UDC 619:614.484:615.28.076.9:576.895.132.8 **DOI 10.36016/JVMBBS-2020-6-2-6**

STUDY OF DISINVASIVE PROPERTIES OF INNOVATIVE ALDEHYDE DISINFECTANT

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Summary. The pollution rate of environment by pathogens of invasive diseases and contamination of manure, soil, water, and other objects by them are constantly changing depending on the prevalence and intensity of invasion among farm animals. Prevention and control of invasive animal diseases are essential to prevent their spread, as well as to obtain high-quality sanitary products for livestock production. The preservation of pathogens in the environment depends on the intensity of exposure to natural and artificial factors and their resistance to chemicals that are used for disinfection. A large number of disinfectants, both domestic and foreign, have been proposed for disinfection, but they are not always effective under industrial conditions for conducting forced or preventive disinfestation. The introduction of disinfectants into practice is impossible without a preliminary laboratory assessment of their disinvasive properties. The aim of our work was to study the disinvasive properties of a new aldehyde disinfectant on the test models of *Ascaris suum* eggs. The studies were carried out in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in accordance with the methodological recommendations 'Methods to Identify and Evaluate Safety Parameters and Quality of Disinfectants, Detergent-Disinfectants Used During Production, Storage, Transportation and Sale of Products of Animal Origin' (2010). As a result of the studies, it was found that the aldehyde disinfectant exhibits disinvasive properties to the test culture of *Ascaris suum* eggs when applied at a concentration of 4.0% at room temperature (18–20 ± 0.5°C) and exposure of 3 h. The disinfectant can be used for preventive and forced disinvasions of animal holding facilities and other veterinary control facilities

Keywords: disinfectant, disinvasive properties, *Ascaris suum*, test culture, concentration, exposure

Introduction. Biological pollution of environment is increasingly being attributed to many major veterinary and ecological problems of our time, one of the aspects of which is transfer and wide spread of helminth eggs and larvae in the environment (Daugschies, Bangoura and Lendner, 2013). Inspection of rural soil in the Kharkiv Region and soil in Kharkiv and Balakliia has revealed its contamination with different morphotypes' helminth eggs. The average level of rural soil pollution is 12.5%. The soil in urban park zones is contaminated with helminth exogenous stages from 5.0 to 55.5%, and urban residential zones — from 20 to 23.3% (Paliy et al., 2019).

Helminthiases of animals in livestock farms and complexes are spread among the susceptible population when non-compliance with veterinary and sanitary regulations and norms is observed (Paliy et al., 2018b), which in turn requires the use of highly effective disinfectants that have a wide range of biocidal effects (Paliy et al., 2015; Paliy, 2018).

Today, composite preparations consisting of several active substances from different groups of chemical compounds are preferred, which, due to the synergism of the components, have a broader range of activity and are effective at much lower concentrations, are more economically advantageous (Zavgorodniy et al., 2013; Koski, Anttila and Kuusela, 2015; Paliy and Paliy, 2019).

However, it should be noted that not all disinfectants, including commercial preparations, are effective against the helminth exogenous stages. Some agents are able to delay or stop the embryogenesis of helminth eggs, but do not completely destroy them (Oh et al., 2016). Other disinfectants are highly effective in contact with a pure helminth egg culture (Bessat and Dewair, 2019). Therefore, before widespread use of disinfectants in order to destroy the causative agents of animal helminthiasis in the environment, it is imperative to determine their disinvasive properties in the laboratory (Campbell et al., 1982; Shalaby et al., 2011).

One of the key conditions that affect the validity of the results obtained from the test of the disinfectant is the use of reference test cultures. In order to evaluate the disinvasive effect of drugs, it is advisable to use the eggs of *Ascaris suum* (Pecson and Nelson, 2005; Yu et al., 2014). This test culture is used to test not only chemicals but also to evaluate the ovicidal activity of physical factors (Brownell and Nelson, 2006).

Glutaraldehyde is most common active substance of most modern disinfectants (Rajan and Ripple, 2009) used in both human medicine (Akamatsu et al., 1997; Burgess et al., 2017) and veterinary practices (Paliy et al., 2016; Zazharskyi et al., 2018).

