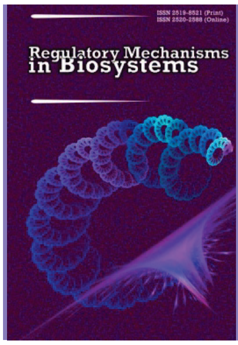

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(4), 559–563
doi: 10.15421/022381

The influence of chemicals on the development and viability of *Trichuris vulpis* nematode eggs isolated from infested dogs

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Article info

Received 15.09.2023
Received in revised form 28.10.2023
Accepted 07.11.2023

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Yevstafieva, V., Dolhin, O., Melnychuk, V., Dedukhno, A., Pishchalenko, M., & Krasota, O. (2023). The influence of chemicals on the development and viability of Trichuris vulpis nematode eggs isolated from infested dogs. Regulatory Mechanisms in Biosystems, 14(4), 559–563. doi:10.15421/022381

Preventive measures against invasive diseases should be carried out taking into account the peculiarities of the life cycle of helminths. This is especially relevant for geohelmintiases, where the infection is mainly transmitted via environmental objects contaminated with the pathogen at the exogenous stages of the parasite's development. It has been proven that among the causative agents of nematodoses of the gastrointestinal tract of animals, the eggs of geohelminths of the genus *Trichuris* are the most resistant to the action of disinfectants. The aim of the research was to establish in laboratory conditions the ovicidal effect of chemicals on the development and viability of *Trichuris vulpis* nematode eggs isolated from infested dogs. Two disinfectants were tested *in vitro*, Arquades-plus (dialkyl dimethyl ammonium chloride, dodecyl dimethyl ammonium chloride, tetrasodium salt) and Brovadez-plus (alkyl dimethyl benzyl ammonium chloride, dodecyl dimethyl ammonium chloride). Arquades-plus disinfectant showed a high level of ovicidal efficiency (94.0%) only at the maximum concentration and exposure (2.0%, 60 min), and 4.7% of nematode eggs in the test culture remained viable. Brovadez-plus disinfectant was less effective and did not provide a high level of ovicidal efficiency against *Trichuris* eggs in the studied concentrations and exposures. Satisfactory and unsatisfactory levels of its ovicidal effect (63.1–77.1% and 2.5–58.9%) were established when using the product in concentrations of 2.0% and 0.5–1.5%, respectively, for exposures of 10–60 min. The disinfestation activity of the preparations was characterized by metric changes in experimental test cultures compared to the control test culture. Under the influence of Arquades-plus at all concentrations, the length of the eggs was greater (by 0.4–2.0%), and the width was smaller (by 0.7–4.9%). Under the influence of Brovadez-plus in concentrations of 1.0–2.0%, the length of the eggs was greater (by 0.5–0.9%), the width was smaller (by 0.3–4.1%). The results of the conducted studies allow us to recommend the Arquades-plus disinfectant in a 2.0% concentration (exposure of 60 minutes) for effective control and prevention of infection of dogs with the causative agent of trichurosis.

Keywords: trichurosis; dogs; exogenous development; disinfection; environment; ovocidal activity.

Introduction

At the current stage of society's development, protecting the environment from various types of pollution is the most important task of mankind. Special attention is drawn to the problem of biological pollution of the environment, one of the forms of which is parasitic. This is due to the high resistance of exogenous forms of helminth pathogens (eggs and larvae) to the influence of external environmental factors, as a result of which they persist, contaminating objects of the external environment, and, subsequently, can infect animals and humans. This feature of causative agents of helmintiases is an important link of their epizootic process (Maikai et al., 2008; Bojar & Kłapeć, 2012; Simonato et al., 2019; Cociancic et al., 2020). This is especially true for geohelmintiasis pathogens, in which case dogs are an important reservoir of intestinal nematodes, and are potential sources of infestation for both humans and other animals. Thus, in Poland, eggs of geohelminths *Ascaris* spp. (95.0%), *Toxocara* spp. (37.5–96.0%), *Trichuris* spp. (37.5–60.0%) (Bojar & Kłapeć, 2018; Zdybel et al., 2019; Kowalczyk & Kłapeć, 2020) were found. In water samples from rivers in South Africa, eggs of *Ascaris* spp., *Toxocara* spp., *Trichuris* spp. and *Taenia* spp. were found (Amoah et al., 2020). In certain regions of Japan, 107 sandboxes in public parks were examined showing their contamination with eggs of *Toxocara* spp. and *Capillaria* spp. (Matsuo & Nakashio, 2005).

