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Efficacy of Some Disinfectants on Embryonated Eggs of *Toxocara canis*

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Abstract: The aim of this study was to evaluate the efficacy of various disinfectants in different concentrations against embryonated *Toxocara canis* eggs. For this purpose, 2.5%, 5%, 7.5%, and 10% iodine, 2% glutaraldehyde, 10% benzalkonium chloride, 7% sodium hypochloride, 1% potassium permanganate, 70% ethyl alcohol, 10% potassium hydroxide and 3% phenol solutions were used as the disinfectants. This study was performed both in vitro and in-vivo. In the in vitro experiment, *T. canis* eggs were treated with disinfectant solutions in different time intervals, and larval motility was observed under X400 magnification with an inverted microscope. Microscopic examinations revealed that *T. canis* eggs treated in 2.5%, 5%, 7.5%, and 10% iodine solutions were completely non-motile at 120, 60, 40, and 40 minutes post-treatment respectively, whereas the eggs treated in the all other disinfectants were still motile after 24 hours. In the in-vivo study, 1000 embryonated eggs treated with disinfectants were inoculated each mouse via the oral route, and their brain tissues were examined for larval presence on the 7th day post-inoculation. In addition, a control group was set up for comparison with the study groups. No *T. canis* larvae were observed in mice inoculated with eggs treated with any of the iodine solutions. However, larvae were observed in the other study groups inoculated with eggs treated with the other disinfectants.

These results showed that only the iodine disinfectants produced a statistically significant difference ($p<0.001$) according to the Chi square (X^2) test. Thus, iodine disinfectant solutions were found to be effective against embryonated eggs of *T. canis*.

Key Words: *Toxocara canis*, disinfectants, efficiency

Introduction

Toxocara canis and *T. cati* are nematodes of the family *Ascaridae*, whose adult forms inhabit the proximal small intestine of their mammalian definitive hosts, namely, canids and felids respectively. The infection of humans with *T. canis* can produce a clinical condition known as visceral larva migrans, which generally manifests itself by a number of different symptoms that include fever, coughing, wheezing, lassitude, anorexia, hepatomegaly, and eosinophilia (1). *T. canis* eggs are usually found in soil contaminated by dog or cat faeces. Humans acquire the infection by ingesting infective eggs of the dog (primarily) or cat ascarid *T. canis* or *T. cati*. Direct contact with infected dogs and cats plays a secondary role in transmission due to the required extrinsic incubation period before eggs become infective. Interest in this parasitic infection reflects a growing awareness of the possible human health problems associated with large numbers of pet animals in urban areas (2-5).

T. canis eggs passed in faeces are unembryonated and ineffective. These eggs are subglobose, 75x85 µm in size and have a thick pitted shell. When first passed, they are embryonated and must undergo further development in conditions of suitable temperature and humidity. The process takes 3-4 weeks, and the eggs remain infective for a long period. Maturation ceases at temperatures below 10ºC and the eggs are killed at –15ºC. They are also sensitive to desiccation and sunlight. The eggs of *T. canis* are extremely resistant to chemical and climatic agents, and it is thought that they can survive in a suitable external environment for about 6 years, which is similar to the related nematode *Ascaris lumbricoides* (2, 6).

The shell of *T. canis* eggs' shell comprises five layers. Starting from the outside, these are: a) a thin uterine membrane with occasional small bulges; b) a vitelline layer represented by a thin membrane which follows the contour of the crests and ribs of the subjacent layer; c) a thick and homogeneous chitinous coat (maximum

thickness 6.3 µm); d) an electron-dense granular layer 0.35 µm in average width, the exterior of which is regular, but whose internal surface is jagged; and e) a lamellar zone formed by the superposition of 4 or 5 fibrous layers 0.6 µm in average width. With the help of this strong shell structure, *T. canis* eggs become very resistant to all environmental conditions and chemical agents (7).

This study was carried out in two stages: in vitro and in vivo. In the in-vitro stage of this study, the eggs of *T. canis* were treated with different disinfectants. In the in-vivo stage of the study, these eggs were given to mice orally (1000 eggs for each mouse). Seven days after infection, all the mice were subjected to necropsy and their brains were examined for *T. canis* larvae. At the end of the study, it was shown that different solutions of iodine halted the motion of larvae without damaging the egg shell. No larvae were seen in the brains of the mice in the necropsies. This shows that this disinfectant is very effective against infective *T. canis* eggs comparing to other agents.

In this study we tried to evaluate the efficacy of some routinely used disinfectants against infective *T. canis* eggs.

Materials and Methods

Collection and preparation of eggs: Adult *T. canis* worms were collected from puppy' faeces. The eggs were obtained from the uteri of adult female worms and stirred in a magnetic stirrer for 10 minutes with 1% sodium hypochloride. This suspension was filtered through a sieve with 150-µ pores. The filtrate was centrifuged at 500 g for 3 minutes 3 times with 0.9% NaCl solution in order to remove sodium hypochloride. The eggs were incubated in 0.5% formalin at 26ºC for 4 weeks in order to allow embryonic development (8).

