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Action of selected disinfectants on Toxocara canis eggs

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Summary

The goal of the work was to monitor the occurrence of helmintologic disorders of dogs in the district of Košice-surroundings. Moreover, the authors wished to evaluate the potential devitalising effect of the following selected chemical substances and disinfective agents: NaOH (5%, 70°C), Savo (10%), Saniten (10%), H1(100%) on eggs of T. canis under laboratory conditions. Using coprologic examination the authors realized that the most common eggs in dog feces are of T. canis (41.4%). Then follow eggs of Trichuris sp. (21.8%), T. leonina (11.5%), eggs from the Ancylostomatidae group (9.2%) and Capillaria sp. (2.3%). Under laboratory conditions, the ovocide effectiveness of NaOH on nonembryonated eggs of T. canis by an exposition of 180 minutes was 23.98 ± 4.33%. The disinfective agent SAVO also had low effectiveness; by an exposition of 180 minutes it devitalized 24.77 ± 5.33% of eggs of T. canis. A higher effectiveness was evidenced by the disinfective agent Saniten. Using the disinfective agent H1, 98.04 ± 1.77% eggs of T. canis were devitalized. Based on the testing of each agent under laboratory conditions the authors can recommend the usage of the disinfective agent Hviezda, as it evidenced the best devitalizing effect on the eggs of T. canis.

Keywords : ovocide, disinfective agents, T. canis

The rearing of companion animals, particularly dogs, is popular and wide-spread. Approximately 500 000 dogs are kept in Slovakia at the present. The total number of newborn puppies per year has been assessed at 90 000.

The numbers presented indicate a close contact between people and dogs. Obviously, such close coexistence of humans and dogs raises some risks. There exist a range of ecological and health problems that may affect people (8, 20). Attention should be particularly focused on ascaris of the genus *Toxocara*, which can induce a very unpleasant disease in humans – larval toxocarosis. High infestation of dogs and cats with *Toxocara* and the close contact of these animals with humans and their environment is a prerequisite of their frequent occurrence also in humans. The disease occurs in humans in two forms: *larva migrans visceralis*, a generalised disease affecting various organs, and *larva migrans ocularis*, an ocular form. Frequent occurrence of *Larva migrans visceralis* has been observed particularly in pre-school children. This is understandable as these children frequently play in playgrounds with sand and loose soil, the favourite defecation places for cats and dogs. This environment is very suitable for maturation of the eggs of parasites and their survival for several months, even years. The access of infected dogs and cats to children's playgrounds and poor hygiene habits of children increase the probability of their infections. The ocular form is more common in older children and adults (3, 9, 10).

Disinfection of the external environment could considerably decrease such risk but the effectiveness of the presently used disinfectants against parasites is insufficient and in the case of carnivore ascaris is also inadequately studied.

With regards to the above mentioned questions, this study focused on the testing of the ovocidal effect of selected chemicals, disinfectants and preparations on non-embryonated eggs of *T. canis* under laboratory conditions.

Material and methods

Eggs of the helminth *Toxocara canis* obtained from various locations in Slovak Republic. The following disinfectants were evaluated in the study: sodium hydroxide (96% NaOH); Savo – a chlorine preparation containing 35-45% active chlorine; Saniten – a preparation based on quaternary ammonium compounds; H1 – contains hydrogen peroxide, tenzides and sodium hydroxide (the agens in development).

Isolation and purification of helminth eggs was carried out by a modified method according to Anon. (1).

Determination of the vitality of exposed, non-embryonated eggs of *Toxocara canis*. The vitality of non-embryonated eggs of *Toxocara canis* was determined by their incubation up to the invasive stage in a thermostat at 26°C for 21 days (13). The developmental ability of eggs was compared to the control group maintained in distilled water under aerobic-anaerobic conditions.

The ovocidal effect of selected disinfectants (Savo 10%, Saniten 10%, H 1 concentrated and NaOH 5% heated to 70°C) on non-embryonated eggs of *T. canis* was tested under laboratory conditions by a suspension test, using 60, 90 and 180 min of exposure time. The effectiveness (ovocidal effect) of the preparations tested was evaluated by comparing the number of devitalised non-embryonated eggs of *T. canis* (%) in experimental groups after their exposure to the solutions tested with the number of devitalised non-embryonated *T. canis* eggs in the control group.

