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Effect of Disinfectants on Exogenous Stages of the Panther Chameleon (Furcifer Pardalis) Parasites

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Abstract: Disinfection of terrariums in which reptiles are kept is quite problematic. Most terrariums are made of glass and therefore do not always withstand the effects of high temperatures. At the same time, the use of disinfectants is important for breaking the epizootic chain of parasites, because their exogenous stages of development are quite resistant to the action of disinfectants. In this regard, the effect of disinfectants at various concentrations and exposures on helminth eggs and larvae and oocysts of the panther chameleon oysters were investigated. The test egg culture of Trematoda gen spp., Spinicauda freitasi, Hexametra angusticaecoides, Pharyngodonidae gen. spp., Thubunaea spp., larvae of Rhabdiasidae gen. sp. and oocyst Eimeria sp. were used for the study. The effectiveness of the disinfectants Virocid, Biodez-R, Neochlor, Brovadez 20 and hot caustic soda (80–90 ºC) were determined. It has been investigated that exogenous forms of parasites are resistant to conventional concentrations and exposures of disinfectants. Therefore, for a sufficient level of disinvasion, 4-5 % concentration of disinfectants with 24-48 hours exposure should be used. The most resistant to disinfectants are Trematoda gen eggs. spp. and oocysts of Eimeria sp. At the same time, they are not resistant to disinfectants of the larvae of Rhabdiasidae. gen. spp.

Keywords: disinfectants, panther chameleon, parasites, efficiency

I. INTRODUCTION

Parasitic diseases cause considerable damage to reptiles and their owners. They significantly complicate the syndrome of disadaptation undergoing reptiles for transportation and relocation. It should be noted that during disadaptation, death among reptiles can reach 60 %, while in reptile groups held in terrariums - up to 40 % per year [5].

For effective control of reptile parasites it is proposed to carry out a complex of veterinary and sanitary measures. First and foremost step is deworming of a reptiles and prevention of a parasitic diseases in them. One of such measures is disinfection. Many researchers have been interested in the issues of disinfection. However, few of them raised the issue of disinfection of terrariums [2, 3, 7].

At present, a wide range of disinfection methods are available in veterinary medicine for animals and the environment. These methods are divided into physical, chemical and biological [11, 13].

It should be noted that Losev L. O. (1934) recommended for disinfection the use of boiling water, which treated the premises for 2–3 minutes [8]. Bubnov V. D. (1963) recommended at one time to use soldering lamps or other devices that produce a large amount of heat for disinfection. However, such treatments cannot be used in terrariums. It should be noted that most modern terrariums are made of glass. Therefore, when treated with boiling water or at high temperatures, the glass of the terrariums can be easily damaged [4].

The use of chemical disinfectants is considered to be the most convenient method of disinfection. However, exogenous stages of parasite development are quite resistant to disinfectants. A number of researchers have long proved that ascarid eggs are resistant to 3 % creolin emulsion, 2 % sodium hydroxide solution, many organophosphorus compounds, iodine compounds, saturated saline solutions, and inorganic acids [1, 3, 7]. A 2 % solution of sulfur carbon, formalin and its derivatives, aldehydes, have a weak effect on ascarid eggs, 0.6–0.8 % emulsion of sulfanol, 4 % of alkaline solutions and ammonia. At the same time, organochlorine compounds, hydrogen sulfide, carbamates and phenols have a strong ovocidal effect [10].

II. MATERIAL AND METHODS

Studies were conducted in the laboratory of the Department of Parasitology and Tropical Veterinary Medicine Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine during 2016–2018 years. Fecal samples from the panther chameleons (Furcifer pardalis Cuvier, 1829) were used. The animals were kept at the Bion Nature Center (Kiev).

Fecal samples were collected with tweezers, which were washed and disinfected in 70 % alcohol after each sample was taken. The faeces were placed in a disposable plastic bag, signed and logged in to register primary studies. The test material was transported to the study site in a cold bag with a temperature of 4–9 °C. The studies were carried out on the day of fecal sampling and not later than three hours after their selection [9].

A test culture of the trematode Trematoda gen spp., nematode *Spinicauda freitasi* Olfers, 1919, *Hexametra angusticaecoides* Chabaud & Brygoo, 1960, Pharyngodonidae gen. sp., *Thubunaea* sp., larvae of nematodes Rhabdiasidae. gen. sp. and oocyst *Eimeria* sp. eggs was prepared for the study.

