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Research Article

Effect of Nano Disinfectant and Commercially Available Disinfectant Classes on SPF-Egg Experimentally Infected with *E. coli* and *Salmonella* Species

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ABSTRACT

This study was carried out in order to compare between the used commercially available disinfectants on fertile egg sanitation with new Nano technology based molecule in terms of safety and potency against bacterial causes of late embryonic death in hatcheries. In the present study, five days old 225 specific pathogen free embryonated chicken eggs (SPF-ECE) were divided into 9 equal groups. Egg surface infection was applied for groups 1, 3, 5 and 8 by *E. coli* and for groups 2, 4, 6 and 9 by *Salmonella enteritidis* (*S. enteritidis*). Groups 1 and 2 were fumigated by formaldehyde gas, while groups 3 and 4 were disinfected with Nano disinfectant preparation by dipping method. Groups 5 and 6 were disinfected using hydrogen peroxide (H_2O_2) 5% by spraying methods. Group 7 was considered as control negative group. While groups 8 and 9 were considered control positive experimentally infected with *E. coli* and *S. enteritidis*, respectively. Embryonic mortalities in addition to total bacterial counts from egg shell swaps were reported post disinfection. After hatching, weights of one day old chicks as well as liver and spleen to body weight ratios of each group were calculated. Among all the tested disinfectant groups, the highest embryonic mortalities were appeared in groups 1 and 2, followed by groups 3 and 4, and finally groups 5 and 6. Results of total bacterial counts from egg shell swaps revealed that lowest bacterial count was detected in groups 3 and 4, followed by groups 5 and 6, then groups 1 and 2. Results of effect of tested disinfectants on the hatched chicks weights at one day old revealed that the highest body weight hatched chicks was appeared in groups 5 and 6, followed by groups 3 and 4, then finally groups 1 and 2. The lowest liver to body weight ratio was detected in groups 5 and 6, while the lowest spleen to body weight ratio was found in groups 1 and 2. It could be concluded that disinfection of incubated egg is of great value in order to control egg shell surface contamination for production of good quality one day old chicks. H_2O_2 is still effective and safe disinfectant till now. Nano technique is considered a useful tool when compared with traditional prepared disinfectant in term of rapid control of bacterial contaminant, but more investigations are recommended specially in condition of safety on animal, birds and human beings.

Key words: Specific pathogen free embryonated chicken eggs, *E. coli*, *Salmonella enteritidis*, Egg shell swaps

INTRODUCTION

Many bacterial agents including *E. coli* and *Salmonella* spp. found to be the main cause of what is called dead-in-shell embryos and one day culled chicks (Rezk, 2010; Abo El Yazeed *et al.*, 2015; Amer *et al.*, 2017), this give rise for persistence need for better control of this pathogens by strict hygienic measures including disinfection of hatcheries together with production of clean fertile egg free from bacterial contamination.

Disinfection of fertile egg become new concept in modern poultry production not only for production of high quality new hatched one day old chicks but also for decrease embryo mortality in hatchery due to reducing egg shell microbial load. Disinfection of fertile egg carried out either by fumigation, spraying or dipping in order to control contamination by pathogenic microorganisms. Formaldehyde gas is considered as an excellent antimicrobial agent not affected by organic matter and is widely used for egg and hatchery disinfection (Fabrizio *et al.*, 2002). Fumigation by formaldehyde gas is influenced by many factors as the concentration of formaldehyde, duration of exposure, relative humidity, temperature in addition to organic matter that contaminate egg shell surface (Cadirci, 2009). Unfortunately, control of these parameters together is difficult as well as toxicity of formaldehyde gas has been proved not only for birds but also to human beings