Therefore, the successful elimination of helminthic diseases, as well as their further prevention, is achievable only with a complex of organizational and economic, veterinary and sanitary and special anti-parasitic measures, which must necessarily include the disinfestation of environmental objects with the use of effective, modern and safe means (Burg & Borgsteede, 1987; Massara et al., 2003; Oh et al., 2016). At the same time, there are currently no special disinfectants that would act only on parasite eggs or larvae. Therefore, it is recommended to carry out research on determining the disinfestation properties of this or that disinfectant or other means in order to effectively use these in the fight against and for prevention of helminth infections (Morrondo et al., 2006; Shalaby et al., 2011; Melnychuk & Yuskiv, 2018; Boyko et al., 2020; Boyko & Brygadyrenko, 2022).

The most resistant of all eggs of helminths to abiotic environmental factors are the eggs of roundworms. Eggs of the nematode *Ascaris suum* can survive in the soil and retain their invasive properties for 7 to 15 years. Hence, scientists determined the disinfestation effectiveness of chemical agents on test cultures of *Ascaris* nematode eggs (Thitasut, 1961; Krishnaswami & Post, 1968; Labare et al., 2013). However, some authors prove that over time the effectiveness of these chemical agents has decreased and embryonic stages of helminths that are more resistant to negative effects appear (Yuskiv & Melnychuk, 2015; Khorolskyi, 2022). Therefore, in recent years, a number of studies have been conducted to determine the disinfectant properties of preparations against individual helminthiasis in relation to the embryonic stages of its development. In particular, nine disinfectants and chemicals were tested on *Heterakis gallinarum* eggs, among which only NaOCl had pronounced disinfectant properties (Cupo & Beckstead, 2019). When studying the effect of 6 commercial disinfectants on larval development in *Toxascaris leonina* eggs, scientists found that only Dettol® and Virkon® caused 100% larval death ($P < 0.05$) (El-Dakhly et al., 2018). Also, the influence of the chlorocresol-based disinfectant Neopredisan®135-1 (NP) on the embryonic development of *T. canis* eggs *in vitro* at concentrations of 0.25%, 0.50%, 1%, 2% and 4% was determined, where the authors demonstrated a significant ovicidal effect regardless of exposure (30, 60, 90 or 120 min). The ovicidal activity increased depending on the concentration and exposure, with a maximum efficiency of 95.8% at 4% concentration and 120 min of exposure (Zhang et al., 2020).

Recently, scientists have noted the high viability and resistance to negative effects of eggs of *Trichuris* nematodes. Thus, the viability of *T. skrjabini* eggs in laboratory conditions, depending on the temperature, ranged from 75.3% to 80.3%, and that of *T. globulosa* eggs from 68.0% to 76.3% (Yevstafieva et al., 2020, 2023). Also, the authors showed that the eggs of *T. suis* released by animals into the external environment are more adapted to the conditions of the external environment (survival was 96.6 ± 0.3%) than eggs isolated from the gonads of female nematodes (survival was 89.3 ± 0.3%) (Yevstafieva et al., 2016). Therefore, it is relevant to conduct research on the disinfestation effect of modern disinfectants in relation to the embryonic stages of development of nematodes of the genus *Trichuris*.

The aim of the work was to establish in laboratory conditions the ovicidal effect of chemicals on the development and viability of *Trichuris vulpis* nematode eggs isolated from infested dogs.

Materials and methods

The work was carried out in the Laboratory of Parasitology and Veterinary and Sanitary Examination of the Poltava State Agrarian University (Ukraine) in 2023. The research was carried out within the framework of the initiative topic of scientific work "Monitoring, implementation of improved methods of diagnosis, treatment and prevention of invasive animal diseases" (state registration No. 0121U100644).

