

COMPARATIVE EVALUATION OF THE EFFICACY OF VARIOUS SANITIZERS IN A POULTRY HATCHERY

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Summary

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Disinfection procedures in hatcheries are an important part of the general set of anti-epidemic measures in poultry breeding and therefore justify the necessity of studies to evaluate the achieved disinfection effect. This study presents the results of a controlled trial with disinfectants from three chemical groups – the commercial preparations “Dezinfect-B,” “Sanifort,” and sodium hydroxide – on the efficacy of disinfection in a poultry hatchery. Control was performed through microbiological tests on samples from surfaces, obtained before and after their disinfection. The effect of the performed disinfection procedures was evaluated through the achieved microbial count reduction, residual microflora, and detection of the presence of indicator bacterial species. A higher efficacy was established for treatment with 2% solution of sodium hydroxide and 0.025% solution of Sanifort (99% sodium dichloroisocyanurate dihydrate) compared to disinfection with 3% solution of Dezinfect-B, containing 1.6% iodine with exposure times of 60 min.

Key words: Dezinfect-B, disinfection, hatchery, Sanifort, sodium hydroxide

INTRODUCTION

One of the primary factors contributing to achieving best results in poultry practice is the good health condition of the birds. Risk analysis data designate hatcheries to be a major risk factor in proper health safeguarding in the poultry industry, due to the possibility of incubating eggs contaminated with pathogenic microorganisms (Ayubi & Karadzhov, 1994).

During incubation, there are conditions for occurrence and maintenance of microbism in the hatcheries and the development of the so-called incubator infections. When cleaning and disinfection procedures are not performed properly, conditions for infection agent transfers among the different batches of newly hatched

chickens are present. This is related to significant epidemiological and economical risks.

All these facts emphasize the importance of disinfection of hatcheries as a part of general set of antiepidemic measures in agriculture.

The disinfection process is complex and multifaceted, as well as influenced by a number of factors and conditions. Some of them are related to the properties of the used disinfection agent, others to the type and resistance of microorganisms or the environmental conditions in the area where the disinfection takes place (Russell & Russell, 1995; Karadzhov *et al.*, 2004). Of particularly high significance is the

presence of organic and non-organic contaminants, the presence of biofilm, as well as the characteristics of surfaces – hydrophobicity, porosity, pH etc. The environmental influence on the disinfection process is various and sometimes decisive for disinfection efficacy (McDonnel & Russell, 1999; Slavchev *et al.*, 2005; Angelov *et al.*, 2006; Baychev & Karadzhov, 2006).

The aim of the current study was to determine and compare the effect of disinfection in a hatchery through the usage of disinfectants of different chemical groups, under conditions typical for such an environment.

MATERIALS AND METHODS

A controlled study was performed in one Bulgarian poultry hatchery. Disinfections were performed by spraying the surfaces with a coarse aerosol at 0.5 L/m², using a mechanical sprinkler device. The total area for disinfection was 172 m². Room temperature during the time of disinfection was 21 °C, and air humidity – 78%. The exposure to the disinfection agent was 60 min. The following sanitizers were used:

- Sanifort – chlorine-releasing disinfectant from the group of chloramines, containing 99% sodium dichloroisocyanurate dehydrate, with minimum 56% active chlorine (Zhivas Ltd., Sofia, Bulgaria). For the study, a 0.025 % water solution was used;
- Dezinfekt-B – iodine-releasing disinfectant from the group of iodophors, containing 1.6% active iodine and 7% surfactants (Iod Inc., Varna, Bulgaria). For the study, 3 % water solution was used;
- Sodium hydroxide – alkaline disinfectant, containing 98% sodium hydro-

xide (Vionas Ltd., Pazardzhik, Bulgaria). For the study, 2% water solution was used.

The microbiological control on the effect of performed disinfections was done through the following methods: achieved microbial count reduction, establishment of residual microflora, and detection of indicator bacterial species presence (Iliev *et al.*, 1982; Urban *et al.*, 2003; Slavchev *et al.*, 2005; Angelov *et al.*, 2006).

The samples for determination of the extent of microbial contamination of surfaces, prior to disinfection were obtained after mechanical cleaning of the room and inventory. The samples for establishing the extent of microbial contamination of surfaces after disinfection were collected after the end of the 60 min exposure period.

Three samples from each of the control surfaces of the room and inventory were obtained and tested microbiologically:

- walls – hatchery (including crevices);
- floor – hatchery (including corners);
- hatcher – wall;
- hatcher – floor;
- hatcher baskets.

The samples from tested surfaces were obtained by the microbiological swab method (by rubbing a sterile cotton swab, soaked with sterile physiological solution, against the surfaces) of a 20 cm² surface (4×5 cm in size, measured with a sterile metal model).

After disinfection, samples were obtained with sterile cotton swabs, previously soaked in disinfectant-specific neutralizing solution (stop solution) – 0.5 % sodium thiosulphate for the chlorine- and iodine-releasing sanitizers (Sanifort and Dezinfekt-B) and 0.5 % solution of sodium hydrogen carbonate for the sodium hydroxide. Then, swab samples were placed

in tubes with 2 mL sterile 0.9% saline, kept in a refrigerating bag and tested microbiologically within 4 h from collection.