Evaluation of devitalisation effects of selected disinfectants and preparations by suspension test. One millilitre aliquot of parasite egg culture was transferred by a pipette into a centrifugation tube. After the sedimentation of eggs on the bottom of the tube the supernatant was aspirated and the sediment was exposed to the tested disinfectants. Eggs with the tested solution were mixed by shaking. After respective exposure times the supernatant was aspirated and the sedimented eggs were washed several times with distilled water. Then the tubes were incubated at 26°C for 21 days and the non-developed eggs were counted under a microscope.

Statistical evaluation. Results of the experiments are presented as mean values ± standard deviation (SD). The significance of differences between experimental and control samples were evaluated by Anova and Dunnett Multiple Comparison test at significance levels $p < 0.05$ and $p < 0.01$ (Statistica 6.0, StatSoft Inc., USA).

Results and discussion

Altogether 87 samples of dog feces from the Košice district surroundings (Slovac Republic) were examined coprologically for the presence of helminths. Of these samples 53 (60.9%) showed the presence of helminth eggs. The most frequent were eggs of *T. canis* followed by eggs of *T. leonina*, *Trichuris* sp., *Capillaria* sp. and eggs of helminths of the *Ancylostomatidae* family (*Ancylostoma* sp. and *Uncinaria stenocephala*) – tab. 1.

Tab. 1. Coprological examination of dogs from the district Košice--surroundings

Species	Prevalence (%)
T. canis	41.4
T. leonine	11.5
Trichuris sp.	21.8
Capillaria sp.	2.3
Ancylostomatidae	9.2

In the control samples, evaluated in parallel with the experimental disinfectant-exposed samples, the proportion of devitalised *T. canis* eggs reached 11.25 ± 2.69% on average.

Tab. 2. Ovocidal effect of disinfectants studied on non embryonated eggs of *T. canis*

Exposure time (min.)	Devitalised eggs ($\bar{x}$ % ± SD)			
	NaOH 5% (70°C)	Savo 10%	Saniten 10%	H1 100%
60	11.21 ± 1.24***	9.93 ± 2.27**	56.32 ± 6.83**	94.18 ± 0.48***
90	11.49 ± 5.30***	9.42 ± 1.28**	63.07 ± 7.70**	97.96 ± 1.93***
180	23.98 ± 4.33***	24.77 ± 5.33**	56.01 ± 8.05**	98.04 ± 1.77***
Control	11.25 ± 2.69	11.25 ± 2.69	11.25 ± 2.69	11.25 ± 2.69

Explanations: * – difference in proportion of devitalised eggs of *T. canis* was significant at the level $p < 0.05$; ** – difference in proportion of devitalised eggs of *T. canis* was significant at the level $p < 0.0$; *** – difference in proportion of devitalised eggs of *T. canis* was significant at the level $p < 0.001$

pH of the tested 5% NaOH solution at a temperature of 70°C was 9.52. The developmental abilities of non-embryonated eggs of *T. canis* exposed to the tested disinfectants (exposure time 60, 90 and 180 min) were not affected significantly. The number of devitalised eggs of *T. canis* increased with increasing time of exposure to the respective disinfectant (tab. 2). The lowest number of devitalised eggs of *T. canis* was recorded after 60 min exposure (11.21 ± 1.24%) and the highest after 180 min exposure (23.98 ± 4.33%).

Testing of the disinfectant preparation Savo provided similar results, i.e. the absence of a marked ovocidal effect of this preparation on non-embryonated eggs of *T. canis.* In this preparation intended for surface disinfection, the active chlorine is bound to alkaline chemical substances which is reflected in the pH (11.73). After 60 min exposure, 9.93 ± 2.27% of non-embryonated *T. canis* eggs were devitalised. An increase in exposure time only negligibly increased the number of devitalised eggs. The highest number of devitalised eggs was recorded after 180 min exposure to this disinfectant (tab. 2). Tests with disinfectants Savo and NaOH showed no significant differences in the number of devitalised non-embryonated eggs of *T. canis* in experimental groups in comparison with the control group when using the specified exposure times.

More pronounced devitalisation effect on eggs of *T. canis* in comparison with NaOH and disinfectant preparation Savo was observed when using the disinfectant preparations Saniten and H1 (fig. 1).

Already 60 min exposure of eggs to the disinfectant Saniten (tab. 2) on average resulted in the devitalisation of 56.32 ± 6.83% of non-embryonated eggs of *T. canis.* An increase in exposure time to 90 min caused that the mean number of devitalised eggs of *T. canis* increased to 63.07 ± 7.70%. After 180 min exposure, 56.01 ± 8.05% of *T. canis* eggs were devitalised. The tested solution was acidic (3.66). When comparing the experimental and control groups, the difference in the number of devitalised non-embryonated eggs of *T. canis* was significant at the level of $p < 0.01$ at all exposure times.

