



## Evaluation of effectiveness of disinfectants against exogenous stages of development of *Nematodirus spathiger* nematodes

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One of the most common nematode diseases of ruminants is gastrointestinal strongylosis. The pathogen's life cycle partly occurs in the external environment. Its propagative stages are eggs secreted by infested animals, and invasive larvae, which persist in the environment for a long time and form biological pollution. Therefore, an integral part of the effective control and prevention of gastrointestinal strongylosis of ruminants is the implementation of measures aimed at destroying pathogens in the environment to curb their potential distribution. The aim of the research was to investigate the disinvasive effectiveness of chemical preparations against eggs and invasive L<sub>3</sub> larvae of the nematode *Nematodirus spathiger*, isolated from cattle, in laboratory conditions. It is established that the products "Hermecid-VS" (contains didecyldimethylammonium chloride, glutaraldehyde, benzalkonium chloride) and "Virosan" (contains benzalkonium chloride and glutaraldehyde) have ovicidal and larvicidal properties against exogenous stages of development of *N. spathiger*. At the same time, "Hermecid-VS" showed higher disinvasion activity compared to "Virosan". The ovicidal activity of disinfectants was higher than the larvicidal one. High levels of ovicidal efficacy were established when "Hermecid-VS" was used: 90.9% at a 0.1% concentration (exposure 60 min), and 100.0% at 0.25% and 0.5% concentrations (10–60 min). The larvicidal efficiency of "Hermecid-VS" was 90.7% at 0.25% concentration (60 min), and 95.3–100.0% at 0.5% concentration (10–60 min). The preparation "Virosan" showed a high level of ovicidal efficacy when used at a concentration of 0.25% (30–60 min) – 92.6–100.0%, at a concentration of 0.5% (10–60 min) – 100.0%, and a high level of larvicidal efficacy at a concentration of 0.5% (60 min) – 97.0%. The detrimental effect of disinfectants was characterized by morphological changes in the eggs and L<sub>3</sub> larvae of *N. spathiger*, as well as in the metric parameters of *N. spathiger* eggs during their development. The obtained research results allow us to recommend the disinfectants "Hermecid-VS" and "Virosan" with the mentioned usage protocols to increase the effectiveness of prevention and treatment of *N. spathiger* infection in cattle.

**Keywords:** nematodiosis; cattle; nematode eggs; invasive larva; disinfection; ovicidal efficiency.

### Introduction

Parasitic helminths of the order Strongylida (syn. Rhabditida) are the dominant representatives of nematode pathogens, which are recorded in many countries of the world and parasitize almost all domestic animals, as well as humans (Jex et al., 2009; Roeber et al., 2013a; Bacelar et al., 2022). Among them, gastrointestinal strongylids cause one of the most common helminthic diseases in cattle in farms of various types (Mashayekhi et al., 2013; Ruhoollah et al., 2021; Nouri et al., 2022). Infections caused by strongylids cause significant losses to livestock, due to weight loss, slowed growth and development of calves, reduced milk and meat production, death of young animals, as well as significant costs of treatment measures (Knox et al., 2012; Roeber et al., 2013b; Besier et al., 2016). In addition, strongylids, parasitizing in the gastrointestinal tract, mechanically damage epithelial cells, especially the intestinal wall, which reduces its ability to digest and absorb feed and causes symptoms such as anorexia, diarrhea, anemia, weight loss, chewing disorders, dull coat (Zalizar, 2017; Pilla et al., 2019).

The most common representatives of the gastrointestinal strongylids that parasitize cattle include nematodes of the genera *Haemonchus*, *Ostertagia*, *Cooperia*, *Nematodirus*, *Bunostomum*, *Oesophagostomum*, *Trichostrongylus*, and *Chabertia*. The host animals are most often infected with these pathogens on pastures or through forage collected from poor pastures (Lima, 1998; Renwal et al., 2017; Hodda, 2022). Such prevalence of gastrointestinal strongylids among susceptible hosts is due to the fact that these infections, more often in

adult animals, are asymptomatic. This complicates the timely detection of sick animals. Also, the pathogens have a direct life cycle without the participation of intermediate hosts. In particular, infested animals release eggs together with feces into the external environment, and contaminate it. The eggs form larvae of the first (L<sub>1</sub>), second (L<sub>2</sub>) and third (L<sub>3</sub>, invasive) stages, which are capable of horizontal and vertical migration. This contributes to their faster entry into the body of susceptible animals (Roeber et al., 2013c; Utami et al., 2024).

An integral part of effective control of animal helminthiasis, including gastrointestinal strongylid infections, is the implementation of measures aimed at preventing the penetration of parasites into the body of susceptible animals and at destroying parasites in the environment, which helps prevention of outbreaks of these infections and limits the possibility of their spread (Zhang et al., 2020; Arnold Landry et al., 2021; Boyko & Brygadyrenko, 2023a). Despite the research available in the scientific literature on the search and use of disinvasive agents, the issue of effective destruction of the propagative stages of development of nematodes, eggs and larvae, in the external environment remains relevant (Dauguschies et al., 2013; Romero et al., 2020; Liotta et al., 2024).

The majority of disinfectants are represented by chemical substances or compounds, with widely varying larvicidal and ovicidal (OE) efficacy (Bessat & Dewair, 2019; Boyko & Brygadyrenko, 2021; Zhang et al., 2024). Thus, in vitro Deltrin at 3.0% and 5.0% concentrations destroyed 100% of *Nematodirus* eggs, and Citrin at similar concentrations destroyed only 72.2–80.0%. Also, the authors found that in specific test objects the duration of action of chemicals increa-

ses, and the percentage of destruction of helminth eggs decreases: for 3.0% deltrin solution, OE was 93.3%, for 3.0% citrine solution it was 66.7%, for 4.0% phenol solution OE was 60.0% (Nasibov, 2024).