At the laboratory, tubes were filled up to 10 mL with sterile physiological saline (basic dilution). After numerous washings, the swab was removed from the tube, and the washing liquid was used to prepare decimal dilutions – 1:10, 1:100, and 1:1000.

Inoculation on enriched nutrient media (blood agar, containing 5% defibrinated sheep blood) were performed from the primary and decimal dilutions for quantitation of the total number of mesophilic aerobes and facultative anaerobes.

Inoculations on selective nutrient media were done with the primary dilution (MacConkey agar, Sabouraud agar with chloramphenicol, and meat peptone agar containing 10% sodium chloride) using the routine methods, to establish the presence of the following groups of microorganisms in samples: coliforms, staphylococci, and moulds.

Counting of the colonies for determi-

nation of the total number of mesophilic aerobes and facultative anaerobes, was performed in Petri dishes (with Ø=9 cm), with no less than 10 and no more than 300 colonies. Merged colonies were counted as one. Depending on the number of counted colonies and the respective degree of dilution, the counts of living microorganisms on 1 cm² of control surfaces were calculated.

For every control surface, 3 swab samples were examined, and the average number of microorganisms on 1 cm² of the respective control surface was calculated.

Statistical analysis of data was performed by using the statistical software StatMost for Windows. The statistical significance of the results was determined by one-way ANOVA.

RESULTS

The data from the microbiological studies of the control surface samples are presented in Tables 1 and 2.

Table 1. Total number of mesophilic aerobes and facultative anaerobic microorganisms on control surfaces (CFU/cm²) in a hatchery (the results represent the mean values)

Control surface	3 % Dezinfekt-B			2 % sodium hydroxide			0.025 % Sanifort		
	A	B	C	A	B	C	A	B	C
Wall – hatchery	3.10 × 10 ²	0	100.00%	5.00 × 10 ²	2.00 × 10 ¹	96.00 %	2.04 × 10 ²	3.25 × 10 ¹	98.41%
Floor – hatchery	4.63 × 10 ⁴	1.25 × 10 ³	97.31%	7.40 × 10 ³	1.30 × 10 ²	98.24 %	1.65 × 10 ⁵	2.82 × 10 ³	98.30%
Hatcher baskets	4.03 × 10 ³	3.96 × 10 ²	90.20%	5.39 × 10 ⁴	2.86 × 10 ²	99.47 %	1.80 × 10 ⁵	1.27 × 10 ⁴	92.94%
Hatcher–wall	8.40 × 10 ³	1.16 × 10 ³	86.20%	6.00 × 10 ³	1.50 × 10 ²	97.50 %	2.48 × 10 ⁵	1.15 × 10 ²	99.95%
Hatcher floor	4.80 × 10 ⁶	5.76 × 10 ⁵	88.00%	2.53 × 10 ⁶	1.44 × 10 ⁴	99.43 %	1.68 × 10 ⁶	6.32 × 10 ³	99.62%
Mean CFU/cm ²	9.70 × 10 ⁵	1.15 × 10 ⁵	88.10%	5.19 × 10 ⁵	2.99 × 10 ³	99.42 %	4.54 × 10 ⁵	4.39 × 10 ³	99.03%

A – prior to disinfection; B – after disinfection; C – reduction of microbial contamination in %.

Table 2. Number of samples positive for the respective group of microorganisms, prior to and after disinfection with each of the three tested disinfectants (3 samples from each control surface).

	Wall of hatchery		Floor of hatchery		Hatcher baskets		Wall of hatcher		Floor of hatcher		Total	
	A	B	A	B	A	B	A	B	A	B	A	B
3 % Dezinfekt-B												
Staphylococci	2	0	3	1	2	1	2	1	3	2	12	5
Coliforms	3	0	3	0	3	0	3	0	3	1	15	1
Moulds	3	0	3	1	2	1	2	2	3	1	13	5
2 % sodium hydroxide												
Staphylococci	3	0	3	1	2	0	2	0	3	1	13	2
Coliforms	3	0	3	0	3	0	3	0	3	0	15	0
Moulds	3	0	3	1	2	0	3	0	3	2	14	3
0.025 % Sanifort												
Staphylococci	3	1	3	2	2	1	2	0	3	1	13	5
Coliforms	3	0	3	0	3	0	2	0	3	0	14	0
Moulds	3	0	3	1	1	1	2	1	3	1	12	4

A – prior to disinfection; B – after disinfection.

The quantitative microbiological studies established that the different surfaces in the hatchery were contaminated to a different extent (Table 1). In all three studies, the highest microbial contamination was detected on the floor of the hatcher – an average of 3.0×10^6 CFU/cm², followed by the hatcher baskets (7.9×10^4) and the floor (7.3×10^4), while the lowest contamination was observed on the wall of the hatchery (average 3.35×10^2).