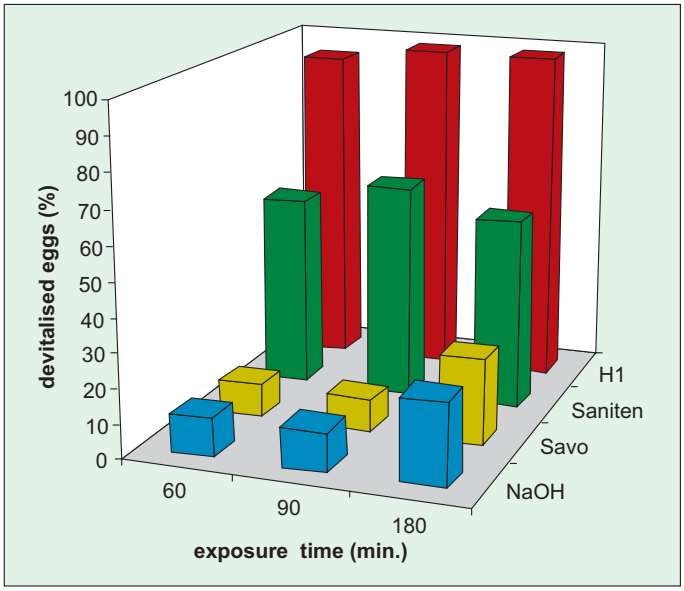

Fig. 1. Comparison of devitalisation effect of the tested disinfectants on non-embryonated eggs of *T. canis* at various exposure times

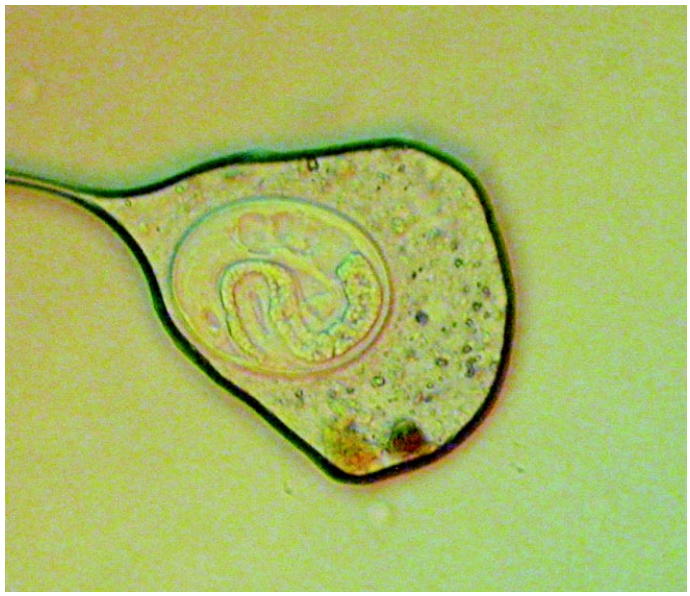

Fig. 2. Devitalised egg of *T. canis* after 180 min exposure to disinfectant preparation exposure to NaOH (magn. 200 ×)

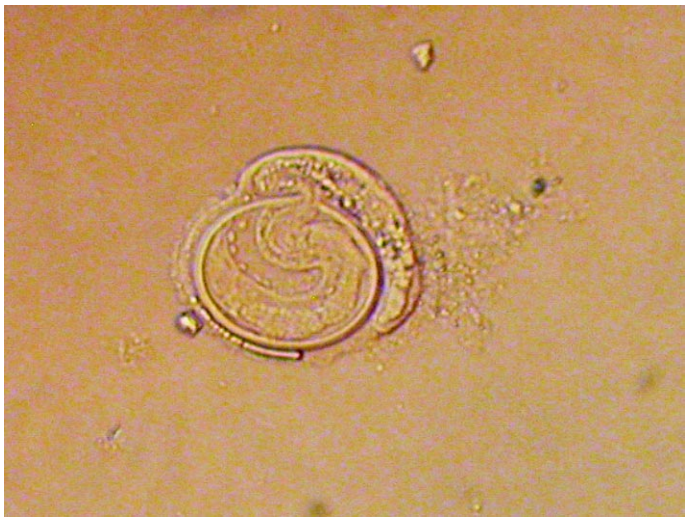

Fig. 3. Devitalised egg of *T. canis* with partially developed larva after 180 min Savo (magn. 200 ×)