In order to determine the ovicidal efficiency of Arquades-plus and Brovadez-plus disinfectants, test cultures of non-invasive nematode eggs of *Trichuris vulpis* isolated from the feces of infested dogs were used.

Arquades-plus contains the active ingredient dialkyl dimethyl ammonium chloride (10%), didecyl dimethyl ammonium chloride (5%), tetrasodium salt (2.5%). Brovadez-plus contains the following active substances: alkyl dimethyl benzyl ammonium chloride (10%), didecyl dimethyl ammonium chloride (5%).

In laboratory conditions, Petri dishes were prepared with a mixture of *T. vulpis* eggs (at least 100 specimens), which were obtained from infested dogs according to Melnychuk (2018). Disinfectants in concentrations of 0.5%, 1.0%, 1.5%, 2.0% were added to each dish. After exposure (10, 30, or 60 min), test cultures of *T. vulpis* eggs were washed in distilled water and transferred to separate Petri dishes. A control test culture of *T. vulpis* eggs was also prepared, in which distilled water was added instead of disinfectants. After that, the experimental and control dishes were placed in a thermostat at a temperature of 27 °C and cultivated for 20 days until the appearance of motile larvae in the eggs of *Trichuris*. They were aerated and moistened every three days. The test for each disinfectant was repeated three times. Ovicidal efficiency (OE, %) was determined on the 20th day according to the method Volkov & Simonov (1977). The morphometric parameters of *T. vulpis* eggs (egg length and width, n = 15) during cultivation were studied using ImageJ for Windows® software (version 2.00) in interactive mode. Microphotography was carried out using a Sigeta M3CMOS 14000 14.0 MP digital camera (China).

Statistical processing of the experimental results was carried out using Statistica 10 (StatSoft Inc., USA) software. Standard deviation (SD) and average values (x) were calculated. Significance of difference between average values was established using one-way analysis of variance and F-test for 95% confidence level.

Results

At a concentration of 2.0% for 60 minutes of exposure, Arquades-plus showed a high level of ovicidal efficiency against *T. vulpis* eggs (94.0%). Under these regimes, 4.7% remained viable (Table 1).

Table 1
Ovicidal activity of Arquades-plus
on the test culture of *Trichuris vulpis* eggs (x ± SD, %)

Time of exposure, min	Parameters	Concentration of disinfectant			
		0.5%	1.0%	1.5%	2.0%
10	Formation of motile larvae	73.7 ± 3.1	54.3 ± 4.9	36.3 ± 2.9	20.3 ± 1.2
	Arrested development, death of eggs	26.3 ± 3.1	45.7 ± 4.9	63.7 ± 2.9	79.7 ± 1.2
	OE, %	3.0	30.6	53.6	74.0
30	Formation of motile larvae	67.3 ± 2.1	49.0 ± 4.6	29.7 ± 1.5	11.3 ± 3.5
	Arrested development, death of eggs	32.7 ± 2.1	51.0 ± 4.6	70.3 ± 1.5	88.7 ± 3.5
	OE, %	14.0	37.4	62.1	85.5
60	Formation of motile larvae	61.0 ± 3.6	43.0 ± 5.6	26.0 ± 1.0	4.7 ± 3.1
	Arrested development, death of eggs	39.0 ± 3.6	57.0 ± 5.6	74.0 ± 1.0	95.3 ± 3.1
	OE, %	22.1	45.1	66.8	94.0

A satisfactory level of ovicidal efficiency was established when using the product at 2.0% concentration for exposures of 10 min (74.0%) and 30 min (85.5%), as well as at 1.5% concentration for exposures of 30 min (62.1%) and 60 min (66.8%). Also, Arquades-plus at a concentration of 1.5% for exposure of 10 min and at a concentration of 0.5–1.0% for exposure of 10–60 min showed an unsatisfactory level of ovicidal efficiency (OE from 3.0% to 53.6%) against *T. vulpis* eggs, resulting in 36.3% to 73.7% of viable eggs.

Brovadez-plus was less effective than Arquades-plus, as it did not provide a high level of ovicidal efficiency against *Trichuris* eggs in the studied concentrations and exposures (Table 2).