Recently, scientists have been studying disinvasive activity of environmentally safe substances of natural origin. Thus, in a laboratory experiment, the mortality rate of larvae of the nematode *Haemonchus contortus* (Rudolphi, 1803) of the order Strongylida was assessed under the influence of different concentrations of eight aromatic acids (formic, tartaric, benzoic, salicylic, stearic, kojic, aminoacetic, and succinic). The minimum LD<sub>50</sub> for L<sub>3</sub> larvae of *H. contortus* was recorded when formic acid was used (Boyko & Brygadyrenko, 2019). In another study, the high larvicidal efficacy of aqueous emulsions of eugenol, isoeugenol, thymol and carvacrol was proved against *H. contortus* L<sub>3</sub> larvae, which are gastrointestinal parasites of goats (Boyko & Brygadyrenko, 2023b). *In vitro* experiments when studying the harmful effects of alkalis (sodium hydroxide, potassium hydroxide), acids (boric acid, phosphoric acid) and salts (potassium chloride, calcium chloride, sodium nitrite, potassium nitrate, sodium nitrate, potassium nitrate, ammonium bicarbonate, sodium bisulfite, sodium bisulfate, sodium sulfate, potassium sulfate, calcium sulfate, sodium thiosulfate, sodium metabisulfite, potassium metabisulfite, copper sulfate pentahydrate, tetrasodium pyrophosphate, sodium triphosphate, sodium borate decahydrate, talc) on *H. contortus* larvae of various stages of development showed that 1% solutions of sodium hydroxide and potassium hydroxide were the most effective, and 69% of *H. contortus* larvae died within 24 hours (Boyko & Brygadyrenko, 2022). Therefore, establishing the features of the ovicidal and larvicidal efficacy of modern disinfectants against certain species of gastrointestinal strongylids that parasitize cattle is relevant.

The aim of the research was to investigate in laboratory conditions the disinvasive efficacy of disinfectants against eggs and invasive L<sub>3</sub> larvae of the nematode *Nematodirus spathiger* isolated from cattle.

**Table 1**  
Disinfectants tested for ovicidal and larvicidal activity against the nematode *Nematodirus spathiger*

Product name, manufacturer	Active substance, %	Formula	Usage
"Hermecid-VS", LLC "Vetsintez", Ukraine	didecyldimethylammonium chloride, 2.25% CAS 7173-51-5	C <sub>22</sub> H <sub>48</sub> ClN	A wide spectrum of action against various kinds of microorganisms: bacteria, viruses, fungi. The preparation has a long-lasting effect and does not cause habituation of microorganisms
	glutaraldehyde, 15% CAS 111-30-8	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	
	benzalkonium chloride (alkyldimethylbenzylammonium chloride), 8% CAS 8001-54-5	[C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub> R]Cl	
"Virosan", LLC "Bio TestLab", Ukraine	benzalkonium chloride (alkyldimethylbenzylammonium chloride), 25% CAS 8001-54-5	[C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub> R]Cl	Has a broad spectrum of activity against various microorganisms: bacteria ( <i>Staphylococcus aureus</i> , <i>Streptococcus faecalis</i> , <i>Pseudomonas aeruginosa</i> , <i>E. coli</i> , <i>Klebsiella pneumoniae</i> , <i>Proteus mirabilis</i> , <i>Listeria monocytogenes</i> , <i>Mycoplasma</i> spp.), viruses ( <i>Newcastle disease virus</i> , <i>Reovirus</i> , <i>Rotavirus</i> , <i>Coronavirus</i> , <i>Paramyxovirus</i> , <i>Poxvirus</i> , <i>Orthomyxovirus</i> , <i>Pestivirus</i> ), fungi ( <i>Candida</i> spp., <i>Aspergillus</i> spp., <i>Fusarium</i> spp., <i>Penicillium</i> spp.)
	glutaraldehyde, 11% CAS 111-30-8	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	

Statistical processing of the experimental results was carried out using Statistica 10 (StatSoft Inc., USA) software. Standard deviation (SD) and average values ( $\bar{x}$ ) were calculated. Differences between the values of the groups were determined using the Tukey test, where the differences were considered significant at  $P < 0.05$ .

## Results

With increasing concentration and exposure of the disinfectant "Hermecid-VS", its ovicidal efficacy against *N. spathiger* eggs increases (Table 2). A high level of ovicidal efficacy was established when "Hermecid-VS" was used at a 0.1% concentration for 60 min exposure (90.9%), at 0.25% and 0.5% concentrations regardless of exposure (100.0%). A satisfactory level of ovicidal efficacy of "Hermecid-VS" was found when it was used at a 0.1% concentration for 10 min exposure (79.6%) and 30 min exposure (85.7%). The disinfectant "Virosan" showed lower values of ovicidal efficacy against the eggs of *N. spathiger*, although with increase in its concentration and expo-

## Materials and methods

The work was carried out in the Laboratory of the Department of Parasitology and Veterinary and Sanitary Examination of the Poltava State Agrarian University (Ukraine), in 2024–2025.

The ovicidal and larvicidal efficacy of the disinfectants "Hermecid-VS" and "Virosan" (Table 1) were determined using test cultures of eggs and invasive L<sub>3</sub> larvae of the nematode species *N. spathiger*. Eggs were obtained from the gonads of female helminths, collected during the dissection of the intestines of cattle coming from farms in the Poltava region. Invasive larvae were obtained by culturing the nematode eggs to L<sub>3</sub> larvae.

