



*Original Contribution*

**EFFICACY OF ULTRA-LOW VOLUME DISINFECTION WITH HYDROGEN PEROXIDE VAPOUR IN A POULTRY HATCHERY**

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**ABSTRACT**

Good hygiene in the hatcheries is one of the most important factors for normal hatching, low embryonic mortality and healthy chickens. The best disinfectant in these sites should combine high antimicrobial efficiency, lack of damages on the eggs and embryos, and has to be harmless for the staff and the environment. One of the few disinfectants which meets all these conditions is hydrogen peroxide. This study presents data on the effectiveness of disinfection with hydrogen peroxide vapour in a poultry hatchery. Nocolyse One Shot disinfectant (Oxy Pharm, France), containing 12% hydrogen peroxide and silver ions was used, which was applied by Nocospray (an ultra-low volume application device, Oxy Pharm, France) in a dose of 5 mL per 1 m<sup>3</sup>. The results obtained show high efficiency of the disinfection treatments. The achieved microbial reduction in the hatcheries was in the range from 95.51% (1.35 log<sub>10</sub>) for the floor to 99.89% (2.95 log<sub>10</sub>) for the air. The reduction of microbial contamination during the processing of hatching eggs was in the rate of 99.75% (2.6 log<sub>10</sub>). The high efficiency of the treatments, the low toxicity and ecological safety of the hydrogen peroxide and the ease way of its application are the reasons to extend the implementation of the method in hatchery hygiene practices.

**Key words:** poultry, hatchery, hatching eggs, hydrogen peroxide vapour, ULV disinfection

**INTRODUCTION**

In healthy parent flocks, the egg surface and the egg contents are sterile at the time of egg laying. Once they have been laid, such eggs suffer secondary contamination on contact with the nest or the transport belts, or as a result of their storage, transportation or handling (1). Apart from the contaminated eggs, other sources of microbial contamination in the hatchery are the staff, equipment, the air flow and especially the residual microbial contamination from the previous batch of chickens (1-2).

Found themselves in the hatchery, microorganisms have optimal conditions for growth-suitable temperature, humidity and nutrients. It has been established that under such

conditions, only within 24 hours one bacterial cell could multiply to 4.7 x 10<sup>24</sup> cells (1).

Hence, within a very short time, microbial contamination in the hatchery may increase drastically. This threatens seriously the embryo health and provokes the spread of contagious diseases. That is why ensuring a good hygiene in the hatchery is one of the most important factors for the trouble-free egg hatching, low embryo mortality rate and production of healthy chickens (2-3).

The disinfection programme should be able to efficiently reduce microbial contamination and maintain low levels of some key microorganisms with significant impact for hatcheries such as *Salmonella* spp., *Pseudomonas* spp., *Proteus* spp., *E. coli*, *Staphylococcus* spp., *Streptococcus* spp. and *Aspergillus* spp. (1, 4).

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It is known that not all of the disinfectants are able to inactivate all types of microorganisms. For example, quaternary ammonium compounds which are widely used, are not able to inactivate bacterial spores and non-enveloped viruses, and some bacteria such as *Pseudomonas* spp. and *Enterococcus* spp. exhibit very high resistance (5).

Other disinfectants possess a very high antimicrobial activity and a broad spectrum but they quite easily damage the equipment (alkalies, acids, hypochlorites) and/or release toxic gases (aldehydes, chlorine-releasing compounds, phenols). Some of them (formaldehyde) are known carcinogens. Their use poses a very serious risk for the health of the hatchery workers (5-8).

The best disinfectant in hatcheries should combine high antimicrobial efficiency, lack of damages on the equipment, eggs and embryos, and has to be harmless for the staff and the environment. One of the few disinfectants which meet all these conditions are oxygen releasing compounds and their representative is hydrogen peroxide (5, 7-8).

Nocolyse One Shot disinfectant (Oxy Pharm, France) is a bio- disinfectant for air and surfaces disinfection, which contains 12% hydrogen peroxide and silver ions. The method of its application is by ultra-low volume (ULV) manner by using an ULV device (Nocospray, Oxy Pharm, France), which emits a mist of the disinfectant with particle size around 5  $\mu\text{m}$  (9).

This disinfection method stands out with a number of advantages such as: 1) broad spectrum: it has bactericidal, virucidal, fungicidal and sporicidal activity; 2) it is harmless to the staff: lack of any toxic or allergenic substances; 3) it is harmless to the environment: it is decomposed to non-toxic products; 4) it is harmless to the equipment: it is suitable for all types of surfaces including electronic ones, without corroding or oxidizing effect; 5) it is easy to apply: automated systems; 6) very small quantity of the product are needed 1 - 5 mL per  $\text{m}^3$  (9-11). Its excellent efficacy has been established by a number of scientific studies in laboratory conditions (12, 13), in an industrial bread factory (14) and even for disinfection of diving suits (15).