Table 2
Ovicidal activity of Brovadez -plus
on the test culture of *Trichuris vulpis* eggs (x ± SD, %)

Time of exposure, min	Parameters	Concentration of disinfectant			
		0.5%	1.0%	1.5%	2.0%
10	Formation of motile larvae	76.7 ± 1.5	60.3 ± 1.5	43.7 ± 6.5	29.0 ± 4.0
	Arrested development, death of eggs	23.3 ± 1.5	39.7 ± 1.5	56.3 ± 6.5	71.0 ± 4.0
	OE, %	2.5	23.3	44.5	63.1
30	Formation of motile larvae	71.3 ± 1.5	56.7 ± 2.1	40.3 ± 6.1	25.3 ± 3.8
	Arrested development, death of eggs	28.7 ± 1.5	43.3 ± 2.1	59.7 ± 6.1	74.7 ± 3.8
	OE, %	9.3	28.0	48.7	67.8
60	Formation of motile larvae	67.7 ± 3.5	47.3 ± 7.0	32.3 ± 6.1	18.0 ± 2.0
	Arrested development, death of eggs	35.3 ± 3.5	52.7 ± 7.0	67.7 ± 6.1	82.0 ± 2.0
	OE, %	17.8	39.8	58.9	77.1

Thus, Brovadez-plus at a concentration of 2.0% for exposures of 10–60 min showed a satisfactory level of ovicidal efficiency, ranged from 63.1% to 77.1%. The unsatisfactory level of ovicidal activity of this disinfectant against *T. vulpis* eggs is evidenced by the results of its test in 0.5–1.5% concentrations during exposures of 10–60 min. Then, OE ranged from 2.5% to 58.9%.

When studying the morphological characteristics of eggs exposed to disinfectants, it was found that using Brovadez-plus was linked to the harmful effect manifested by the destruction of the embryo, as a result of its gradual disintegration and resorption (Fig. 1a). When using Arquades-plus, the harmful effect was manifested by the destruction of egg plugs, as a result of which embryos were released beyond their limits (Fig. 1b). In the control test culture, on the 20th day of cultivation, 81.0 ± 1.2% of eggs with a mobile larva inside were formed (Fig. 1c).

During the experiment, changes in the metric parameters of *T. vulpis* eggs were determined. Thus, in the control test culture, the egg length decreased by 2.7% (79.3 ± 1.8 μm, $P < 0.05$) and the egg width increased by 5.4% on the 20 day of cultivation (38.8 ± 1.0 μm, $P < 0.001$). Using the disinfestant Arquades-plus was associated with changes observed on the 20th day of cultivation, which were characterized by a slight increase in egg length at a concentration of 0.5% by 0.4% (79.6 ± 2.6 μm), at 1.0% by 0.8% (79.9 ± 2.0 μm), and at 1.5% by 1.5% (80.5 ± 2.1 μm) compared to the parameters of the control test culture. A significant increase in the egg length was established under the action of the agent in a 2.0% concentration, by 2% (80.9 ± 2.2 μm, P < 0.0025, Fig. 2).