To create a test culture, feces of panther chameleons (F. pardalis) were examined by native smear for the presence and sufficient number of exogenous stages of endoparasites development. Then the sample of faeces was placed in a centrifuge tube, 5 cm^3 of water was added, centrifuged for 2 min at 800 rpm, the supernatant was drained, water was added and centrifuged again. Washed 4–5 times until transparent fluid became transparent. After the last centrifugation, the supernatant was drained, one precipitate was taken and examined under a microscope (magnification x 100). Under the microscope, the number of exogenous stages of development of endoparasites was determined and 300±15 specimens were placed per hour glasses (d=50 mm). Edges of clock glasses with test culture of *Thubunaea* sp. and Rhabdiasidae. gen. sp. lubricated with petroleum jelly to prevent the larvae from spreading. The test culture was aerated daily for one hour; water was changed, kept in a thermostat at 27±0.5 °C [6].

Experimental solutions of disinfectants of different concentrations (from 1 to 5 %) were poured into the test culture and kept at appropriate exposures (from 1 to 48 hours). At the end of the exposure, the test culture was washed 5 times with dichlorinated water and examined under a microscope. Then investigated whether there were signs of egg death (shell deformation, vacuole formation, uneven crushing layers, displacement of the germ mass to one of the poles, cessation of larvae movement, etc.). The test culture was the control to which distilled water was added [12].

To determine the effectiveness of disinfectants against exogenous stages of parasite development, test cultures were stained with the Maretsky O. J. (1954) method, using methylene blue (0.05 g), lactic acid (15 ml) and caustic soda (0.5 g). After 15–30 min, colored test cultures were examined under a microscope. It should be noted that live eggs of helminths were not stained, and dead - acquired blue color [12]. Ovocidal effectiveness of solutions of disinfectants was determined by the formula 1.

$$OE=100\ \% - (Y_1/Y_2)*100 \qquad (1)$$

where Y_1, Y_2 are the number of live helminth eggs in the test and control cultures [13].

The assessment of disinvasive effectiveness was determined by the indicators: high level of efficiency – 90–100 %, satisfactory – 60–90 %, unsatisfactory - up to 60 %.

The effectiveness of the disinfectants Virocid, Biodez-R, Neochlor, Brovadez 20 and hot caustic soda (80–90 ºC) were tested on the eggs and larvae of helminths and eimeria oocysts were tested.

III. RESULT

The effectiveness of disinfectants on eggs Trematoda gen. spp. at different concentrations and exposures are presented in table 1.

Table 1. Effect of disinfectants on eggs Trematoda gen. spp.

Concen-tration, %	Exposure, hours	Virocide, %	Biodez-R, %	Neochlor, %	Brovadez 20, %	Caustic soda, %	Control, %
1	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
2	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
3	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	8,06	0	0	0
	24	0	0	18,39	0	0	0

	48	0	28,02	36,68	7,61	0	0
4	1	0	0	0	0	0	0
	6	0	0	16,69	0	0	0
	12	0	24,26	32,13	0	0	0
	24	0	36,89	72,49	10,36	0	0
	48	0	94,39	96,69	33,66	40,92	0
5	1	0	0	6,21	0	0	0
	6	0	6,71	21,73	14,38	0	0
	12	0	23,05	75,93	34,58	21,96	0
	24	0	77,33	96	93,93	94	0
	48	0	96,67	100	100	100	0

The obtained data show that disinfectants have a poor effect on trematode eggs. Only with high concentration and exposure time of 1–2 days a sufficient level of disinfection can be obtained. The best manifestation had the Neochlor, which showed a high level of disinfection at a concentration of 5 % at exposures of 24 and 48 hours. Also, Biodez-R, Brovadez 20 and hot caustic soda at 5 % concentration at 48 hours exposure show high levels of disinfection.

The results of the effectiveness of disinfectants on *S. freitasi* eggs are shown in table 2.

Table 2. Effect of disinfectants on nematode *S. freitasi* eggs

Concen-tration, %	Exposure, hours	Virocide, %	Biodez-R, %	Neochlor, %	Brovadez 20, %	Caustic soda, %	Control, %
1	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
2	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	4,22	0	0	0
	48	0	0	14,98	0	0	0
3	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	7,33	0	0	0
	48	0	0	26,8	0	41,92	0
4	1	0	0	0	0	0	0
	6	0	0	16,72	0	7,21	0
	12	0	23,62	53,99	0	26,21	0
	24	0	69,1	92,03	19,43	61,46	0
	48	0	90,26	95,78	45,78	91,56	0
5	1	0	0	10,46	0	0	0
	6	0	22,62	35,74	0	17,05	0
	12	0	46,91	85,99	0	74,59	0
	24	0	83,12	96,1	67,86	91,88	0
	48	0	100	100	94,48	100	0

Eggs of *S. freitasi* nematode are less resistant to disinfectants. Neochlor disinfectants and hot caustic soda at the concentration of 5 % and exposures of 24 and 48 hours showed the best ressult. Biodez-R and Brovadez 20 are highly effective at 5 % concentration over 48 hours of exposure.