The significant advantages of this method mentioned above make it especially suitable for use also in the poultry farming. This gave us a reason to deepen our studies in its disinfection efficacy in the real hatchery practice and under the ambient conditions specific for these type of sites.

## MATERIAL AND METHODS

The studies were carried out in two poultry hatcheries located in South Bulgaria.

*In hatchery 1*, disinfection of a hatching cabinet was carried out. After vacating the cabinet from the newly-hatched chickens, the cabinet was mechanically cleaned and washed using a high-pressure water jet. After drying of all surfaces, ultra-low volume disinfection (ULV) was carried out with Nocolyse One Shot disinfectant (Oxy Pharm, France), applied by Nocospray (an ULV device) in dose of 5  $\text{mL}/\text{m}^3$  and 120 minutes exposure time.

The efficacy of the disinfection was determined by microbiological testing by estimating the percentage of the microbial reduction achieved expressed by the difference between the number of microorganisms on the surfaces tested (floor, wall, equipment) and air before and after the disinfection. With the same purpose, we also estimated the  $\text{Log}_{10}$ -reduction of the microbial contamination using the formula below (16):  $\text{Log}_{10}$  reduction =  $\text{Log}_{10}$  pre-disinfection bacterial count –  $\text{Log}_{10}$  after- disinfection bacterial count.

a) *The samples of the surfaces* were taken by microbiological swab method (17). A sterile moistened cotton swab was rubbed in a surface of 25  $\text{cm}^2$  by using sterile cardboard templates. The samples were put in tubes, containing 10 mL sterile distilled water and refrigerated during transportation to the laboratory. Sample homogenization and content extraction was performed by shaking in a vortex mixer for 3 minutes, after which the tampons were removed from the tubes. Ten-fold dilutions (from  $10^{-1}$  to  $10^{-7}$ ) were prepared in sterile distilled water. For the quantification of the total bacterial count, aliquots of 0.1 mL from the primary dilution and the corresponding decimal dilutions were sowed in Tryptone Soya Agar w/Lecithin and Polysorbate 80 (HiMedia, India). The samples were incubated at 37° C and aerobic atmosphere for 48h. The grown colonies were counted twice - on the 24th and 48th hour of the incubation, and final result was presented as the highest colony amount. The counting was performed by using a digital colony counter (Colony counter LA660, HiMedia, India). The amount of microorganisms per 1  $\text{cm}^2$  of the control surfaces was calculated depending on the number of grown colonies on the agar and the corresponding rate of dilution. For every control surface, 6 cotton swab samples were examined and the average results were presented.

b) *The air samples* were obtained by a passive sedimentation method. Petri dishes, containing Triptone Soya Agar w/Lecithin and Polysorbate 80 (HiMedia, India), with a diameter of 8.5 cm were used. The plates were arranged at different heights in the hatchery cabinet. They were opened for a certain period of time (from 2 to 10 min.) pursuant to the expected microbial contamination. The samples were incubated at 37°C and aerobic atmosphere for 48h. The grown colonies were counted twice-on the 24th and 48th hour of the incubation, and the highest number of bacteria cells was taken as a final result. Depending on the number of grown colonies on the agar and the exposure of the Petri dishes, a total bacterial count of 1 m<sup>3</sup> was determined (18). Six Petri dishes were placed on different levels and the average results were taken.

**In hatchery 2**, disinfection of the hatching eggs was carried out. After placement of the egg batch in the fumigation chamber, ULV disinfection with Nocolyse One Shot disinfectant, applied by

Nocospray in dose of 5 mL/m<sup>3</sup> and 60 minutes exposure time took place.

The efficacy of the disinfection was determined by estimating the achieved reduction of microbial contamination on the egg surface (CFU per egg). With this purpose, using sterile latex gloves, the eggs were placed in a sterile polyethylene bag containing 10 mL sterile distilled water and they were vigorously rubbed. From rinsed fluids ten-fold dilutions were prepared by the method described in paragraph a) to determine the total bacterial count on the egg surface before and after disinfection.

The statistical data processing was performed using GraphPad software. Comparison of the results before and after disinfection was performed by unpaired t-test. The differences were considered as statistically significant when P value was less than 0.05 (P < 0.05).

## RESULTS

The results of the microbiological examinations of the samples are presented in **Table 1 and 2**.

**Table 1.** Efficacy of ULV disinfection of hatching cabinet with Nocolyse One Shot disinfectant (Oxy Pharm, France) in dose of 5 mL/m<sup>3</sup> and 120 minutes exposure time.