The purpose of our work was to study the disinvasive properties of a new aldehyde disinfectant on *A. suum* eggs as the test model.

Material and methods. The study of the disinvasive properties of the disinfectant was carried out in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in accordance with the methodological recommendations 'Methods to Identify and Evaluate Safety Parameters and Quality of Disinfectants, Detergent-Disinfectants Used During Production, Storage, Transportation and Sale of Products of Animal Origin' (2010).

The object of research was a disinfectant comprising a mixture of quaternary ammonium compounds (25.0%), glutaraldehyde (11.0%), isopropyl alcohol, nonionic surfactants. The sanitizer is a liquid transparent yellowish liquid with a characteristic odor, well soluble in water. 5.0% sodium hydroxide solution was used as a negative control.

To obtain the *A. suum* egg culture, feces of spontaneously invaded pigs were used, in which at least 2–3 eggs in the field of view of the microscope at low magnification were detected by the flotation. One part of the obtained test material was stored in a refrigerator at a temperature of 3°C, and the other part was cultured in an incubator at a temperature of 26–28°C for 20–30 days. In laboratory experiments used helminth eggs that were at protoplast (before blastomer cleavage) and larval stages. To confirm the invasiveness of *A. suum* larvae, a biological test was performed on white rats.

Results and discussions. Preliminary experiments to determine the disinvasive properties of the disinfectant were performed on *A. suum* egg test culture using concentrations of 0.5, 1.0, 2.0, 3.0, 3.5, and 4.0% at a temperature of 20 ± 0.5°C and exposures 1, 3, 6, and 24 h. The obtained results are presented in Table 1.

Analyzing the results in Table 1, it should be noted that the treatment of the test culture with disinfectant solutions with 0.5, 1.0, and 2.0% concentrations at a temperature of 20 ± 0.5°C and exposures 3, 6, and 24 h did not affect *A. suum* egg development.

Treatment of the test culture with 2.0% solution within 24 h caused the death of eggs only on the 28th day.

Along with this, the concentrations of 3.0, 3.5, and 4.0% influenced the delay in the development of test culture eggs and caused their death, that is, they showed a disinvasive property.

The disinfectant exhibited a higher disinvasive activity with 3.5% concentration at exposures 6 and 24 h.

Table 1 — Disinvasive activity rate of the disinfectant to *Ascaris suum* egg culture

Concentration of the disinfectant, %	Terms for determining the viability of *Ascaris suum* eggs, days														
	3			6			14			21			28		
	Exposures, h														
	3	6	24	3	6	24	3	6	24	3	6	24	3	6	24
0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
3.0	+	+	+	+	+	–	+	+	–	+	–	–	–	–	–
3.5	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–
4.0	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Negative control	+	+	+	+	+	–	+	+	–	+	+	–	+	+	–

Notes: '–' — egg death, '+' — egg development.

The results of the disinfectant ovicidal activity determination are presented in Table 2.

Analyzing the results in Table 2, it should be noted that the ovicidal efficacy of the disinfectant depends on the exposure. Thus, on the protoplast stage of *A. suum* eggs it is 96.00% at a concentration of 3.5% and an exposure of 3 h, and 99.22% — at a exposure of 24 h. The disinvasive effect of the disinfectant on *A. suum* eggs at the larval stage was slightly higher (97.64%) at a concentration of 3.5% and an exposure of 3 h. With increasing concentration and the same exposure, *A. suum* eggs at the larval stage were more vulnerable to disinfectant and died 7 days earlier; disinvasive efficacy was also higher (97.64%).