Petri dishes were prepared with different concentrations of "Hermecid-VS" (0.1%, 0.25% and 0.5%) and "Virosan" (0.1%, 0.25% and 0.5%), to study anthelmintic efficacy at different exposures (10, 30, 60 min) in laboratory conditions. The same volume of a tested chemical solution of a certain concentration was added to a previously prepared mixture of eggs and larvae (not less than 50 specimens per dish). After the appropriate exposure, the culture of eggs and larvae of *N. spathiger* was washed in distilled water and transferred to separate Petri dishes. A control test culture of *N. spathiger* eggs was also prepared, into which distilled water was added instead of disinfectants. After that, the experimental and control dishes were placed in a thermostat at a temperature of 30 °C. Test cultures of eggs were observed for 10 days until the appearance of L<sub>3</sub> larvae. Test cultures of L<sub>3</sub> larvae were observed for five days. The cultures were aerated and moistened every two days. The experiment for each disinfectant was repeated three times. Ovicidal and larvicidal efficacy (%) were determined according to the method (Volkov & Simonov, 1977).

Morphometric parameters of eggs and larvae of *N. spathiger* ( $n = 15$ ) were studied using the ToupView program version × 64, 4.10.17015.20200426 (Hangzhou ToupTek Photonics Co., Ltd, China). Microphotography was performed using a SIGETA M3CMOS 14000 14.0 MP digital camera (China).

sure, similarly to using "Hermecid-VS", its effectiveness increased (Table 3).

**Table 2**  
Ovicidal efficacy of "Hermecid-VS" disinfectant against the eggs of *Nematodirus spathiger* ( $\bar{x} \pm SD$ ,  $n = 50$ , %)

Parameters of development	Time of exposure, min	Concentration of preparation		
		0.1%	0.25%	0.5%
Formation of L <sub>3</sub> in eggs	10	9.0 ± 1.0 <sup>c</sup>	0.0 ± 0.0 <sup>ab</sup>	0.0 ± 0.0 <sup>a</sup>
	30	6.3 ± 0.6 <sup>c</sup>	0.0 ± 0.0 <sup>ab</sup>	0.0 ± 0.0 <sup>a</sup>
	60	4.0 ± 1.0 <sup>c</sup>	0.0 ± 0.0 <sup>ab</sup>	0.0 ± 0.0 <sup>a</sup>
Arrested development, egg death	10	41.0 ± 1.0 <sup>c</sup>	50.0 ± 0.0 <sup>ab</sup>	50.0 ± 0.0 <sup>a</sup>
	30	43.7 ± 0.6 <sup>c</sup>	50.0 ± 0.0 <sup>ab</sup>	50.0 ± 0.0 <sup>a</sup>
	60	46.0 ± 1.0 <sup>c</sup>	50.0 ± 0.0 <sup>ab</sup>	50.0 ± 0.0 <sup>a</sup>
Ovicidal efficacy, %	10	79.6 ± 3.0 <sup>c</sup>	100.0 ± 0.0 <sup>ab</sup>	100.0 ± 0.0 <sup>a</sup>
	30	85.7 ± 0.6 <sup>c</sup>	100.0 ± 0.0 <sup>ab</sup>	100.0 ± 0.0 <sup>a</sup>
	60	90.9 ± 2.6 <sup>c</sup>	100.0 ± 0.0 <sup>ab</sup>	100.0 ± 0.0 <sup>a</sup>

Note: different letters in the table within each line indicate significant ( $P < 0.05$ ) differences between groups according to Tukey's test results.

High ovicidal efficacy levels of "Virosan" were established when it was used in a 0.25% concentration for exposures of 30 min (92.6%) and 60 min (100.0%), as well as in a 0.5% concentration regardless of exposure (100.0%). Satisfactory levels of its ovicidal efficacy were found when the product was used in a 0.1% concentration for exposures of 30 min (60.8%) and 60 min (70.5%), as well as in a 0.25% concentration for exposures of 10 min (81.8%). At a 0.1% concentration for exposures of 10 min, "Virosan" showed an unsatisfactory level of ovicidal efficacy, which was 39.9%.

The ovicidal action of "Hermecid-VS" and "Virosan" was characterized by morphological changes in the eggs of experimental test cultures, such as arrest of embryo development and its loosening (Fig. 1a), thinning and destruction of the shell, wrinkling and death of the embryo (Fig. 1b), loosening and disintegration of the structural elements of the egg (Fig. 1c); death of eggs at the stage of formation of the L<sub>1</sub> larva (Fig. 1d).