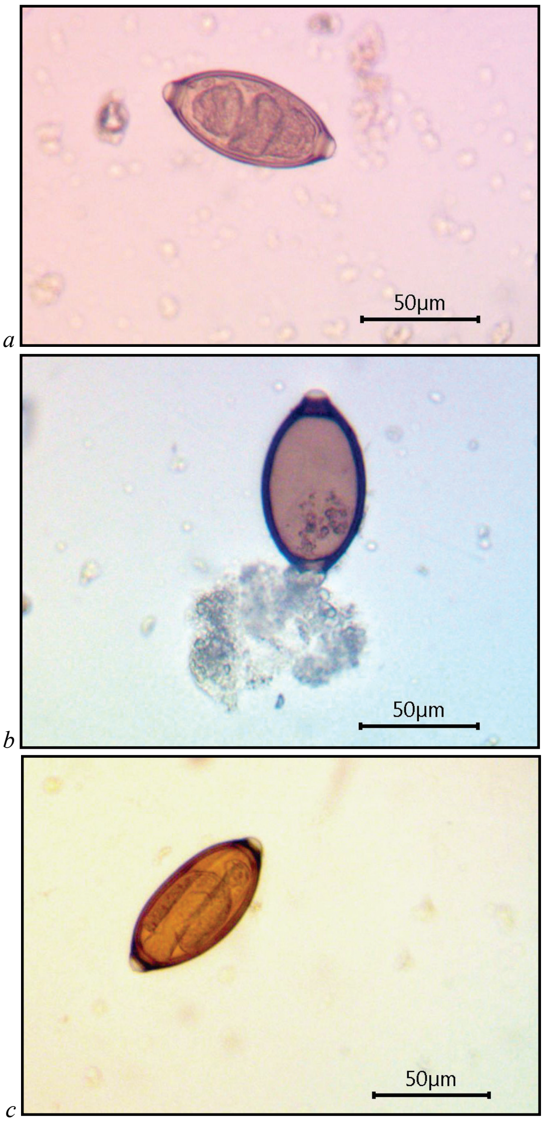

Fig. 1. Morphological changes in *Trichuris vulpis* eggs on the 20th day of cultivation: *a* – destruction and disintegration of the embryo under the action of the Brovadez-plus disinfectant; *b* – the destruction of the egg plug and the emittance of the remaining embryo outside its boundaries under the action of the Arquades-plus disinfectant; *c* – formation of motile larvae in eggs in a control test culture

The width of the eggs under the influence of Arquades-plus turned out to be smaller by only 0.7% (38.5 ± 1.1 μm) when using a 0.5% concentration of the disinfectant. When using the agent in concentrations of 1.0–2.0%, a significant decrease in the width parameter by 3.4–4.9% (37.5–36.9 μm, P < 0.00025) was observed compared to the control test culture.

When using the disinfectant Brovadez-plus, on the 20th day of cultivation, a slight decrease by 0.1% (79.2 ± 2.3 μm) was observed in the egg length of eggs at a concentration of 0.5% compared to the indicators of the control test culture. In concentrations of 1.0–2.0%, a slight increase in the egg length was established by 0.5–0.9% (79.7–80.0 μm, Fig. 3).

The width of eggs under the influence of 0.5% Brovadez-plus solution was smaller by only 0.3% (38.7 ± 1.0 μm). When using the agent in concentrations of 1.0–2.0%, a significant decrease in the width parameter by 2.6–4.1% (37.8–37.2 μm, P < 0.00025–0.0025) was observed compared to the control test culture. Such changes indicate a negative effect of disinfectants on *Trichuris* eggs, which is consistent with their ovicidal efficiency.

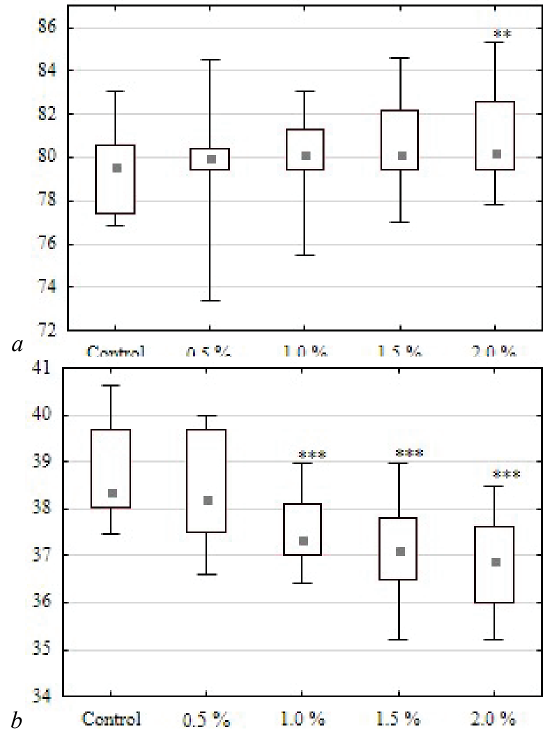

Fig. 2. Metric parameters of *Trichuris vulpis* eggs in test cultures when using Arquades-plus disinfectant in different concentrations for 60 min exposure: *a* – length of eggs, *b* – width of eggs (μm); the small square in the centre corresponds to the median, the lower and upper borders of the large 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 = 20); ** – P < 0.0025; *** – P < 0.00025 – compared to eggs indexes in control test-culture (with Bonferroni correction)