Zone	Sample №	After washing /Initial contamination/		After disinfection		
		CFU/cm <sup>2/3</sup>	Average	Microbial contamination		Microbial reduction
				CFU/cm <sup>2/3</sup>	Average	
Floor	1	2.51 x 10 <sup>3</sup>	1.4 x 10 <sup>3</sup> (315 Log <sub>10</sub> ) SD=7.16 x 10 <sup>2</sup>	8.21 x 10 <sup>1</sup>	6.28 x 10 <sup>1</sup> (1.8 Log <sub>10</sub> ) SD= 3.4x 10 <sup>1</sup>	95.51%* (1.35 Log <sub>10</sub> )
	2	1.47 x 10 <sup>3</sup>		0		
	3	0.39 x 10 <sup>3</sup>		6.46 x 10 <sup>1</sup>		
	4	1.75 x 10 <sup>3</sup>		6.87 x 10 <sup>1</sup>		
	5	1.26 x 10 <sup>3</sup>		6.23 x 10 <sup>1</sup>		
	6	0.99 x 10 <sup>3</sup>		9.88 x 10 <sup>1</sup>		
Wall	1	7.52 x 10 <sup>2</sup>	6.78 x 10 <sup>2</sup> (2.83 Log <sub>10</sub> ) SD=3.88 x 10 <sup>2</sup>	0	1.3 x 10 <sup>1</sup> (1.11 Log <sub>10</sub> ) SD=1.83 x 10 <sup>1</sup>	98.08%** (1.72 Log <sub>10</sub> )
	2	12.54 x 10 <sup>2</sup>		0		
	3	8.15 x 10 <sup>2</sup>		0		
	4	4.33 x 10 <sup>2</sup>		4.56 x 10 <sup>1</sup>		
	5	7.17 x 10 <sup>2</sup>		0.98 x 10 <sup>1</sup>		
	6	0.98 x 10 <sup>2</sup>		2.23 x 10 <sup>1</sup>		
Equipment	1	18.44 x 10 <sup>2</sup>	9.25 x 10 <sup>2</sup> (2.97 Log <sub>10</sub> ) SD=4.91 x 10 <sup>2</sup>	0	2.34 x 10 <sup>1</sup> (1.37 Log <sub>10</sub> ) SD=1.97 x 10 <sup>1</sup>	97.47 %*** (1.6 Log <sub>10</sub> )
	2	9.32 x 10 <sup>2</sup>		0		
	3	4.16 x 10 <sup>2</sup>		4.44 x 10 <sup>1</sup>		
	4	8.88 x 10 <sup>2</sup>		4.22 x 10 <sup>1</sup>		
	5	8.54 x 10 <sup>2</sup>		3.08 x 10 <sup>1</sup>		
	6	6.14 x 10 <sup>2</sup>		2.27 x 10 <sup>1</sup>		
Air	1	7.48 x 10 <sup>4</sup>	7.24 x 10 <sup>4</sup> (4.86 Log <sub>10</sub> ) SD=6.84 x 10 <sup>3</sup>	8.81 x 10 <sup>1</sup>	8.08 x 10 <sup>1</sup> (1.91 Log <sub>10</sub> ) SD=1.52 x 10 <sup>1</sup>	99.89%**** (2.95 Log <sub>10</sub> )
	2	6.4 x 10 <sup>4</sup>		7.93 x 10 <sup>1</sup>		
	3	6.41 x 10 <sup>4</sup>		7.05 x 10 <sup>1</sup>		
	4	8.07 x 10 <sup>4</sup>		7.93 x 10 <sup>1</sup>		
	5	7.49 x 10 <sup>4</sup>		6.17 x 10 <sup>1</sup>		
	6	7.61 x 10 <sup>4</sup>		10.58 x 10 <sup>1</sup>		

Legend: SD – Standard Deviation; \* Indicate statistical significance of the differences (p<0.05): \* p = 0.0011; \*\* p = 0.0019; \*\*\* p = 0.0012; \*\*\*\* p = 0.0001.

**Table 2.** Efficacy of ULV disinfection of hatching eggs with Nocolyse One Shot disinfectant (Oxy Pharm, France) in dose of 5 mL/m<sup>3</sup> and 60 minutes exposure time.