Based on the preliminary experience, to determine the disinfecting concentrations the sanitizer were tested at concentrations of 3.5 and 4.0% on the surfaces of the test objects made of tile, not painted wood, and metal plates (Table 3). When conducting studies to determine the disinvasive properties of the disinfectant relative to *A. suum* egg culture applied on the test objects, it was found that 3.5 and 4.0% solutions had a disinvasive effect on all surfaces. It should be noted that when studying the susceptibility of *A. suum* eggs to the disinfectant during treatment, it is necessary to take into account the physical characteristics of the surfaces, which determines the efficacy of decontamination. In particular, according to

the results (Table 3), the disinfectant at 3.5 and 4.0% concentrations for all exposures showed disinvasive properties on the surfaces made of tile and metal plates and had lower disinvasive activity on the unpainted wood. That is, the rougher the surface of the object being processed, the lower the efficacy of disinvasion.

Table 2 — Ovicidal activity of the disinfectant

Test culture	Concentrations, %	Exposures, h	Terms for death of eggs in experimental cultures, day	Ovicidal efficacy, %
Ascaris suum at the protoplast stage	3.0	3	28	90.00
		6	21	90.86
		24	21	91.10
	3.5	3	21	96.00
		6	6	98.78
		24	6	99.22
	4.0	3	14	99.60
		6	6	99.64
		24	6	99.70
Ascaris suum at the larval stage	3.0	3	21	91.64
		6	14	96.22
		24	6	96.64
	3.5	3	14	97.64
		6	6	98.92
		24	6	99.64
	4.0	3	6	99.22
		6	3	99.64
		24	3	99.86

Table 3 — Disinvasive activity rate of the disinfectant to *Ascaris suum* egg culture applied on the test objects (6^{th} day after treatment)

Concentrations, %	Test object	Exposures, h		
		3	6	24
		Average ovicidal activity for three experiments		
3.5	tile	80.44 ± 0.01	80.89 ± 0.01	84.33 ± 0.01
	wood	67.22 ± 0.01	79.78 ± 0.01	80.67 ± 0.01
	metal	84.33 ± 0.01	85.33 ± 0.01	93.87 ± 0.01
4.0	tile	91.10 ± 0.01	99.64 ± 0.01	99.70 ± 0.01
	wood	80.44 ± 0.01	90.60 ± 0.02	90.85 ± 0.02
	metal	99.55 ± 0.01	99.64 ± 0.01	99.67 ± 0.01
Positive control	tile	–	–	–
	wood	–	–	–
	metal	–	–	–
Negative control	tile	–	–	90.70 ± 0.01
	wood	–	–	53.70 ± 0.02
	metal	–	–	90.70 ± 0.01

Notes: '–' — no ovicidal efficacy; $p < 0.05$.

Comparing the results of the experiments, it should be noted that the exposure affects the disinvasive activity of the sanitizer, that is, the level of inhibition of the development of test cultures and their death. At least 3 h will be required during disinfection measures.

The efficacy of the disinvasive activity of the disinfectant on *A. suum* egg test culture was tested by bioassay on white rats. Animals were divided into three groups of 15 rats each: a research group and two control groups.

White rats of the research group were given 100 eggs of *A. suum* at the larval stage in suspension with physiological saline treated with a disinfectant (4.0%, 3 h). Animals of the 1^{st} control group were given 100 eggs of

A. suum at the larval stage from a culture obtained from invaded pigs. The 2nd control group received physiological saline. Observations of animals were carried out for 6 days. Five rats were euthanized in each group on 1st, 3rd, and 6th days, and the contents of the intestines, intestines, liver, and lungs were examined. In the study of the internal organs of laboratory animals by the compressor method obtained the results presented in Table 4.

When analyzing the results in Table 4, it was found that in animals of the experimental group, eggs and larvae of *A. suum* were found only in the intestinal contents for up to 3rd day, which means that the larvae lost invasiveness. In animals of the 1st control group, live larvae were found in the intestinal mucousa, liver, and lungs, which confirms their invasiveness. In the 2nd control group, the animals remained intact.

Table 4 — Results of bioassay test on white rats (n = 15)

Day	Group of animals								
	Experimental			Control 1			Control 2		
	Number of live *A. suum* larvae in the internal organs of animals								
	intestines	liver	lungs	intestines	liver	lungs	intestines	liver	lungs
1	–	–	–	25.0 ± 0.6	8.2 ± 0.6	–	–	–	–
3	–	–	–	6.2 ± 0.4	12.4 ± 1.4	–	–	–	–
6	–	–	–	–	4.0 ± 0.6	16.0 ± 0.6	–	–	–

Note. '–' — no larvae and eggs.

