consideration should be given not only to the soil to be removed but also the materials of construction that the foam detergent will come into contact with.

Foam preparations have the following advantages: a high efficiency and dynamic impact, a sufficiently long contact time of the foam with a surface, minimal consumption of chemicals and water, readily removed, only a small amount of waste, complete coverage and the homogeneity of the layer is easily seen and there is minimal drenching of surfaces. In addition foams may be used in places with difficult access, to disinfect complex ragged surfaces and to disinfect vertical smooth surfaces, Foams provide another method of applying disinfectant under laboratory and field conditions [1].

Foams are formed from water solutions of {surfactants through dispersion with suitable disinfectant in appropriate technical equipment [2, 3].

Chlorinated foams are alkaline based with a chlorine "donor". They are usually very powerful detergents effective on removing fats, heavy protein deposits and fruit or vegetable staining. They are utilized in primary processing sectors such as red meat, poultry, fish,

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vegetables and fruit. The introduction of hypochlorite in the formulation is to assist with protein removal rather than acting as a biocide.

Commercial poultry production is one of the fastest growing sectors of the animal agricultural industries. Many poultry diseases cause substantial economic losses to the industry each year due to increased mortality or impaired growth. Among bacterial diseases affecting broiler production, necrotic enteritis (NE) is one of the most important diseases in poultry that destroys the intestinal lining of the digestive tract, outbreaks occurring in broilers from 2-5 weeks of age. Mortality is usually between 2-10% but can be as high as 40-50%, symptoms can also resemble coccidiosis and may be miss-diagnosed [4] Necrotic enteritis is the most common and financially devastating bacterial disease in broilers and the sub-clinical form is by far the most damaging for producers [5].

Avian necrotic enteritis is caused by specific strains of *C. perfringens* and costs the world poultry industry an estimated $2 billion annually largely due to the costs of antimicrobial prophylaxis and inefficient feed conversion [6,7].

The incidence of *Clostridium perfringens*-associated necrotic enteritis in poultry has increased in countries that stopped using antibiotic growth promoters. Necrotic enteritis and the subclinical form of *C. perfringens* infection in poultry are caused by *C. perfringens* type A, producing the alpha toxin [8].

Management and hygiene are key to maintaining poultry performance. Problems still exist of finding suitable disinfectants with more advantageous parameters as well as better ways of application.

Wirtanen and Salo [9] revealed that, the suspension tests do not give adequate information and reliable carrier tests, which mimic surface growth, are needed. Microbial adhesion to surfaces is a complex process. Studies have shown that bacteria readily adhere to and colonize the surface of any man-made material [2].

This study was undertaken to assess the efficacy of foam mixtures with a base of calcium hypochlorite 5% or glutraldehyde 1% and with addition of 1% urea against *C. perfringens* after 5, 15 and 30 minutes of contact using cement coupons resemble poultry house floor under dirty condition.

MATERIALS AND METHODS

Building Materials: Cement coupons were manufactured in Arab Contractors Company in Egypt to resemble poultry house floor with dimensions 2 x 2x 1cm^3. All coupons were sterilized in 15-cm glass petri dishes by autoclaving them for 45 min, using a dry cycle, prior to use [10].

Yeast Extract: 3% yeast extract powder solution was prepared by adding 3g yeast extract to 100ml bi- distilled water. (Meron- India- patch number MYEP/03/KJ12)

Sterile Hard Water: 0.304 (g) anhydrous calcium chloride and 0.065 g anhydrous magnesium chloride were dissolved in glass-distilled water and made up to one liter. The final concentration is 2.7 mM $CaCl_2$, 0.7 mM $MgCl_2$.Were dispensed into glass containers and was sterilized by autoclaving at 121 ± 1° C for 15 minutes

Chemical Disinfectants: All dilutions of disinfectants were prepared freshly at the day of work using sterile hard water.