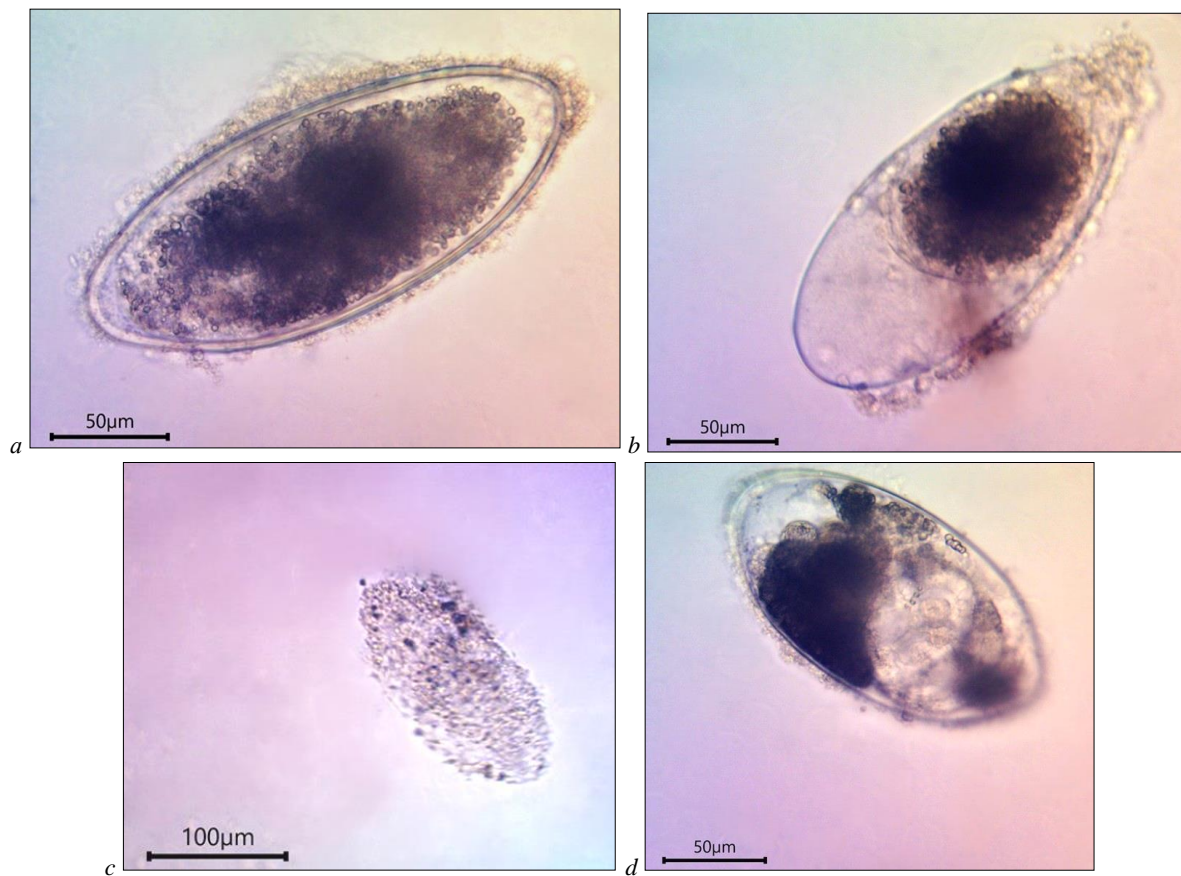
When determining the larvicidal efficacy of the disinfectant "Hermecid-VS", its more destructive effect was found when acting on

L<sub>3</sub> larvae of *N. spathiger*. As well as the ovicidal efficacy indicators, the larvicidal activity of the agent increased with increasing concentration and exposure (Table 4).

**Table 3**  
Ovicidal efficacy of "Virosan" disinfectant against the eggs of *Nematodirus spathiger* ( $\bar{x} \pm SD$ , n = 50, %)

Parameters of development	Time of exposure, min	Concentration of preparation		
		0.1%	0.25%	0.5%
Formation of L <sub>3</sub> in eggs	10	26.7 ± 1.5 <sup>c</sup>	8.0 ± 2.0 <sup>b</sup>	0.0 ± 0.0 <sup>a</sup>
	30	17.3 ± 3.1 <sup>c</sup>	3.3 ± 1.5 <sup>ab</sup>	0.0 ± 0.0 <sup>a</sup>
	60	13.0 ± 2.0 <sup>c</sup>	0.0 ± 0.0 <sup>ab</sup>	0.0 ± 0.0 <sup>a</sup>
Arrested development, egg death	10	23.3 ± 1.5 <sup>c</sup>	42.0 ± 2.0 <sup>b</sup>	50.0 ± 0.0 <sup>a</sup>
	30	32.7 ± 3.1 <sup>c</sup>	46.7 ± 1.5 <sup>ab</sup>	50.0 ± 0.0 <sup>a</sup>
	60	37.0 ± 2.0 <sup>c</sup>	50.0 ± 0.0 <sup>ab</sup>	50.0 ± 0.0 <sup>a</sup>
Ovicidal efficacy, %	10	39.9 ± 1.1 <sup>c</sup>	81.8 ± 5.2 <sup>b</sup>	100.0 ± 0.0 <sup>a</sup>
	30	60.8 ± 7.9 <sup>c</sup>	92.6 ± 3.1 <sup>ab</sup>	100.0 ± 0.0 <sup>a</sup>
	60	70.5 ± 5.6 <sup>c</sup>	100.0 ± 0.0 <sup>ab</sup>	100.0 ± 0.0 <sup>a</sup>

Note: see Table 1.