Sample №	Microbial contamination before disinfection		Sample №	Microbial contamination after disinfection		Microbial reduction
	CFU/Egg	Average		CFU/Egg	Average	
1	1.44 x 10 <sup>6</sup>	2.45 x 10 <sup>5</sup> CFU/Egg (5.39 Log <sub>10</sub> /Egg) SD = 3.89 x 10 <sup>5</sup>	13	3.2 x 10 <sup>2</sup>	6.1 x 10 <sup>2</sup> CFU/Egg (2.79 Log <sub>10</sub> /Egg) SD = 6.11 x 10 <sup>2</sup>	99.75% <sup>□</sup> (2.6 Log <sub>10</sub> )
2	3.0 x 10 <sup>4</sup>		14	1.4 x 10 <sup>3</sup>		
3	8.0 x 10 <sup>4</sup>		15	9.42 x 10 <sup>1</sup>		
4	2.4 x 10 <sup>5</sup>		16	4.5 x 10 <sup>2</sup>		
5	1.0 x 10 <sup>5</sup>		17	3.2 x 10 <sup>1</sup>		
6	5.0 x 10 <sup>4</sup>		18	8.87 x 10 <sup>2</sup>		
7	1.0 x 10 <sup>5</sup>		19	4.55 x 10 <sup>2</sup>		
8	1.22 x 10 <sup>5</sup>		20	3.66 x 10 <sup>2</sup>		
9	3.2 x 10 <sup>5</sup>		21	8.7 x 10 <sup>1</sup>		
10	3.0 x 10 <sup>5</sup>		22	6.61 x 10 <sup>2</sup>		
11	2.09 x 10 <sup>4</sup>		23	4.44x 10 <sup>2</sup>		
12	1.4 x 10 <sup>5</sup>		24	2.13 x 10 <sup>3</sup>		

Legend: SD - Standard Deviation; <sup>□</sup> Indicate statistical significance of the differences(p<0.05) : p=0.0405.

## DISCUSSION

The high antimicrobial activity and the broad spectrum of the hydrogen peroxide vapour have been proven by a number of scientific studies (19). Boyce *at al.*, (20) and Barbut *at al.*, (21) have found that hydrogen peroxide vapour has good sporicidal activity and leads to complete inactivation of *Clostridium difficile* spores regardless of strain and/or surface. Berrie *at al.*, (22) and Bentley *at al.*, (23) have found that it has good virucidal activity even in case of highly environment resistant viruses such as adenoviruses and feline caliciviruses. Hydrogen peroxide vapor inactivates even the most resistant microorganisms: prions (24). This is why this method is recommended for decontamination of contaminated sites in case of infectious outbreaks or nosocomial infections (25-26). Fu *at al.* (27) conclude that this is a safe, fast and effective method for decontamination. According to Klapes & Vesley (28) hydrogen peroxide vapour is a real alternative of the fumigation with ethylene oxide or formaldehyde vapours, without their adverse side effects. Opposed to ethylene oxide and formaldehyde, hydrogen peroxide vapour has very low toxicity, it is not cancerogenic and it has no residual smell in the disinfected premises.

Ayubi & Karadzhov (29) performed aerosol disinfection in egg hatcheries using iodophor disinfectant. They achieved 93.9% average reduction of the microbial contamination of the

air and 88.6 % of the different control surfaces (floor, wall, equipment). Carrying out ULV disinfections of the hatching eggs and hatching cabinets with Nocolyse One Shot disinfectant, we have achieved a better disinfection efficacy of the treated surfaces. For all control zones a high statistically significant reduction (>95%, >1.3 log<sub>10</sub>) of the microbial contamination has been established. The highest percentage of the microbial reduction achieved is that of the air in the hatching cabinet 99.89% (2.95 log<sub>10</sub>) and the surface of the fumigated eggs 99.75% (2.6 log<sub>10</sub>). Sheldon & Brake (3) also established a high level of efficacy of the disinfection of hatching eggs with hydrogen peroxide which is comparable to that achieved by fumigation with formaldehyde. Authors proved that treatment of the eggs with hydrogen peroxide does not damage the egg shell and the embryo, but even significantly increases their hatching rate and the quality of the newly hatched chickens.

With regard to ULV disinfection carried out using Nocolyse disinfectant, Orlando *at al.*, (12) established a significant difference in the efficacy of the method in terms of the horizontal and vertical surfaces. They observed significantly better efficacy on the vertical surfaces. In comparison to this, at the time of our studies we have not registered any statistically significant difference in the method efficacy between the vertical and horizontal surfaces. With this method of disinfection (ULV) the disinfectant is emitted in the form of very small particles (approx. 5 µm)

which evenly fill up the entire disinfected volume and ensure even contact with all surfaces disinfected. That is why we believe that if there are any differences in the disinfection efficacy; those are rather attributable to the impact of the different types of materials (metal, paint or plastic) as they possess different properties and characteristics, directly affecting the disinfection efficacy, and not that much to their orientation.

## CONCLUSIONS

Based on the results obtained, as well as the summary of the published scientific data, it can be concluded that:

1. ULV disinfection of hatching eggs and hatching cabinets with hydrogen peroxide vapour (Nocolyse One Shot disinfectant, applied by Nocospray device (Oxy Pharm, France) in a dose of 5 mL per 1 m<sup>3</sup>) features a high level of disinfection efficacy.
2. The established high level of efficacy, easy application and low toxicity, make fumigation with Nocolyse One Shot disinfectant (Oxy Pharm, France) a suitable disinfection method in the hatching practice and a real alternative of the fumigation with the significantly more toxic formaldehyde.

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