Disinfectant solutions: Eight disinfectants in different concentrations, namely 2.5, 5, 7.5 and 10% iodine, 2% glutaraldehyde, 10% benzalkonium chloride, 7% sodium hypochloride, 1% potassium permanganate, 70% ethyl alcohol, 10% potassium hydroxide and 3% phenol were prepared; 15 ml of each, in different petri dishes, in order to evaluate their efficacy in terms of the motility of embryonated *T. canis* eggs. The same amount of 0.9% NaCl solution was used in the control group.

Treatment of embryonated *T. canis* eggs with disinfectants: Before treatment, the embryonated egg suspension was centrifuged at 500 g for 3 minutes and washed 3 times in sterile distilled water in order to remove the formalin. At the end of this process distilled water was added to the remaining sediment in order to obtain a concentration of approximately 5000 embryonated eggs in 0.5 ml with a final volume of 6 ml. For each study and control group, 0.5 ml of this egg suspension was mixed in a petri dish.

The motility of the larvae was evaluated after 10, 20, 30, 40, 60 minutes, and 24 hours with a X400 magnification inverted microscope. At each time interval, 10 randomised fields were examined so as to determine the motile larvae percentage.

In vivo study: The viability of disinfectant-treated eggs was evaluated in an in-vivo study. Eggs treated with iodine (2.5, 5, 7.5 and 10%) solutions were centrifuged at 500 g for 5 minutes, after which no motile larvae were detected, at 120, 60, 40, and 40 minutes post-treatment respectively. Eggs treated with the other disinfectants and 0.9% NaCl solution were centrifuged at 500 g for 5 minutes 24 hours later. All the sediments were centrifuged at 500 g for 5 minutes 3 times with distilled water so as to remove the disinfectant solutions.

For each disinfectant solution one study group of 5 healthy 4-week-old Swiss-albino mice was used. There was also a control group with the same number of mice for the %0.9 NaCl solution group. Therefore, we had 11 study groups and one control group.

In each study and control group, after the washing process, distilled water was added to sediment containing approximately 5000 embryonated eggs in order to obtain a concentration of approximately 2000 embryonated eggs/ml with a final volume of 2.5 ml. In all the 12 groups, each mouse was infected with 0.5 ml of suspension containing approximately 1000 embryonated eggs by the oral route. All the mice were sacrificed under anaesthesia on the 7^{th} day post-inoculation. For the counting of the larvae in the brain tissue, the brains were removed and sliced into sections aproximately 3x3x3 mm in size. These sliced specimens were pressed between a 22x22 mm cover slip and slide, and all the fields were examined under a light microscope of x50 magnification in order to count the larvae (Table 2).

Results

In-vitro study: In all the iodine-treated groups, although there were no morphological changes, it was determined that the motility of the larvae had ceased. In all the other disinfectant groups and in the control group, there were neither morphological changes nor any cessation of the motility of the larvae. Thus the efficacy percentage of each disinfectant against embryonated *T. canis* eggs was evaluated in vitro (Table 1).

These data showed that the only statistical significance was observed in the iodine groups when compared to the control group ($p<0.001$) using the Chi square (X^2) test.

In-vivo study: No larvae migrans were observed in the brain in any of the iodine groups whereas they were detected with all the other disinfectants and in the control group. Statistically significant differences were found only in the iodine groups ($p<0.001$) according to the Mann Whitney U test (Table 2).

Discussion

A large percentage of the world's several hundred million domestic dogs are infected with the parasitic nematode *T. canis*. Adult worms reside in the gastrointestinal tract and produce eggs which are subsequently shed with faeces into the environment (9). One *T. canis* adult female may produce 20000 eggs per day, and since intestinal parasite burdens range from one to several hundred worms, infected animals contaminate the environment with millions of eggs per day (2).

T. canis eggs, found widespread in the environment, are very hazardous to humans, who are paratenic hosts. These eggs are very resistant to environment conditions and chemical agents.

There are few studies on this subject, and these deal with parasites other than *T. canis*, such as *Ascaris* spp., *Hymenolepis nana*, *Ostertagi ostertag*i and *Cooperia oncophora* (10). Of these parasites, *Ascaris* spp. eggs have a similar structure to *T. canis* eggs (6). For this reason these studies are included in this discussion.

Juris and Breza studied (11) the efficacy of eight disinfectants against non-embryonated and embryonated eggs of *Ascaris suum* in vitro. Kreosolum saponatum, a Czech product containing 51.6% tricresol, at a concentration of 5% killed all eggs within 30 minutes. Its effect was similar to that of the other phenol disinfectants tested. Orsanol BF 12, potassium and sodium

Table 1. Efficacy percentages of disinfectants on embryonated eggs of *T. canis*.