The effect of disinfectants on eggs of *H. angusticaecoides* ascaris at different concentrations and exposures is presented in table 3.

Table 3. Effect of disinfectants on the eggs of the nematode *H. angusticaecoides*

Concen-tration, %	Exposure, hours	Virocide, %	Biodez-R, %	Neochlor, %	Brovadez 20, %	Caustic soda, %	Control, %

1	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
2	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
3	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	1,31	0	2,29	0
	24	0	0	7,41	0	21,21	0
	48	0	17,79	31,88	0	50,34	0
4	1	0	0	0	0	0	0
	6	0	6,51	5,54	0	19,87	0
	12	0	25,16	29,08	12,09	51,96	0
	24	0	40,67	86	39	83	0
	48	0	94,67	100	74,67	100	0
5	1	0	0	0	0	0	0
	6	0	18	14,33	0	10,67	0
	12	0	45,51	50,17	14,29	78,07	0
	24	0	80,66	89,51	51,8	91,48	0
	48	0	100	100	100	100	0

The best disinfectant for *H. angusticaecoides* nematode eggs was hot caustic soda at 5 % concentration and exposures for 24 and 48 hours. Also, 100 % efficacy was noted in the use of Biodez-R, Neochlor and Brovadez 20 disinfectants at 5 % concentration and 48-hour exposure.

Effectiveness of disinfectants on eggs of nematodes Pharyngodonidae gen. sp. are shown in table 4.

Table 4. Effect of disinfectants on the eggs of the nematode Pharyngodonidae gen. sp.

Concen-tration, %	Exposure, hours	Virocide, %	Biodez-R, %	Neochlor, %	Brovadez 20, %	Caustic soda, %	Control, %
1	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
2	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
3	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	5,76	0	0	0
	48	0	0	34,33	8,67	23	0
4	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	45,45	40,91	0	0
	24	0	55,84	62,99	72,4	53,57	0
	48	0	94,48	100	90,58	92,86	0
5	1	0	0	4,19	0	0	0
	6	0	6,05	18,15	24,84	0	0
	12	0	54,15	73,09	64,12	53,88	0

	24	0	75,08	89,64	96,76	79,61	0
	48	0	95,48	100	100	91,29	0

Oxyurate eggs are less resistant to disinfectants than ascarid eggs. Therefore, to destroy the eggs of the nematode Pharyngodonidae gen. sp. a sufficient 4 % concentration of Neochlor, Brovadez 20 and hot caustic soda disinfectants at 48 hours exposure. Biodez-R is effective at 5 % concentration for 48 hours.

It should be noted that the eggs of the nematodes *Thubunaea* sp. are interesting for the study of disinfectants because their shell is thin and the larva is inside. The data obtained are shown in table 5.

Table 5. Effect of disinfectants on nematode eggs *Thubunaea* sp.

Concen-tration, %	Exposure, hours	Virocide, %	Biodez-R, %	Neochlor, %	Brovadez 20, %	Caustic soda, %	Control, %
1	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
2	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	2,67	15,33	0	0	0
	24	7,17	19,22	28,01	42,35	0	0
	48	12,82	46,47	56,09	54,49	24,68	0
3	1	0	0	0	0	0	0
	6	0	9,29	0	0	0	0
	12	0	17	4	27	0	0
	24	15,53	55,02	42,39	52,43	37,22	0
	48	37,38	72,13	83,28	65,25	74,09	0
4	1	0	0	0	0	0	0
	6	0	14,77	30,2	0	8,72	0
	12	29,97	52,12	81,43	31,92	79,48	0
	24	81,97	84,26	92,79	91,15	89,51	0
	48	100	100	100	100	100	0
5	1	0	16,23	19,81	12,01	0	0
	6	0	56,49	65,26	58,12	30,52	0
	12	64,94	89,61	91,23	90,26	90,64	0
	24	89,29	100	100	100	100	0
	48	100	100	100	100	100	0

It was noted that the eggs of *Thubunaea* sp. not resistant to disinfectants at 4 % concentration and 48 hours exposure. Also the disinfectants "Neochlor" and "Brovadez 20" at 4 % concentration and 24-hour exposure showed a rather high level of efficiency. At the same time, Biodez-R, Neochlor, Brovadez 20 and hot caustic soda disinfectants showed satisfactory efficacy levels even at 3 % concentration and 48-hour exposure. It should also be noted that with the addition of disinfectants in mature helminth eggs, the larvae began to move actively and emerged from the shell and died gradually.