According to the research results, a method was developed for disinvasing surfaces contaminated with *A. suum* eggs, including mechanical cleaning, disinvasion by irrigation with a disinfectant comprising a mixture of quaternary ammonium compounds (0.5–1.0%), glutaraldehyde (0.22–0.44%), isopropyl alcohol (0.16–0.32%), nonionic surfactants (0.1–0.2%), deionized water (99.02–98.04%) with an exposure of 3–24 h at a consumption rate of 500 ml/m^2 (Paliy et al., 2018a).

Conclusions. During laboratory tests, the disinvasive properties of the innovative aldehyde disinfectant in relation to *Ascaris suum* egg test culture were determined.

The innovative aldehyde disinfectant exhibits disinvasive properties with respect to *Ascaris suum* egg test culture when applied at a concentration of 4.0% at room temperature (18–20 ± 0.5°C) and exposure for 3 hours.

The disinfectant can be used for preventive and forced disinvasions of animal holding facilities and other veterinary control facilities.

References

Akamatsu, T., Tabata, K., Hironaga, M. and Uyeda, M. (1997) 'Evaluation of the efficacy of a 3·2% glutaraldehyde product for disinfection of fibreoptic endoscopes with an automatic machine', *Journal of Hospital Infection*, 35(1), pp. 47–57. doi: 10.1016/S0195-6701(97)90167-5.

Bessat, M. and Dewair, A. (2019) 'Assessment of the inhibitory effects of disinfectants on the embryonation of *Ascaridia columbae* eggs', *PLoS ONE*, 14(5), p. e0217551. doi: 10.1371/journal.pone.0217551.

Brownell, S. A. and Nelson, K. L. (2006) 'Inactivation of single-celled *Ascaris suum* eggs by low-pressure UV radiation', *Applied and Environmental Microbiology*, 72(3), pp. 2178–2184. doi: 10.1128/AEM.72.3.2178-2184.2006.

Burgess, W., Margolis, A., Gibbs, S., Duarte, R. S. and Jackson, M. (2017) 'Disinfectant susceptibility profiling of glutaraldehyde-resistant nontuberculous mycobacteria', *Infection Control and Hospital Epidemiology*, 38(7), pp. 784–791. doi: 10.1017/ice.2017.75.

Campbell, I., Tzipori, A., Hutchison, G. and Angus, K. (1982) 'Effect of disinfectants on survival of cryptosporidium oocysts', *Veterinary Record*, 111(18), pp. 414–415. doi: 10.1136/vr.111.18.414.

Daugschies, A., Bangoura, B. and Lendner, M. (2013) 'Inactivation of exogenous endoparasite stages by chemical disinfectants: Current state and perspectives', *Parasitology Research*, 112(3), pp. 917–932. doi: 10.1007/s00436-013-3324-4.

Koski, P., Anttila, P. and Kuusela, J. (2015) 'Killing of *Gyrodactylus salaris* by heat and chemical disinfection', *Acta Veterinaria Scandinavica*, 58, p. 21. doi: 10.1186/s13028-016-0202-y.

Oh, K.-S., Kim, G.-T., Ahn, K.-S. and Shin, S.-S. (2016) 'Effects of disinfectants on larval development of *Ascaris suum* eggs', *The Korean Journal of Parasitology*, 54(1), pp. 103–107. doi: 10.3347/kjp.2016.54.1.103.

Paliy, A. P. (2018) 'Antibacterial effect of 'Ecocide C' disinfectant against mycobacteria' [Efektyvnist antybakterialnoi dii dezinfikuiuchoho zasobu «Ekotsyd S» shchodo mikobakterii], *Ukrainian Journal of Ecology*, 8(1), pp. 141–147. doi: 10.15421/2018_198. [in Ukrainian].