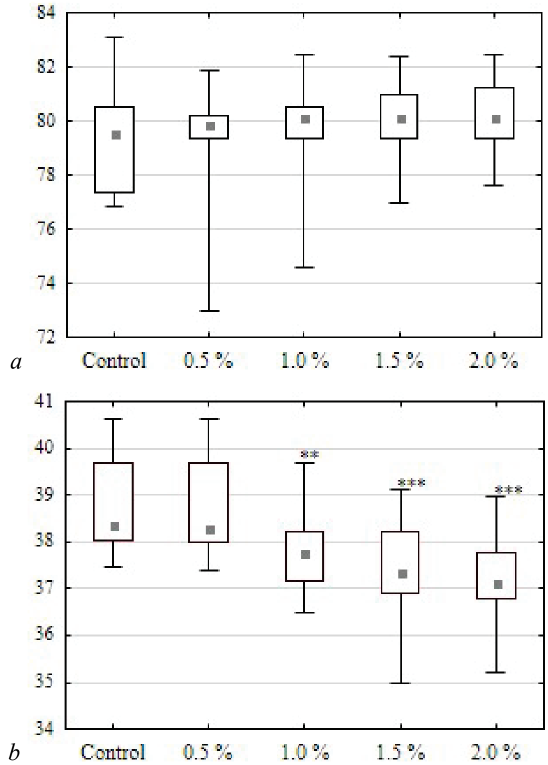

Fig. 3. Metric parameters of *Trichuris vulpis* eggs in test cultures using Brovadez-plus disinfectant in different concentrations for 60 min exposure: *a* – length of eggs, *b* – width of eggs (μm); see Fig. 2

Discussion

It is known that the prevalence of an infectious disease among humans and animals is influenced by its ability to persist at various stages of development in environmental objects, and resistance to adverse factors (Boyko & Brygadyrenko, 2017a, 2021). This is especially evident in geohelminthiases. The causative agent matures in the egg stage in the external environment, acquiring invasive properties (Simonato et al., 2019; Cociancic et al., 2020; Mohaghegh et al., 2021). Hence, environmental objects are factors of transmission and accumulation of these pathogens (Otero et al., 2018; Kowalczyk & Kłapeć, 2020). Therefore, a comprehensive fight against nematodes is impossible without the use of disinfestation in the system of control and prevention measures. The most effective and convenient are chemical means of treatment (Massara et al., 2003; Oh et al., 2016; Hernández et al., 2018; Boyko & Brygadyrenko, 2017b, 2019). When determining the ovicidal properties of this or that drug, using test cultures of nematode eggs of the genus *Ascaris* was suggested (Thitasut, 1961; Krishnaswami & Post, 1968; Labare et al., 2013). However, there are studies that indicate higher resistance of nematodes of the genus *Trichuris*. It is more appropriate to test the disinfestation properties of chemicals on test cultures of the causative agents of these geohelminthiases (Yuskiv & Melnychuk, 2015; Yevstafieva et al., 2023). Thus, we compared the ovicidal efficiency of two chlorine-containing disinfectants, Arquades-plus (a mixture of dialkyl dimethyl ammonium chloride, dodecyl dimethyl ammonium chloride and tetrasodium salt) and Brovadez-plus (a mixture of alkyl dimethyl benzyl ammonium chloride and dodecyl dimethyl ammonium chloride).

According to the instructions, Arquades-plus and Brovadez-plus have a bactericidal and sporicidal effect on most gram-positive and gram-negative bacteria; viricidal against RNA- and DNA-containing viruses; antiprotozoal on *Eimeria*; fungicidal for fungi; algicidal on green algae; also act as deodorants.