In addition to eggs of the ascarid, oxyurate and strongilide, we have studied the effectiveness of disinfectants at the larval stages of parasites. For this purpose, the test culture of larvae of nematodes Rhabdiasidae gen. sp. was made from the faeces of the panther chameleons (F. pardalis). The results of the study are shown in table 6.

Table 6. Effect of disinfectants on the larvae of nematodes Rhabdiasidae. gen. sp.

Concen-tration, %	Exposure, hours	Virocide, %	Biodez-R, %	Neochlor, %	Brovadez 20, %	Caustic soda, %	Control, %
1	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	30,56	51,04	40,28	36,46	0
2	1	0	0	0	0	0	0

	6	0	0	0	0	0	0
	12	0	8,58	0	0	0	0
	24	26,6	46,79	33,33	23,39	5,45	0
	48	42,26	59,03	62,26	58,06	56,13	0
3	1	0	0	0	0	0	0
	6	0	0	0	8,25	0	0
	12	0	13,27	6,46	44,22	28,91	0
	24	35,88	51,83	38,54	72,43	77,74	0
	48	69,74	88,49	91,12	94,08	91,45	0
4	1	0	0	0	0	0	0
	6	45,09	0	87,58	76,47	0	0
	12	84,62	91,99	93,27	98,39	94,87	0
	24	100	100	100	100	100	0
	48	100	100	100	100	100	0
5	1	75	91,33	95	93,93	86	0
	6	95,67	100	100	100	100	0
	12	100	100	100	100	100	0
	24	100	100	100	100	100	0
	48	100	100	100	100	100	0

According to the research larvae Rhabdiasidae gen. sp. were not resistant to disinfectants. So at 4 % concentration and exposures of 24 and 48 hours all experimental disinfectants showed 100 % efficiency. In addition, disinfectants Neochlor, Brovadez 20 and hot caustic soda had high levels of efficiency even at 3 % concentration at 48-hour exposure.

Also the effectiveness of disinfectants was determined on oocysts of Eimeria sp. The results obtained are shown in table 7.

Table 7. Effect of disinfectants on oocysts of *Eimeria* sp.

Concen-tration, %	Exposure, hours	Virocide, %	Biodez-R, %	Neochlor, %	Brovadez 20, %	Caustic soda, %	Control, %
1	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
2	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
3	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
	48	0	0	0	0	50,17	0
4	1	0	0	0	0	0	0
	6	0	0	0	0	0	0
	12	0	0	5,52	7,14	10,71	0
	24	0	0	47,12	17,31	87,18	0
	48	0	88,92	89,9	91,53	89,57	0
5	1	0	0	0	0	0	0
	6	0	0	8,41	0	0	0
	12	0	0	30,62	10,09	20,19	0
	24	0	66,88	76,95	31,82	86,69	0
	48	0	87,33	90,33	98,67	96	0

According to research, eimeria oocysts have proven to be quite resistant to disinfectants. Only the Neochlor, Brovadez 20 disinfectants and hot caustic soda at 5 % concentrations and 48-hour exposures showed a high level of efficiency. It should also be noted that only Brovadez 20 disinfectant showed a high level of efficiency at 4 % concentration and 48-hour exposure.

Therefore, exogenous stages of development of endoparasites are resistant to disinfectants of different chemical groups. The most resistant are the oocysts of *Eimeria* sp. and eggs Trematoda gen. spp. The larval forms of nematodes are most easily destroyed.

IV. CONCLUSION

Research has shown that exogenous stages of parasite development are resistant to disinfectants. Helminth eggs and eimeria oocysts withstand a 3 % concentration of disinfectants. Only high concentrations (4-5 %) of disinfectants over long exposures (24-48 hours) have a sufficient level of disinvasion. The most effective disinfectants were Neochlor and hot caustic soda, less effective – Biodez-R and Brovadez 20. Virocyde disinfectant at various concentrations and exposures has a detrimental effect on the larvae of nematodes Rhabdiasidae gen. sp. but does not affect helminth eggs and eimeria oocysts at all. The most resistant to the action of disinfectants were eimeria oocysts, and the most sensitive are the nematode larvae.

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