Paliy, A. P. and Paliy, A. P. (2019) *Technic and Technological Innovations in Dairy Cattle [Tekhniko-tekhnolohichni innovatsii u molochnomu skotarstvi]*. Kharkiv: Miskdruk. ISBN 9786176192077. [in Ukrainian].

Paliy, A., Zavgorodniy, A., Stegniy, B. and Gerilovych, A. (2015) 'A study of the efficiency of modern domestic disinfectants in the system of TB control activities', *Agricultural Science and Practice*, 2(2), pp. 26–31. doi: 10.15407/agrisp 2.02.026.

Paliy, A., Stegniy, B., Muzyka, D., Gerilovych, A. and Korneykov, O. (2016) 'The study of the properties of the novel virucidal disinfectant', *Agricultural Science and Practice*, 3(3), pp. 41–47. doi: 10.15407/agrisp3.03.041.

Paliy, A. P., Sumakova, N. V., Mashkey, A. M., Stegniy, B. T., Guzhvynska, S. O. and Rodionova, K. O. (2018a) *A Method for Disinvasion of Surfaces Contaminated with Ascaris Suum Eggs [Sposib dezinvazii poverkhon, kontaminovanykh yaitsiamy Ascaris suum]*. Patent no. UA 130430. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=253529. [in Ukrainian].

Paliy, A. P., Sumakova, N. V., Mashkey, A. M., Petrov, R. V., Paliy, A. P. and Ishchenko, K. V. (2018b) 'Contamination of animal-keeping premises with eggs of parasitic worms', *Biosystems Diversity*, 26(4), pp. 327–333. doi: 10.15421/011848.

Paliy, A., Sumakova, N., Petrov, R., Shkromada, O., Ulko, L. and Palii, A. (2019) 'Contamination of urbanized territories with eggs of helmiths of animals', *Biosystems Diversity*, 27(2), pp. 118–124. doi: 10.15421/011916.

Pecson, B. M. and Nelson, K. L. (2005) 'Inactivation of *Ascaris suum* eggs by ammonia', *Environmental Science and Technology*, 39(20), pp. 7909–7914. doi: 10.1021/es050659a.

Rajan, J. S. and Ripple, S. D. (2009) 'Efficacy and safety of glutaraldehyde-based high level disinfectant products', *American Journal of Infection Control*, 37(5), p. e22. doi: 10.1016/j.ajic.2009.04.022.

Shalaby, H. A., Abdel-Shafy, S., Ashry, H. M. and El-Moghazy, F. M. (2011) 'Efficacy of hydrogen peroxide and dihydroxy benzol mixture (disinfectant) on *Toxocara canis* eggs', *Research Journal of Parasitology*, 6(4), pp. 144–150. doi: 10.3923/jp.2011.144.150.

Yu, Y.-M., Kim, J.-W., Na, W.-S., Youn, Y.-N., Choi, I.-W. and Lee, Y.-H. (2014) 'Effects of some pesticides on development of *Ascaris suum* eggs', *The Korean Journal of Parasitology*, 52(1), pp. 111–115. doi: 10.3347/kjp.2014.52.1.111.

Zavgorodniy, A. I., Stegniy, B. T., Paliy, A. P., Gorzheiev, V. M. and Smirnov, A. M. (2013) *Scientific and Practical Aspects of Disinfection in Veterinary Medicine [Naukovi ta praktychni aspekty dezinfektsii u veterynarii]*. Kharkiv: FOP Brovin O. V. ISBN 9789662445596. [in Ukrainian].

Zazharskyi, V. V., Davydenko, P., Kulishenko, O., Chumak, V., Kryvaya, A., Biben, I. A., Tishkina, N. M., Borovik, I., Boyko, O. O. and Brygadyrenko, V. V. (2018) 'Bactericidal, protistocidal and nematodicidal properties of mixtures of alkyldimethylbenzyl ammonium chloride, didecyldimethyl ammonium chloride, glutaraldehyde and formaldehyde', *Regulatory Mechanisms in Biosystems*, 9(4), pp. 540–545. doi: 10.15421/021881.