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(Hayretdağ and Kolankaya, 2008). As hydrogen peroxide (H_2O_2) disinfectant is safe for both human and birds, so it is used as a surface decontaminant. H_2O_2 disinfectant is rapidly evaporated with no residual effect, that's why it is used in breeders hatching eggs with no fear of reducing hatchability unlike fumigation with formaldehyde gas (Hassan *et al.*, 2011). Spraying of fertile egg was also used in different farms and studied by many researchers (Shahein and Sedeek, 2014). Unfortunately, some of the drawbacks for hand spraying include low pressure, incomplete shell surface coverage, and no temperature control for the disinfectant. All disinfectants work better when the solution temperature is high (>110ºF or >40ºC). Moreover, those eggs with adhering organic matter are not properly sanitized with hand spraying (Cooper, 2001). Nowadays, egg dipping is widely used to control egg contamination and produce good quality hatched chicks with low embryo mortality in hatcheries. Egg dipping should be carried out very carefully with many precautions include temperature of used disinfectant to avoid risk of temperature shock, time of dipping as it should be few minutes (one to three times), do not use very dirty eggs in addition to changing the disinfectant solution every time to avoid being source of contamination (Zeweil *et al.*, 2015; Baylan *et al.*, 2018). Essential oil based disinfectant found to control pathogenic bacteria especially those showed multi-drug resistance (Lee *et al.*, 2017; Baptista *et al.*, 2018). Citronella oil is an essential oil prepared as Nano emulsion used in disinfectant preparation found to possess an antibacterial effect together with mosquito repellents activities with less harmful effect than other preparation (Agrawal *et al.*, 2017). Also, thyme oil emulsion found to prevent fungal growth at low concentration (Hassanin *et al.*, 2017) together with possesses antimicrobial activity against many pathogens (Ryu *et al.*, 2018). Nano particle containing preparation found to have more powerful and rapid effect than other preparations, that's why Nano molecules are used now under research in different preparation in medicinal products. Unfortunately, it was found that Nano based materials showing potential adverse effect not only on human health but also among all environment (Servin and White, 2016; Eleftheriadou *et al.*, 2017; Sadeghi *et al.*, 2017), this may be due to physicochemical properties, behavior and interactions inside living body when compared with conventional products (Gallocchio *et al.*, 2015). From the above mentioned data, our study was designated in order to compare commercial available disinfectant against new produced disinfectant with Nano technology techniques in term of control experimentally contaminated specific pathogen free embryonated chicken egg (SPF-ECE) surface.

MATERIALS AND METHODS

Specific pathogen free embryonated chicken egg (SPF-ECE): A total of 225 SPF-ECEs, obtained from governmental SPF farm –Kom Oshem –EL Fayoum.

Bacterial strains

- *E. coli* strain (O78 K80 H11) was used for surface infection of SPF-ECEs. It was supplied from serum and vaccine research institute. It was centrifuged at 3000 rpm for 10 min. Sediment was diluted with sterile buffer saline and adjusted to 10^8 colony forming unit (CFU) *E. coli*/ ml according to El-Boushy *et al.* (2006).
- *S. enteritidis* field strain was previously isolated and characterized by Elbayoumi *et al.* (2016), centrifuged at 3000 rpm for 10 min. Sediment was diluted with sterile buffer saline and adjusted to 10^9 CFU/ml by using McFarland matching tube. The challenge inoculum was prepared according to the method of Timms *et al.* (1990).

Disinfectants used

Nano kill

- Composition: Glutraldehyde – Alkyl dimethyl benzyl ammonium chloride –Octyldecyl dimethyl ammonium chloride – Didecyl dimethyl ammonium chloride – Dioctyl dimethyl ammonium chloride- Citronella oil nano emulsion – Thyme oil nano emulsion.
- Batch No. 6451.
- Expired date 18/3/2021.
- Manufacture: Taba chemical industry.

Formaldehyde gas

- Formaldehyde gas was generated by using 60 ml formalin in 30 ml water and 48 gm potassium permanganate at 37ºC temperature and 80% air humidity according USDA (1985).
- Formalin and potassium permanganate were obtained from El-Gomhoreia Company for lab. Chemicals, Egypt.

Hydrogen peroxide (H_2O_2) 5%

Used for disinfection of embryonated egg groups by egg spraying.