- Fairy ^{R}… Non-ionic surfactants (5%) and anionic surfactants (5-15%) easily were used as foaming agents. It was used in a concentration of 1%.
- Calcium hypochlorite (80%chlorine)
  It was used in a concentration of 5%.
- Glutraldehyde (50%) concentration.
  It was used in a concentration of 1%.
- Urea crystal was used in 1% conc

Neutralizer: Neutralizer testing was done to ensure that the neutralizer would be effective and non-toxic according to ASTM E 1054-02 [11] literature were tested for neutralizer efficacy. All disinfectants were neutralized by a combination of 3% Tween 80 (polysorbate 80), 0.3% lecithin, 0.1% histidine, 0.5% sodiumthiosulphate, 3% saponin and 1% sodium laureth sulphate.

Tested Organism: Field strains of *Clostridium perfringens* were isolated from broiler farm litter samples which were taken and inoculated directly into cooked meat broth medium (Oxoid) and were incubated anaerobically in anaerobic Gas pack jar for 24 h at 37°C. A loopful of growth was then streaked onto 5% sheep blood agar supplemented with neomycin sulphate. The plates were incubated anaerobically for 24 h at 37 oC. The suspected isolates were identified by biochemical tests according to Effat *et al*. [12].

Methods: According to Wood *et al*. [13], Under working laminar air flow, cement coupons was inoculated

Using a micropipette, 10 droplets (Each droplet 10 ul) of the clostridium stock suspension and 1 ml of 3% yeast extract, lifted to dry for at least 2 hours at room temperature (20°C) For porous material. the droplets either wicked into the material or immediately lost surface tension. The number of colony forming unit per coupons was 2.4 x 10^8 /coupon log 8.38, the entire surface of the coupons (Carriers) upon which *Clostridium perfringens* had been inoculated and dried, was covered completely by the test formulation(Freshly prepared foam) foam mixtures with a base of calcium hypochlorite 5% or glutraldehyde 1%were tested. Non-ionic surfactants (5%) and anionic surfactants (5-15%) easily were used as foaming agents. foam is produced by vigorous agitation of the solution containing the active agent and a surfactant. For all tests, the relative humidity was maintained below 70% and the temperature was between 20 and 25C (i.e. ambient conditions). After the appropriate contact time was reached, each coupon was aseptically placed in a sterile 50-ml conical tube containing 10 ml of the extraction-neutralizing solution. Any liquid decontaminant remaining on the horizontally oriented coupons after the contact time was placed into the conical tube along with the coupon itself. Suitable controls were incorporated to check for sterility of media, reagents and carriers; effectiveness of the microbicide neutralization procedure; and carrier population counts.The coupons (Test coupons and any decontaminant runoff) were then subjected to15 min on an orbital shaker at 200 rev min. Following the shaking, 1 ml of the coupon extract liquid was withdrawn and tenfold serial dilutions were prepared. (The number of dilutions varied based on the expectednumber of CFU.) A 0.1 of ml aliquot of each dilution and of the undiluted extract were then spread and seeded on blood agar and incubated anaerobically at 35°C for 48 hours. The number of CFU per coupon was calculated based on the average of the triplicate plates and multiplying by the dilution factor of the plates on which the optimal counting range of 25-300 CFU was observed. The log reduction is calculated by subtracting the mean log density for treated carriers from the mean log density for control carriers.

RESULTS AND DISCUSSION

The use of foams in disinfection is universally advantageous and means a great advance in disinfection technology [14].

There are some advantages of using foam technique which is dissolves grease, soap film and scum on virtually any washable surface, non-abrasive, fast-acting foam won't scratch the surface, proven effective against over 75 pathogenic microorganisms.

General Properties of foam detergent is excellent cleaning and degreasing properties, superior foaming properties, can be used as a foam or a spray, may be used through pressure washers or spraying equipment, suitable for use on all hatchery, processing and farm building surfaces, non staining, non tainting.

C. perfringens is a member of normal intestinal flora that reproduces at high rates and produces toxins [6]. *C. perfringens* type A, which causes infection in chickens, has been reported to cause food poisoning in humans as well [15].

Clostridium perfringens enteritis has been reported in most areas of the world and adversely affects the integrated system of poultry production, resulting in gastrointestinal dysbacteriosis and necrotic enteritis [16,17] *C. perfringens* required a relatively long contact time to achieve significant kill and are therefore often applied as foam [18].