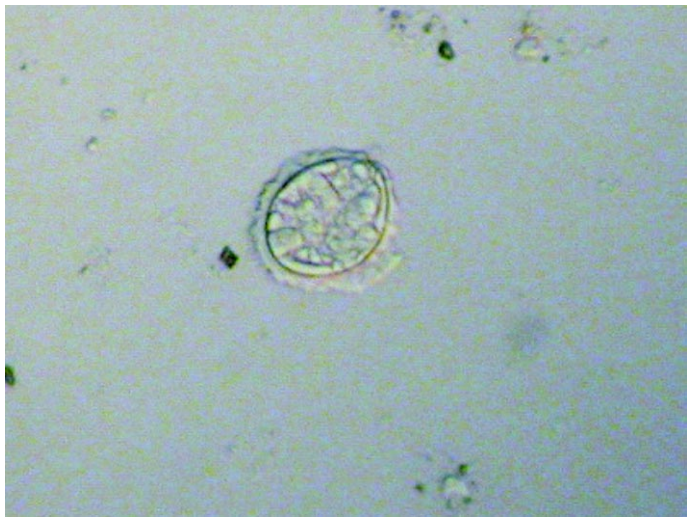

Fig. 4. Devitalised egg of *T. canis* with considerably non-developed larva after 90 min exposure to desinfectant preparation Saniten (magn. 200 ×)

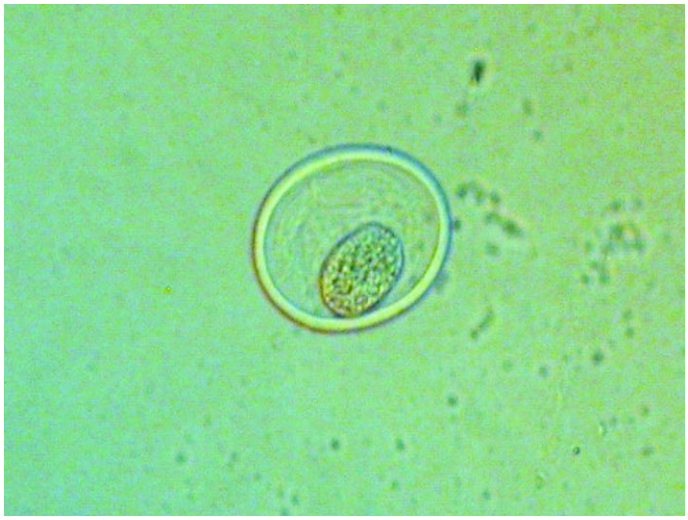

Fig. 5. Devitalised egg of *T. canis* with reduced germinal mass after 60 min exposure to disinfectant preparation H1 (magn. 200 ×)

The most pronounced ovocidal effect was observed when using the disinfectant H1. The exposure times tested caused the devitalisation of more than 90% of non-embryonated eggs of *T. canis* (tab. 2) pH of the tested disinfectant was 10.02. The eggs exposed to the disinfectant preparation H1 showed obvious morphological changes. They were translucent and had a markedly reduced germinal mass. This mass was manifested mostly as a small dot at the internal margin of the egg. When compared with the control group, significant differences in the number of devitalised non-embryonated eggs of *T. canis* were observed at the level of $p < 0.001$ at predetermined exposures to preparation H1.

Helminth zoonoses are diseases caused by parasitic helminths of macroscopic dimensions. They infest humans and animals and show variable degrees of pathogenicity. Helminths are well adapted to parasitise

the host using various means of penetration. They spread all over the world (4, 11). The most frequent canine helminths are nematodes *T. canis*, as it was proved also by our coprological examinations. In comparison with Halanová et al. (12), who reported 15.4% prevalence of *T. canis* in dogs in Košice over the years 1996-1998, our coprological examination indicated a higher prevalence (41.4%) of *T. canis* in dogs from the district of Košice. Antolová et al. (2) and Reiterová et al. (19) reported in their studies that more than 50% of dogs younger than 8 months were infected with *T. canis*.

According to the surveillance of zoonoses in the Slovak Republic in 2003, toxocarosis was diagnosed in as many as 110 out of 908 examined dogs (12.1%) and in 240 of 311 examined cats (77.1%) (22). A similar situation was observed in other European towns. For example, Habluezelt et al. (10) reported that the prevalence of *T. cati* in cats in the Bristol area was as high as 63%. However, these data fail to draw an objective picture of the real infestation of animals with helminths because only a small number of owners brings their pets for examination. Therefore the real prevalence of toxocarosis in dogs and cats is probably considerably higher.