**Fig. 1.** Morphological changes in *Nematodirus spathiger* eggs under the influence of disinfectants: *a* – arrested development of the embryo and its loosening, *b* – thinning and destruction of the shell, wrinkling and death of the embryo; *c* – loosening and disintegration of the structural elements of the egg; *d* – death of the egg at the stage of formation of the L<sub>1</sub> larva

**Table 4**  
Larvicidal efficacy of "Hermecid-VS" disinfectant against the L<sub>3</sub> larvae of *Nematodirus spathiger* ( $\bar{x} \pm SD$ , n = 50, %)

Parameters of development	Time of exposure, min	Concentration of preparation		
		0.1%	0.25%	0.5%
Viable L <sub>3</sub>	10	33.3 ± 2.5 <sup>c</sup>	15.0 ± 2.0 <sup>b</sup>	2.3 ± 1.5 <sup>a</sup>
	30	27.0 ± 1.0 <sup>c</sup>	9.3 ± 1.5 <sup>b</sup>	0.0 ± 0.0 <sup>a</sup>
	60	21.3 ± 1.5 <sup>c</sup>	4.7 ± 1.2 <sup>b</sup>	0.0 ± 0.0 <sup>a</sup>
Dead L <sub>3</sub>	10	16.7 ± 2.5 <sup>c</sup>	35.0 ± 2.0 <sup>b</sup>	47.7 ± 1.5 <sup>a</sup>
	30	23.0 ± 1.0 <sup>c</sup>	40.7 ± 1.5 <sup>b</sup>	50.0 ± 0.0 <sup>a</sup>
	60	28.7 ± 1.5 <sup>c</sup>	45.3 ± 1.2 <sup>b</sup>	50.0 ± 0.0 <sup>a</sup>
Larvicidal efficacy, %	10	33.3 ± 5.0 <sup>c</sup>	70.0 ± 4.0 <sup>b</sup>	95.3 ± 3.1 <sup>a</sup>
	30	46.0 ± 2.0 <sup>c</sup>	81.3 ± 3.1 <sup>b</sup>	100.0 ± 0.0 <sup>a</sup>
	60	57.3 ± 3.1 <sup>c</sup>	90.7 ± 2.3 <sup>b</sup>	100.0 ± 0.0 <sup>a</sup>

Note: see Table 1.

High levels of larvicidal efficacy of "Hermecid-VS" were established when it was used at a 0.25% concentration for 60 min exposure (90.7%) and at a 0.5% concentration regardless of exposure (95.3–100.0%). Satisfactory levels of larvicidal efficacy of "Hermecid-VS" were found when it was used at a 0.25% concentration for 10 min exposure (70.0%) and 30 min (81.3%). An unsatisfactory level of larvicidal efficacy of the product was found when it was used at a 0.1% concentration regardless of exposure (33.3–57.3%).

The agent "Virosan" did not show 100% lethal effect on L<sub>3</sub> larvae of *N. spathiger* during the experiment. Its efficacy, although increasing with increasing concentration and exposure, was lower compared to the larvicidal action of "Hermecid-VS" (Table 5).

A high level of larvicidal efficacy of "Virosan" was established when it was used only at a 0.5% concentration for 60 min exposure (97.0%). Satisfactory levels of larvicidal efficacy were found when

the product was used at a 0.25% concentration for 60 min exposure (70.7%) and at a 0.5% concentration for 10 min exposure (78.0%) and 30 min (84.7%). Unsatisfactory levels of larvicidal efficacy of "Virosan" were found when it was used at a 0.1% concentration regardless of exposure (13.3–32.7%) and at a 0.25% concentration for 10 min exposure (42.0%) and 30 min (59.3%).

The larvicidal action of "Hermecid-VS" and "Virosan" was characterized by morphological changes in L<sub>3</sub> larvae of experimental test cultures in the form of loosening of the structure of the larval body and their gradual resorption (Fig. 2a, 2b).

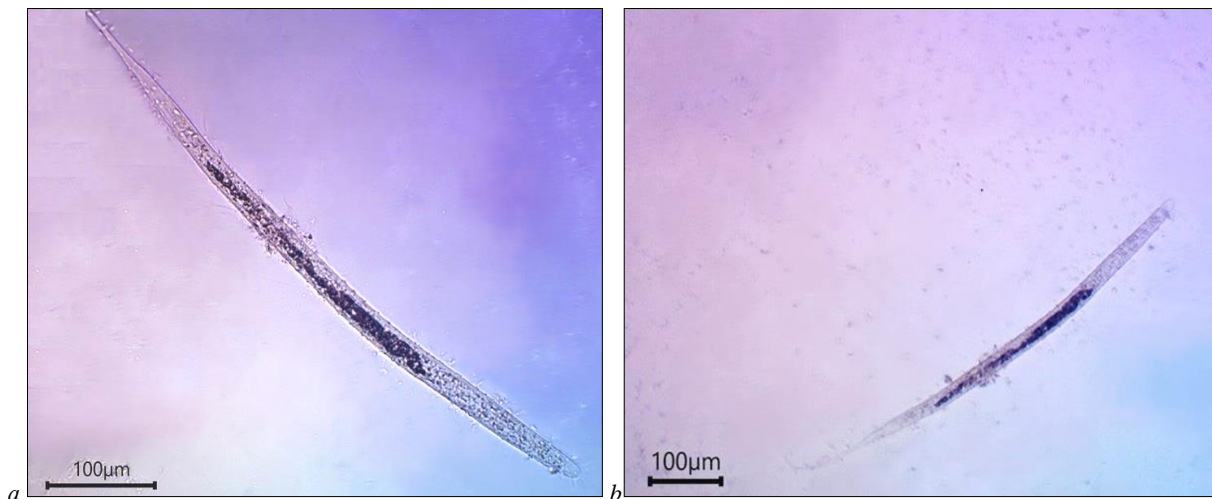
In the control test culture, only 5.7 ± 2.5% of *Nematodirus* eggs died during cultivation, and 44.3 ± 2.5% of eggs developed motile L<sub>3</sub> larvae (Fig. 3a). And in the control test culture of L<sub>3</sub> larvae, 100% of these larvae remained viable (Fig. 3b).