Table 1 showed that, log reduction of *Clostridium perfringens* count after addition of 1% foaming agent only reached 1.48 after 30 minutes and upon addition of 1% urea the reduction improved to 3.38 after 30 minutes

Schmidt 2003 found that, QACs are cationic surfactant sanitizers and also have some cleaning activity,

As the urea has to be degraded to ammonia before it functions as a disinfectant, As additional effects, urea increases the fertilizer value of the treated material and there is no risk of microbial regret [19].

Bojorn Vinneras [20] revealed that, *Clostridium spp* in its dormant state was resistant to urea treatment. As the urea has to be degraded to ammonia before it functions as a disinfectant.

Concerning Table 2, the log reduction of *Clostridium perfringens* count after addition of 1% foaming agent and 5% calcium hypochlorite reached 5.76 after 30 minutes and upon addition of 1% urea the reduction reached 5.94 after 30 minutes this result agreed with that obtained by Chlibeka *et al*. [1] who found that, Foam mixture had perfect antimicrobial activity, including a sporicidal effect. while Kyle Smith *et al*. [21] revealed that,the QAC-based and lower concentration of bleach-based products did not reveal sporicidal activity.

Table 1: Log10 reduction of *Clostridium perfringens* count(log 8.38) due to addition of 1%Foaming agent and with addition of 1% urea

	1%Foaming agent		1%Foaming agent + 1% urea	
	Log	Log reduction	Log	Log reduction
5minutes	7.27	1.11	5.59	2.79
15 minutes	7.2	1.18	5.3	3.08
30 minutes	6.9	1.48	5.0	3.38

Table 2: Log10 reduction *Clostridium perfringens* count (Log 8.38) due to addition of 1%Foaming agent + 5% calcium hypochlorite and with addition of 1% urea

Time	Log	1%Foaming agent + 5% calcium hypochlorite Log reduction	Log	1%Foaming agent + 5% calcium hypochlorite + 1% urea Log reduction
5minutes	5.0	3.38	3.0	5.38
15 minutes	3.34	5.04	2.95	5.43
30 minutes	2.62	5.76	2.44	5.94

Table 3. Log10 reduction *Clostridium perfringens* count(Log 8.38) due to addition of 1%Foaming agent+ 1%glutraldehyde and with addition of 1% urea

Time	Log	1%Foaming agent+ 1%glutraldehyde Log reduction	Log	1%Foaming agent+ 1%glutraldehyde+ 1% urea Log reduction
5minutes	4.3	4.08	3.6	4.78
15 minutes	2.47	5.91	2.2	6.81
30 minutes	2.0	6.38	1.00	7.38

Table 3, showed that, log reduction of *Clostridium perfringens* count after addition of 1% foaming agent and 1% glutraldehyde reached 6.38 after 30 minutes and upon addition of 1% urea the reduction improved and reached 7.38 after 30 minutes. This results nearly in agreement with that obtained with Borick *et al*. [22] who found that, 2% glutraldehyde was capable of killing spores of bacillus and *Clostridium species*, Dyas and Das [23]found that, aerobic species survived for 2 hours but *C.difficile* was killed in under 10 minutes using 2% glutraldehyde.

Vegetative bacteria are readily susceptible to the action of glutraldehyde, 2% glutraldehyde inactivate vegetative *Clostridium perfringens* with in 2-3 hours [24].

Spores of clostridium species can be killed by high level disinfectants such as 2% aqueous glutaraldehyde within 3 hours and 8% formaldehyde [25, 26]. Sreenivasan and Chorny [27] Found a relationship between microbial inactivation and the concentration of biocide in foam (Ranging from 0.1-0.5%) and exposure period were noted ($p<0.05$). Although, lower numbers of viable biofilm bacteria were recovered after treatment with the disinfectant foam than by the cognate aqueous biocide,

CONCLOSION

In conclusion the use of foam mixture in disinfection is great advantageous and provide excellent result in reduction of *Clostridium perfringens* count attached to cement coupons resemble poultry house floor.

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