Experimental design

In the present study, five days old 225 SPF-ECEs were divided into 9 equal groups (25 per each group). Egg surface contamination by *E. coli* was applied for groups 1, 3, 5 and 8. While egg surface contamination by *S. enteritidis* was applied for groups 2, 4, 6 and 9. After incubation for two hours, disinfection process was applied according to the used disinfectant. Groups 1 and 2 were fumigated by formaldehyde gas for 20 minutes inside setter (at 37ºC temperature and 80% air humidity), while groups 3 and 4 were disinfected with Nano disinfectant preparation by dipping method according manufacturer recommendation. Groups 5 and 6 were disinfected using H_2O_2 5% by spraying methods on surface of experimentally infected eggs. Group 7 was considered as control negative group (not treated). While groups 8 and 9 were considered control positive experimentally infected with *E. coli* and *S. enteritidis*, respectively as showed in Table 1. SPF-ECEs were incubated and candled for embryo mortality at 10th, 13th, 15th and 18th days of incubation. Total bacterial counts were taken place on plate count agar medium (PCA) by swaps randomly collected from five eggs per each group at 24 and 48 hours post disinfection according to Fardiaz (1993). After hatching, one day old chicks' weights in addition to liver and spleen to body weight ratios were calculated for each group.

Table 1: Treatment protocol for each group.

Group	Disinfectant used	Method of disinfectant application	Experimental surface infection
1	Formaldehyde gas	fumigation	*E. coli*
2	Formaldehyde gas	fumigation	*S. enteritidis*
3	Nano kill	Egg dipping	*E. coli*
4	Nano kill	Egg dipping	*S. enteritidis*
5	H_2O_2 5%	spray	*E. coli*
6	H_2O_2 5%	spray	*S. enteritidis*
7	-ve		-ve
8	-ve		*E. coli*
9	-ve		*S. enteritidis*

RESULTS

Results of embryonic mortalities in Table 2 showed that no mortalities till hatching in control negative group 7. The highest embryonic mortalities were observed in control positive bacterial contaminated groups 8 and 9. These results revealed that all disinfectant classes are effective to control *E. coli* and *S. enteritidis* experimentally contaminated egg surface.

Table 2: Effects of disinfectants on embryonic mortalities.

Group	Embryonated chicken egg age before hatching				Total mortality	Mortality %
	10^{th} day	13^{th} day	15^{th} day	18^{th} day		
1	0	0	3	2	5	20
2	0	0	2	3	5	20
3	0	0	1	2	3	12
4	0	0	2	1	3	12
5	0	0	1	1	2	8
6	0	0	1	1	2	8
7	0	0	0	0	0	0
8	6	4	2	2	14	56
9	6	5	2	2	15	60

Among all tested disinfectants, groups 1 and 2 disinfected by fumigation with formaldehyde gas showed the highest embryo mortalities, followed by groups 3 and 4 that received Nano produced disinfectant by dipping, while groups 5 and 6 received H_2O_2 5% showed the lowest embryo mortalities.

Results of total bacterial counts from egg shell swaps per each group at 24 and 48 hours post treatment (Table 3) revealed that lowest bacterial count after 48 hours in groups 3 and 4 treated with Nano based disinfectant, followed by groups 5 and 6 treated with H_2O_2 5%, then groups 1 and 2 fumigated with formaldehyde gas, while the highest bacterial count was appeared in group 9 experimentally surface contaminated with *S. enteritidis*, followed by group 8 experimentally surface contaminated with *E. coli*, while group 7 showed no bacterial growth.

Results of effect of applied disinfectants on the hatched chicks weights and liver and spleen relative weights on day one after hatching are shown in Table 4. Control negative group 7 showed the highest body weight of hatched chicks, followed by groups 5 and 6 disinfected with H_2O_2, then groups 3 and 4 treated by Nano based disinfectant, followed by groups 1 and 2 treated by formaldehyde gas fumigation. Finally, positively infected groups with *S. enteritidis* (group 9) and *E. coli* (group 8) showed the lowest body weight of new hatched chicks.

Table 3: Total bacterial count from egg shell swaps 24 and 48 hours post treatment.