Because of this we had to focus our attention on the principal source of infection: the feces of dogs and cats. Almost 70% of dog feces are deposited in green areas close to human dwellings. Considering the assumption that each dog produces on average 0.25 kg feces daily, a considerable quantity of these wastes accumulate on grasslands. A bad or even critical situation exists in large towns and cities were the quantities of dog and cat feces become excessive. The collection of dog feces from public places is common in the EU member countries. Three quarters of this waste in Germany is removed by dog owners in a safe way. In a number of Czech towns the collection of dog feces has become a routine issue of town administration care about the cleanness and tidiness of public places.

One of the most serious problems is the high resistance of infectious stages of helminths (eggs, oocysts) in the environment. Especially eggs of toxocara – an endoparasite of dogs and cats – are among the helminth eggs most resistant to external environmental factors. Their cellular wall is composed of an outer layer formed by acidic polysaccharide and proteins; the middle layer consisting of chitin-protein complex; and the internal (viteline) layer is composed of proteins (25%) and lipids (75%, particularly alpha-glycosides). This thick and resistant cellular wall protects the eggs against the action of routinely available disinfectants. Eggs of helminths are found in the outer environment mostly in sand or soil to which they pass with feces, because sand and soil are also materials which dogs and cats prefer for the elimination of their wastes. In this environment helminth eggs may remain infectious for months and, under optimum conditions even for 3-5 years.

One of the population risk groups are children in which toxocarosis shows an increasing trend. The highest prevalence of toxocarosis (in the form of *larvae migrans visceralis*) has been observed in children from 18 months to 3-4 years, associated with physiologically increased hand-mouth activity. These children tend to put sand, grass, soil, stones, feces, plaster and similar matter in their mouth. The danger of infection also increases in children affected by geophagy – i.e. ingesting sand and soil (7, 14).

Environmental hygiene is one of important preventive measures related to the elimination of the adverse influence of parasitic nematodes on human health. Disinfection is performed regularly in relation to infectious diseases, but little or no attention is paid to disinfection as far as parasitic diseases are concerned.

In order to resolve the problems related to the sanitation of locations contaminated by dog excrements, the present study investigated, under laboratory conditions, the effects of NaOH and disinfectant preparations Savo, Saniten and H1 on non-embryonated eggs of *T. canis*, the causative agents of the zoonotic disease toxocarosis.

The results obtained allow the authors to state that of the four laboratory-tested disinfectants and chemicals the highest devitalisation effect on non-embryonated eggs of *T. canis* was observed after their exposure to the disinfectant H1. Already 60 min exposure to this preparation was sufficient to devitalise more than 90% of the exposed eggs.

When testing the other disinfectants a relatively high disinfectant effect was also exhibited by the disinfectant Saniten, based on quaternary ammonium salts. However, the ovocidal effects of Saniten were lower in comparison with H1. After 180 min exposure of *T. canis* eggs to this disinfectant, $56.01 \pm 8.05\%$ of the eggs were devitalised. The fact that helminth eggs are susceptible to ammonia-containing preparations was reported also by 15, 17, 18.

When using the disinfectant Savo, based especially on active chlorine but also with NaOH, one of the chemicals used for disinfection, no marked ovocidal effect on helminth eggs was observed similar to the results reported by 16.

Our results agree with those obtained by Burg and Borgsteede (5) who tested the devitalisation effect of 11 disinfectants based on active chlorine, phenol, cresol, NaOH and KOH, quaternary ammonium salts, glutaraldehyde and formaldehyde on non-embryonated and embryonated eggs of *A. suum* and on eggs and infectious larvae of *Cooperia oncophora* and *Ostertagia ostertagi*. The authors mentioned observed that none of the preparations tested was effective on non--embryonated and embryonated eggs of *A. suum* and *O. ostertagi*. The disinfectants based on phenol were effective on eggs of *C. oncophora*. Infectious larvae of *C. oncophora* and *O. ostertagi* were effectively devitalised by NaOH.

According to a number of authors, the vitality of enteronematode eggs is markedly affected by preparations based on phenol and cresol. Cotteleer and Fameree (6) tested the ovocidal activity of cresol derivatives Creolin and Tecrezol on *A. suum* eggs. The preparations mentioned devitalised the eggs after 8 to 11 days when used in concentrations 10^{-2} and in 20 days in concentrations 10^{-3}.

Of the tested disinfectants and chemicals (Savo, Saniten, H1 and sodium hydroxide) the disinfectant preparation H1 appears promising for the devitalisation of propagative stages of endoparasites in concentrations that have no apparent negative effect on the environment. As this is a novel preparation, it is still necessary to monitor its potential impact on the environment and on the basis of the respective tests prepare recommendations for the practical nature of its use.

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