**Table 5**

Larvicidal efficacy of "Virosan" disinfectant against the L<sub>3</sub> larvae of *Nematodirus spathiger* ( $\bar{x} \pm SD$ , n = 50, %)

Parameters of development	Time of exposure, min	Concentration of preparation		
		0.1%	0.25%	0.5%
Viable L <sub>3</sub>	10	43.3 ± 3.1 <sup>c</sup>	29.0 ± 4.4 <sup>b</sup>	11.0 ± 2.7 <sup>a</sup>
	30	37.0 ± 2.7 <sup>c</sup>	20.3 ± 2.1 <sup>b</sup>	7.7 ± 2.5 <sup>a</sup>
	60	33.7 ± 4.0 <sup>c</sup>	14.7 ± 2.5 <sup>b</sup>	3.0 ± 2.0 <sup>a</sup>
Dead L <sub>3</sub>	10	6.7 ± 3.1 <sup>c</sup>	21.0 ± 4.4 <sup>b</sup>	39.0 ± 2.7 <sup>a</sup>
	30	13.0 ± 2.7 <sup>c</sup>	29.7 ± 2.1 <sup>b</sup>	42.3 ± 2.5 <sup>a</sup>
	60	16.3 ± 4.0 <sup>c</sup>	35.3 ± 2.5 <sup>b</sup>	47.0 ± 2.0 <sup>a</sup>
Larvicidal efficacy, %	10	13.3 ± 6.1 <sup>c</sup>	42.0 ± 8.7 <sup>b</sup>	78.0 ± 5.3 <sup>a</sup>
	30	26.0 ± 5.3 <sup>c</sup>	59.3 ± 4.2 <sup>b</sup>	84.7 ± 5.0 <sup>a</sup>
	60	32.7 ± 8.1 <sup>c</sup>	70.7 ± 5.0 <sup>b</sup>	97.0 ± 4.0 <sup>a</sup>

Note: see Table 1.



**Fig. 2.** Morphological changes in L<sub>3</sub> *Nematodirus spathiger* larvae under the influence of disinfectants: a, b – loosening of the structure and gradual resorption

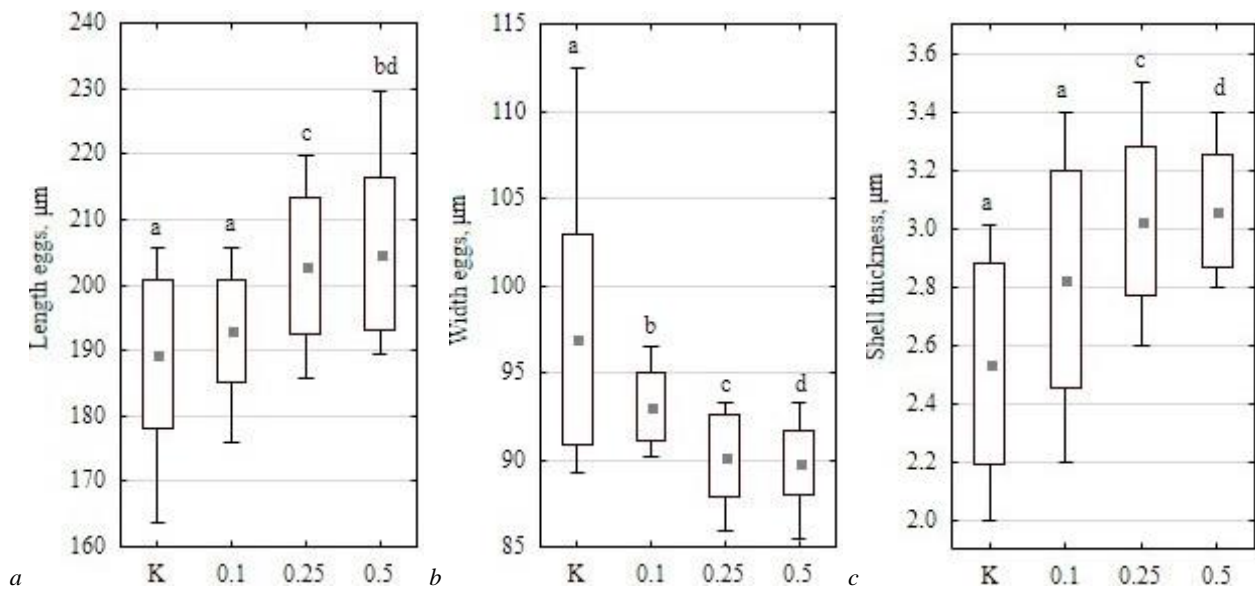


**Fig. 3.** Exogenous stages of development of the nematode *Nematodirus spathiger* in experimental test cultures: a – formation of L<sub>3</sub> larva in the egg; b – L<sub>3</sub> larva