Group	Total bacterial count 24 hours post disinfection (CFU per egg) (Log±SD)	Total bacterial count 48 hours post disinfection (CFU per egg) (Log±SD)
1	3.57±0.18	2.72±0.13
2	3.82±0.31	2.81±0.12
3	1.56±0.02	0.71±0.02
4	1.44±0.02	0.51±0.02
5	3.31±0.20	2.1±0.01
6	3.29±0.23	1.99±0.23
7	No bacterial growth	No bacterial growth
8	4.21±0.40	6.9±1.02
9	4.33±0.44	7.03±1.31

Table 4: Effects disinfectants on the hatched chicks' weights, liver and spleen relative weights at day one after hatching (mean±SD).

Group	One day old chick body weight	Liver to body weight ratio	Spleen to body weight ratio
1	39.11±0.29	2.80±0.07	0.125±0.002
2	39.72±0.29	2.81±0.07	0.124±0.002
3	40.22±0.30	2.43±0.07	0.134±0.002
4	40.41±0.30	2.42±0.07	0.133±0.002
5	41.54±0.30	2.41±0.07	0.135±0.002
6	41.95±0.30	2.41±0.07	0.136±0.002
7	42.01±0.30	2.40±0.07	0.136±0.002
8	39.02±0.29	2.88±0.07	0.138±0.002
9	38.87±0.29	2.89±0.07	0.139±0.002

Regarding to liver to body weight ratio results, the lowest ratio was observed in control negative group 7, followed by groups 5 and 6 treated with H_2O_2, then groups 3 and 4 treated with Nano based disinfectant, followed by groups 1 and 2 treated by formaldehyde gas fumigation, while the highest liver to body weight ratio were noticed in experimentally infected groups with *S. enteritidis* (group 9) and *E. coli* (group 8). Concerning to spleen to body weight ratio results, groups 1 and 2 which treated by fumigation with formaldehyde gas showed the lowest ratio, followed by groups 3 and 4 treated with Nano based disinfectant, followed by groups 5 and 6 treated by H_2O_2. Control negative group 7 showed the same results as group 6 treated with H_2O_2 and finally the highest spleen to body weight ratio was appeared in experimentally infected groups with *S. enteritidis* (group 9) and *E. coli* (group 8).

DISCUSSION

Sanitization of hatching egg has a great positive impact on hatchability and production of good quality one day old chick (Bialka *et al.*, 2004) and this achieved by good hatchery sanitation together with good quality fertile egg free from both surface contamination and vertical transmitted diseases (Rehkopf *et al.*, 2017; Melo *et al.*, 2019).

In our study, we compared between the effects of three different commercial disinfectants applied by different methods for sanitation of experimentally infected SPF-ECEs. The highest embryo mortalities were noticed in groups disinfected by formaldehyde gas fumigation. This may be related to fumigation process itself, as it may

cause cuticle damage and also has negative impact on embryo especially when it takes place during first 9 days of embryonic life (Cadirci, 2009). In contrary, Zeweil *et al.* (2015) reported that fumigation with formaldehyde gas showing lower embryonic mortality but unfortunately it possesses teratogenicity and toxicity in newly hatched chicks. On the other hand, our groups treated with H_2O_2 5% showed lowest embryo mortality similar to that obtained by Fichet *et al.* (2007) who reported that H_2O_2 used for hatching egg sanitation not only effective for decrease bacterial contamination but also is safe on embryonated chicken egg. Nano based disinfectant did not cause high embryonic mortalities as formaldehyde gas.

Many researchers reported that disinfectant contains glutraldehyde and quaternary ammonium compound has little impact on embryo mortalities the same as that found by H_2O_2 (Zeweil *et al.*, 2015). Moreover, thyme oil and citronella oil were safe and not causing mortalities (Dahama *et al.*, 2015). Batkowska *et al.*, (2017) reported that the use of Nano based disinfectant resulting in lower loss of moisture content of fertile egg and better hatchability with low embryo mortality. These results did not match with our results, this may be explained by the usage of egg dipping itself as a method of application of Nano based disinfectant in our trial as this method was proved to cause cuticle loss (Shafey, 2002; Ghonim *et al.*, 2008; Khairy *et al.*, 2011) which increase possibilities of embryo mortalities (Peebles *et al.*, 1987; 1998).