We found that Arquades-plus in a concentration of 2.0% after exposure for 60 minutes showed a high level of ovicidal efficiency against *T. vulpis* eggs, 94.0%. Then, 95.3% of *Trichuris* eggs died. As the concentrations and exposures decreased, the efficiency gradually fell. OE was 74.0–85.5% at 2.0% concentration for exposures of 10–30 min; 53.6–66.8% at 1.5% concentration during exposures of 10–60 min; 30.6–45.1% in 1.0% concentration during exposures of 10–60 min; and 3.0–22.1% in a concentration of 0.5% for exposures of 10–60 min. Brovadez-plus was less effective and in the tested concentrations and exposures did not provide a high level of ovicidal efficiency against *Trichuris* eggs. Thus, OE of Brovadez-plus was 63.1–77.1% at 2.0% concentration for exposures of 10–60 min; 44.5–58.9% at 1.5% concentration for exposures of 10–60 min; 23.3–39.8% at 1.0% concentration during exposures of 10–60 min; and 2.5–17.8% in a concentration of 0.5% for exposures of 10–60 min. Previously, five surface disinfectants and four temperature regimes have been tested on *T. vulpis* eggs (Kines et al., 2021). In the study, exposure to 10% povidone-iodine and 95% ethanol for ≥ 5 min inactivated 100% of these eggs. Freezing at ≤ –20°C for ≥ 24 h also inactivated *Trichuris* eggs. Other authors, when testing the disinfestation activity of Arquades-plus on *T. skrjabini* nematode eggs, found that at 1.5% concentration and exposure for 60 minutes and 2.0% concentration and exposure for 10–60 minutes, the product had a high level of ovicidal efficiency of 94.2% and 98.3–100.0%, respectively (Petrenko & Kharchenko, 2023). Also, the chlorine-containing disinfectant Dezsan has been highly efficient against the non-invasive and invasive egg test cultures of *T. ovis* (90.6–100.0%), *T. skrjabini* (91.0 – 100.0%), and *T. globulosa* (90.9–100.0%) (Melnychuk et al., 2022).

Morphological features in nematode eggs that were exposed to disinfectants manifested as the destruction of the embryo through gradual disintegration and absorption when using Brovadez-plus, and as the destruction of egg plugs, as a result of which the embryos were released beyond their limits when using Arquades-plus. These changes were accompanied by metric changes in the eggs of the test cultures. Thus, under the influence of Arquades-plus at all concentrations, the egg length was greater (by 0.4–2.0%), and the width was smaller (by 0.7–4.9%) compared to the control test culture ($P < 0.00025–0.0025$). Under the influence of Brovadez-plus in concentrations of 1.0–2.0%, the egg length was greater (by 0.5–0.9%), and the width was smaller (by 0.3–4.1%) compared to the control test culture ($P < 0.00025–0.0025$) (Petrenko & Kharchenko, 2023). Similar data were obtained by the authors, who note a decrease in the parameters of the length of *T. skrjabini* eggs by 3.8–3.9% and an increase in the parameters of the width of the eggs by 5.0–5.2% under the influence of Arquades-plus, which, in their opinion, confirms the violation of the development of nematode eggs.

Conclusion

When establishing the disinfestation properties of two chemical agents on *Trichuris vulpis* eggs isolated from the feces of infested dogs, it was found that the mixture of dialkyl dimethyl ammonium chloride, didecyl dimethyl ammonium chloride and tetrasodium salt (Arquades-plus) was more effective than the mixture of alkyl dimethyl benzyl ammonium chloride and didecyl dimethyl ammonium chloride (Brovadez-plus). Arquades-plus showed a high level of ovicidal efficiency, 94.0% at 2.0% concentration and exposure for 60 minutes, as a result of which 95.3% of *Trichuris* eggs died. Brovadez-plus was insufficiently effective: at 2.0% concentration and exposures of 10–60 min, it showed a satisfactory level of ovicidal efficiency (63.1–77.1%), and at 0.5–1.5% concentrations with exposures of 10–60 min, the level of ovicidal efficiency was unsatisfactory (2.5–58.9%). The harmful effect of the agents was confirmed in metric studies of *T. vulpis* eggs. At the most effective exposures and concentrations of the tested disinfectants on the 20th day of the experiment, egg length increased (by 0.9–2.0%) and egg width decreased (by 4.1–4.9%) compared to similar indicators in the control test culture.

The authors state that there is no conflict of interest.

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