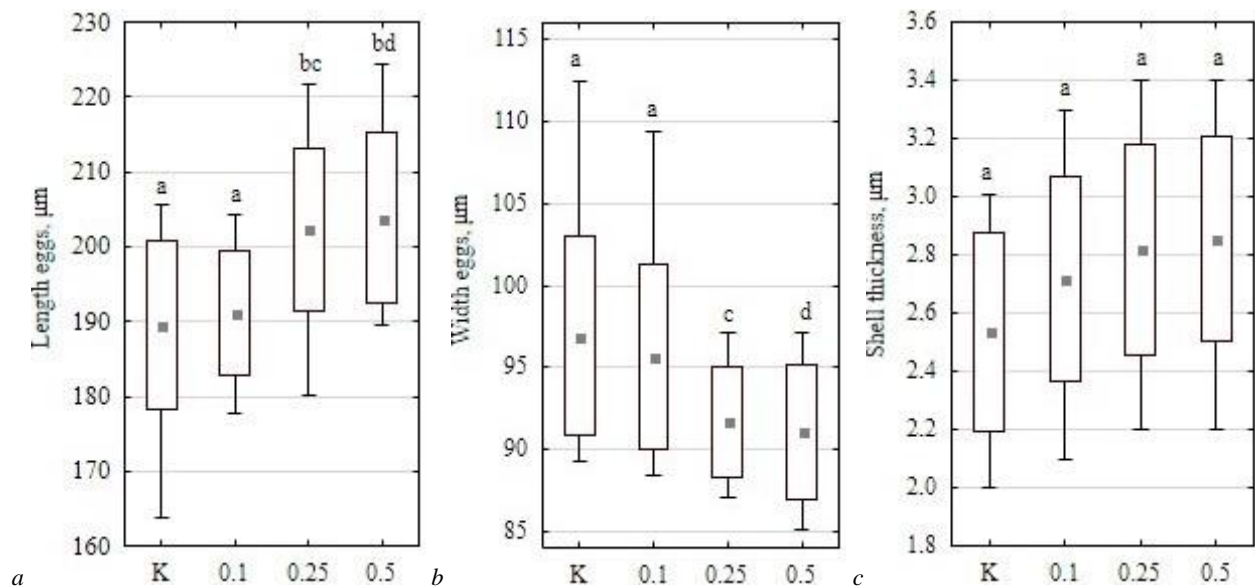
Along with morphological changes in *Nematodirus* eggs under the influence of disinfectants, the metric parameters of the eggs changed. In particular, when the "Hermecid-VS" agent was used at increasing concentrations, changes in the metric parameters of the eggs included increase in their length and shell thickness at 0.25% concentration by 7.1% and 20.0% (202.9 ± 10.3 and 3.0 ± 0.3 µm, P < 0.05), and at 0.5% concentration by 8.0% and 24.0% (204.7 ± 11.6 and 3.1 ± 0.2 µm, P < 0.05) compared to similar values in the control test culture (Fig. 4a, 4c). At the same time, egg width decreased at 0.1% concentration by 3.9% (93.1 ± 2.0 µm, P < 0.05), at 0.25% concentration by 6.9% (90.2 ± 2.3 µm, P < 0.05), and at 0.5% concentration by

7.2% (89.9 ± 1.8 µm, P < 0.05) compared to the control test culture (Fig. 4b).

Similar changes, only in the length and width of eggs, were observed in the metric indicators of *N. spathiger* eggs under the action of "Virosan". Under the action of the drug, an increase in the egg length and a decrease in the egg width were observed at 0.25% concentration by 6.8% and 5.4% (202.3 ± 10.8 and 91.7 ± 3.4 µm, P < 0.05), at 0.5% concentration by 7.6% and 5.9% (203.9 ± 11.4 and 91.1 ± 4.2 µm, P < 0.05) compared to similar values in the control test culture (Fig. 5a, 5b). No significant changes were found in the shell thickness (Fig. 5c).



**Fig. 4.** Metric parameters of *Nematodirus spathiger* eggs in test cultures under the effect of "Hermecid-VS" at various concentrations and 60 min of exposure: *a* – length of egg, *b* – width of egg, *c* – shell thickness ( $\mu\text{m}$ ); *K* – control; the small square in the center corresponds to the median, the lower and upper borders of the large rectangular correspond to the first and the third quartiles, respectively, vertical line segments, directed up and down from the rectangular, correspond to minimum and maximum values ( $n = 15$ ); different letters in the figure indicate significant ( $P < 0.05$ ) differences between groups according to the Tukey test results



**Fig. 5.** Metric parameters of *Nematodirus spathiger* eggs in test cultures under the effect of "Virosan" at various concentrations and 60 min of exposure: *a* – length of egg, *b* – width of egg, *c* – shell thickness ( $\mu\text{m}$ ); see Fig. 4

In the control test culture, the egg length decreased by 7.9% ( $189.5 \pm 11.2 \mu\text{m}$ ), the egg width increased by 8.1% ( $96.9 \pm 6.1 \mu\text{m}$ ), and the shell thickness decreased by 21.9% ( $2.5 \pm 0.3 \mu\text{m}$ ) during the cultivation process.

## Discussion

*Nematodirus* infection is highly prevalent among wild and domestic ruminants, its infestation rates can reach 100% (Oliver et al., 2014; Hyuga & Matsumoto, 2016; Jelinski et al., 2017). Such a significant spread of these pathogens is associated with their special development cycle: all three larval stages ( $L_1$ – $L_2$ – $L_3$ ) are formed in the external environment in eggs.  $L_3$  larvae that emerge from the eggs are sufficiently resistant to the effects of adverse factors. This ensures their significant survival in the environment and the possibility of infecting a significant number of susceptible animals (Boulenger, 1915; Dikmans & Andrews, 1933; Liu et al., 2022). Thus, the complex of measures for maintaining relevant epizootic situations on livestock

farms, as well as measures to combat this invasion, need to ensure the destruction of exogenous stages of nematode development using highly effective agents, the activity of which has been proven in experimental studies (Arnold Landry et al., 2021; Boyko & Brygadyrenko, 2023a; Liotta et al., 2024). Therefore, we determined the disinvasive efficacy of two modern disinfectants, "Hermecid-VS" (active ingredients: didecyldimethylammonium chloride, glutaraldehyde, benzalkonium chloride) and "Virosan" (contains benzalkonium chloride and glutaraldehyde) against eggs and invasive  $L_3$  larvae of nematodes of the species *N. spathiger*, which parasitize cattle.