Regarding to total bacterial count, our results of all disinfectant groups showed significant reduction of bacterial growth on experimentally infected egg surface when compared with control groups. These results were agreed with that recorded by Zeweil *et al.* (2015) and Badran *et al.* (2018). On the other hand, bacterial growth was higher in fumigated groups with formaldehyde gas than all disinfectant groups under investigation. Similar results were obtained by Fabrizio *et al*., (2002) who found that formaldehyde gas eliminate partially some bacteria from all population of egg shell contaminated surface. It was reported that H_2O_2 with different concentrations together with disinfectant based glutraldehyde and quaternary ammonium compounds were efficient in decreasing surface contamination of egg shell (Wells *et al.*, 2010; 2011). Lowest bacterial growth was reported in our groups treated with Nano based disinfectant may be due to synergistic effect of disinfectant components as each component works with different mode of action for inhibiting bacterial growth (Brantner *et al.*, 2014; Battersby *et al.*, 2017; Castro Burbarelli *et al.*, 2017; Jiang *et al.*, 2018). Similar results were reported by Batkowska *et al.* (2017) who found that the usage of Nano based disinfectant causing reduction of number of bacterial colonies of all bacterial species when compared with formalin. Also, Ibrahim *et al.* (2018) reported that disinfection of quail fertile egg with Nano based disinfectant in different concentration leading to decrease bacterial load of egg shell without any adverse effect on hatchability.

Our results related to weights of one day old chicks showed that the lowest body weights were reported in formaldehyde treated groups than other treated groups. Similar results were detected by different researchers as Khan *et al.* (2005); Nasr El-Deen (2009); Johnson (2018) who reported decreased weights of newly hatched chicks from fumigated embryonated chicken eggs when compared with control negative group.

Among all our disinfected groups, the highest liver to body weight ratio was observed in groups treated by formaldehyde fumigation. This may be due to negative effect of formaldehyde gas on hepatic function. Similar results were obtained by Khan *et al.* (2006) who reported that formaldehyde gas may cause hepatotoxicity resulting in hepatomegaly. Pandy *et al.* (2000) reported hepato – renal toxicity in rat exposed to formaldehyde gas. Moreover, many researchers reported that formaldehyde gas has suppressive effect on plasma protein produced in liver which indicate negative impact of fumigation on liver function (Babar *et al.*, 2001). Unlike, H_2O_2 or glutraldehyde quaternary ammonium compound based disinfectants have no negative impact against liver to body weight ratio (Khan *et al.*, 2003; Badran *et al.*, 2018). Also, disinfectant produced by Nano technique has no effect on hepatic structure and function of newly hatched chick's (Ibrahim *et al.*, 2018), which is matched with our results.

Concerning to results of spleen to body weight ratio, the lowest ratio was in fumigated groups with formaldehyde gas. This result was parallel to that obtained by Nasr El-Deen (2009) who concluded that pre-incubation formaldehyde gas fumigation of embryonated chicken egg resulting in negative impact not only on spleen and hemogram but also on performance, liver and kidney of newly hatched chicks, the same results were reported by Badran *et al.* (2018). No harmful effect was noticed on spleen in our groups treated with Nano based disinfectant when compared with control group, this was matched with results of Joshua *et al.* (2016) who reported that in ovo feeding Nano particles through amniotic sac does not harm not only internal organ but also one day old chick body weight.

Conclusions

It could be concluded that disinfection of incubated egg is of great value in order to control contamination of egg shell surface for production of good quality one day old chicks, this is achieved by different methods either fumigation, egg dipping or egg spraying, the latest method is not recommended due to many drawbacks. H_2O_2 5% still acts as an effective and safe disinfectant till now. As Nano technique is considered a useful tool when compared with traditional prepared disinfectant in term of rapid control of bacterial contaminant, but more investigations are recommended specially in condition of safety on animal, birds and human beings.

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