After disinfection with the three disinfectants, a significant drop in microbial contamination rates was observed. As seen from the data in Table 1, the reduction in microbial counts on the various surfaces, after application of the three disinfectants, varied within 88–100%, while the residual microflora on the surfaces after disinfection was below 15000 CFU/cm². An exception was the hatcher floor, where the residual microflora after treatment with Dezinfekt-B was 5.7×10^5 CFU/cm².

The analysis of the results from disinfection with 3% Dezinfekt-B showed on

the average 88.1% reduction in microbial contamination rate for all control surfaces. Contamination reduction was the highest on the wall (100%) and the floor (97.31%) of the hatchery, and the lowest on hatcher surfaces – wall (86.2%), floor (88%), hatcher baskets (90.2%). After disinfection with 0.025 % solution of Sanifort, an average reduction in microbial contamination of 99.03% was established for all control surfaces. The lowest reduction was observed on hatcher baskets – 92.94%. After treatment with 2% sodium hydroxide solution, the results showed the highest mean value of contamination reduction – 99.42%, for all control surfaces.

Before treatment with Dezinfekt-B, all samples were positive for coliforms, and after the procedure – there was only 1 positive out of 15 samples (Table 2). Before treatments with Sanifort and sodium hydroxide, there were 15 and 14 positive samples respectively, and after the procedure, no coliform-positive samples were

detected. There was a statistically significant difference in efficacy ($P < 0.01$) between Dezinfekt-B and either sodium hydroxide and Sanifort. No significant difference between the efficacy of sodium hydroxide and Sanifort was established.

DISCUSSION

It is well known that disinfection includes a set of methods and measures of neutralizing (dilution, removal, or extermination) pathogenic or non-pathogenic microorganisms on living and non-living matter (Arsov *et al.*, 1988).

Disinfection can be considered optimal when it achieves complete elimination of unwanted microorganisms. This goal is impossible to reach with the existing contemporary methods and means.

In Bulgaria, there are standards for evaluation of the efficacy of disinfection procedures, according to which disinfection of animal breeding facilities can be considered good enough if more than 80% reduction in the final amount of bacteria can be achieved (achieved microbial count reduction method); the acceptable residual microflora after disinfection is up to 15,000 microorganisms/cm² (residual microflora method), and the number of coliform-positive samples should be no more than 10% of all samples collected (detection of the presence of indicator bacterial species method) (Karadzhov *et al.*, 2004).

The quantitative microbiological studies established that the different surfaces in the hatchery were contaminated to different extents (Table 1). In all three studies, the highest microbial contamination was detected on the floor of the hatchery, followed by the hatchery baskets and the floor. The high extent of microbial contamination on the surfaces in hatcheries

indicates that there were mistakes during the process of sorting and removing the non-fertilized eggs, which tend to “explode” during incubation, thus contaminating the surfaces.

After performing disinfection with the three sanitizers, a significant drop in microbial contamination rate was observed with reduction in microbial counts within 88%–100%. The percentage of coliform-positive samples obtained after disinfection was 6.67% after treatment with “Dezinfekt-B,” while for sodium hydroxide and Sanifort it was zero. The results showed that the effect of the performed disinfection procedures was very good.

The analysis of the results from disinfection with 3% Dezinfekt-B showed on the average 88.1% reduction in microbial contamination rate, as a mean value for all control surfaces. Contamination reduction was the highest on the wall (100%) and the floor (97.31%) of the hatchery, and the lowest on hatchery surfaces – wall (86.2%), floor (88%), hatchery baskets (90.2%). The lower efficacy of disinfection in the hatchery could be explained with the high microbial contamination due to reasons described above, and the lack of thorough cleaning before disinfection. The contamination stems from the presence of organic residues on the surfaces, which can quickly “deplete” the applied solution and reduce its disinfection capacity.

After disinfection with 0.025 % solution of Sanifort, the lowest reduction was observed on hatchery baskets – 92.94%. The possible cause for this is their design, which incorporates many hard to clean corners that impede the disinfectant’s action.

After sanitization with 2% sodium hydroxide solution, the results showed the highest mean value of contamination reduction – 99.42%, for all control surfa-

ces. The high efficacy of disinfection of the hatcher surfaces – the floor (99.43 %), the wall (97.5 %), and the hatcher baskets (99.47 %) is probably due to the hydrolytic effect of sodium hydroxide and its ability to dissolve organic residues, which allows a good in-depth penetration (Arsov *et al.*, 1988).

The data from presented study shows that reduction in microbial population after disinfection affected to the highest extent coliforms, accepted as the primary hygiene indicator for preventive disinfections (Karadzhev *et al.*, 2004). These results are a good reason to ascertain the higher efficacy of disinfection procedures with 2% sodium hydroxide and 0.025 % Sanifort, compared to disinfection with 3 % Dezinfekt-B with exposure time of 60 min.

Due to the established differences in the microbial contamination of different surfaces in the hatchery before disinfection took place (Table 1), a differentiated approach to disinfection procedures may be appropriate. A more thorough mechanical cleaning is required for strongly contaminated surfaces, application of more concentrated solutions, longer exposure times, which would increase the efficacy of sanitization procedures.

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