The conducted studies have established that the disinfectants "Hermecid-VS" and "Virosan" have ovicidal and larvicidal properties against the propagative stages of development of the nematode *N. spathiger*. The efficacy indicators of the preparations depended on the modes of application of the agents. With increasing concentration and exposure, the efficacy of drugs increased. The agent most effective against eggs and  $L_3$  larvae was "Hermecid-VS". High levels of its ovicidal activity (90.9–100.0%) were established when used in con-

centrations 0.1% (60 min), 0.25% and 0.5% (10–60 min). Its larvicidal efficacy was somewhat lower, a high level (90.7–100.0%) of this indicator was established when “Hermecid-VS” was used in concentrations 0.25% (60 min) and 0.5% (10–60 min).

The disinfectant “Virosan” showed lower values of ovicidal and larvicidal efficacy against the nematode *N. spathiger*. High levels of its ovicidal efficacy (92.6–100.0%) were established when it was used in concentrations 0.25% (30–60 min) and 0.5% (10–60 min). Also, “Virosan” did not show a 100% destructive effect on L<sub>3</sub> larvae of *N. spathiger* during the experiment. Thus, a high level of its larvicidal efficacy (97.0%) was established only when it was used in a 0.5% concentration (60 min).

There is no data in the available scientific literature on the study of the ovicidal and larvicidal efficacy of chemical disinfectants which contain the active ingredients didecyldimethylammonium chloride, glutaraldehyde, benzalkonium chloride. However, the ovicidal activity of the chemicals deltrin, citrine and phenol has been described *in vitro*, and their efficacy, depending on the concentration and the test object, ranged from 60.0 to 100.0% (Nasibov, 2024).

Our studies also confirmed the detrimental effect of “Hermecid-VS” and “Virosan” on eggs and L<sub>3</sub> larvae of *N. spathiger* by morphological changes that occur in them, as well as through establishing the metric parameters of eggs during cultivation. We found that, in accordance with the ovicidal efficacy of the agents, corresponding changes occur in the length, width and thickness of the shell of *Nematodirus* eggs. Thus, in the control test culture, during the normal process of development, the length of eggs decreased (by 7.9%), the width increased (by 8.1%), and the thickness of shell decreased (by 21.9%). When the product “Hermecid-VS” was used, changes in the metric indicators of eggs were accompanied by an increase in their length (by 7.1–8.0%,  $P < 0.05$ ), shell thickness (20.0–24.0%,  $P < 0.05$ ) and a decrease in egg width (3.9–7.2%,  $P < 0.05$ ). When the drug “Virosan” was used, changes in the metric indicators of *Nematodirus* eggs were characterized by an increase in their length (by 6.8–7.6%,  $P < 0.05$ ) and a decrease in their width (5.4–5.9%,  $P < 0.05$ ). Changes in the metric and morphological parameters of helminth eggs under the influence of disinfectants, as confirmation of their harmful effect, have been evidenced by the works of scientists. Thus, chemical agents “Brovades-plus”, “Bi-dez” and “Dezsans” caused morphometric changes in the eggs of the nematode *Aonchothea bovis* (Melnychuk & Yuskiv, 2018). The drug “Arquadez-plus” caused similar changes in the eggs of the nematode *Trichuris skrjabinii*, which were isolated from sheep (Petrenko & Kharchenko, 2023).

Therefore, the obtained research results allow us to recommend the disinfectants “Hermecid-VS” and “Virosan” in certain regimens to increase the effectiveness of measures to combat and prevent *Nematodirus* infection in cattle.

## Conclusion

It was established that the disinfectants “Hermecid-VS” and “Virosan” have ovicidal and larvicidal effect on eggs and L<sub>3</sub> larvae of the nematode *Nematodirus spathiger*. Their disinvasive efficacy depends on the concentration of the agent and the application regimens. “Hermecid-VS” showed high ovicidal efficacy (90.9–100.0%) at concentrations of 0.1% (60 min), 0.25% and 0.5% (10–60 min), as well as larvicidal efficacy (90.7–100.0%) at concentrations of 0.25% (60 min), 0.5% (10–60 min). A satisfactory level of ovicidal efficacy of “Hermecid-VS” (79.6–85.7%) was established when it was used at a concentration of 0.1% (10–30 min), and that of larvicidal efficacy (70.0–81.3%) at a concentration of 0.25% (10–30 min). “Virosan” showed lower disinvasive activity against eggs and L<sub>3</sub> larvae of *N. spathiger*. Its high levels of ovicidal efficacy (92.6–100.0%) were established when it was used at a concentration of 0.25% (30–60 min), 0.5% (10–60 min), larvicidal efficacy (97.0%) at a concentration of 0.5% (60 min). Satisfactory levels of ovicidal efficacy of “Virosan” (60.8–70.5%) were established when it was used in concentrations of 0.1% (30–60 min), 0.25% (10 min), larvicidal efficacy (70.7–84.7%) – in concentrations of 0.25% (60 min), 0.5% (10–30 min). The detrimental effect of disinfectants was accompanied by changes in mor-

phological and metric indicators. With increasing concentration of the agent, the changes become more pronounced. When the agent “Hermecid-VS” was used on *Nematodirus* eggs, their length and thickness of the shell were greater by 7.1–8.0% and 20.0–24.0%, and the width was smaller by 3.9–7.2%. When the drug “Virosan”, was used changes in the metric indicators of *Nematodirus* eggs were characterized by higher values of their length by 6.8–7.6% and lower values of width by 5.4–5.9%.

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The authors state that there is no conflict of interest.

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