Disinfectants	Motility percentage by time									
	10 m*	20 m	30 m	40 m	1 h	2 h	4 h	8 h	16 h	24 h
Iodine (2.5%)	48	37	29	11	4	NM*	NM	NM	NM	NM
Iodine (5%)	41	32	11	5	NM	NM	NM	NM	NM	NM
Iodine (7.5%)	36	26	14	NM	NM	NM	NM	NM	NM	NM
Iodine (10%)	28	21	7	NM	NM	NM	NM	NM	NM	NM
Glutaraldehyde (2%)	100	100	100	100	100	100	100	100	100	100
Benzalkonium chloride (10%)	100	100	100	100	100	100	100	100	100	100
Sodium hypochloride (7%)	100	100	100	100	100	100	100	100	100	100
Potassium permanganate (1%)	100	100	100	100	100	100	100	100	100	100
Ethyl alcohol (70%)	100	100	100	100	100	100	100	100	100	100
Potassium hydroxide (10%)	100	100	100	100	100	100	100	100	100	100
Phenol (3%)	100	100	100	100	100	100	100	100	100	100
NaCl 0.9% (Control)	100	100	100	100	100	100	100	100	100	100

m* : minute NM : No motility detected

Table 2. Evaluation of Larva Migrans in vivo.

Disinfectants	Number of larvae in brain
Iodine (2.5%)	ND*
Iodine (5%)	ND
Iodine (7.5%)	ND
Iodine (10%)	ND
Glutaraldehyde (2%)	52
Benzalkonium chloride (10%)	49
Sodium hypochloride (7%)	48
Potassium permanganate (1%)	53
Ethyl alcohol (70%)	56
Potassium hydroxide (10%)	47
Phenol (3%)	51
NaCl 0.9% (control)	55

* ND: Not Detected

hydrochlorides, Jodonal B and liquid Jodisol were either ineffective or had a considerably lower level of efficacy.

In a study by Burg and Borgsteede (10), in which a 90% mortality rate was considered to be effective, none of the disinfectants (chlorine, phenol, cresol, sodium and potassium hydroxide, quaternary ammonium compounds, glutaraldehyde or paraformaldehyde) was found to be effective against non-embryonated and embryonated eggs of *A. suum*.

In another study, Avramova et al. (12) studied the effect of some disinfectants in a culture of *A. lumbricoides* in vitro. In this study, the best results were obtained with iodophorous preparation 4% solution, which killed 95% of the ova in 2 hours, perhydrol 6% solution and performic acid killed 95% of *A. lumbricoides* ova in 4 hours.

Ozone treatment was found by Ooi et al. to have no effect on the development and viability of *T. canis* eggs (13).

Barutzki (14) found that unembryonated and embryonated eggs of *T. canis* and *A. suum* were damaged by 5% Incicoc after 10 minutes, with the same effect on unembryonated *T. canis* eggs after only 2 minutes, whereas the other agents (6% Dekaseptol, 5% Lomasept, 5% Lysococ) exhibited inadequate effects.

Akao et al. (15) evaluated the effectiveness of benzalkonium-ion intercalated aluminium triphosphate (BIAT) in vitro as a larvicide against fertilised eggs of *T. canis*. Akao et al. revealed that BIAT had a larvicidal effect when eggs were incubated with BIAT from the start of development. However, this effect was not observed in eggs that had already matured into infective larvae. We produced almost the same results as Akao et al., in that in our study 10% benzalkonium was ineffective against embryonated of *T. canis*.

In the present study, all the iodine solutions (2.5, 5, 7.5, 10%) were found to be effective against embryonated *T. canis* eggs, whereas all the other disinfectants were ineffective. These data were also confirmed by in-vivo study, as no larvae were only observed in mice inoculated orally with iodine-treated eggs. Based upon the above findings, our results are in agreement with other previous studies, in that iodine solution may be a good choice of disinfectant against *T. canis* eggs. Very few chemical agents are effective against *T. canis* eggs according to the literature. There have been very few studies on *T. canis*. Therefore, the data obtained in this study was compared to other studies of ascarids. Our results confirm the results of Avramova et al. (12), who examined the effects of chlorophenol, creosol, NaOH, KOH, and quarternary ammonium derivatives on *A. suum* eggs. Sodium hypochloride had no effect on *T. canis* eggs, and this confirms the results of Juris and Breza (11), who studied the effect of sodium hypochloride on *A. suum* eggs.

Sodium hypochloride was always recommended as a disinfectant agent against *T. canis* eggs in textbooks and articles. Contrary to this, our study shows that the use of iodine solutions is the best way to destroy *T. canis* eggs. Different solutions of iodine are effective both in vitro and in vivo. We can conclude that iodine preparations should be used in animal shelters and cages to eliminate *T. canis* eggs, and also to prevent contamination in laboratories. Further studies are needed to obtain a better understanding of the effect of iodine on embryonated eggs of *T. canis*.

References

1. Lynch NR, Wilkes LK, Hodgen AN, Turner KJ. Specificity of Toxocara ELISA in tropical populations. Parasit Immunol 10: 323-337, 1988.
2. Lawrence TG, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol Rev 3: 230-250, 1981.
3. Schantz,PM, Parasitic zoonoses in perspective. Int. J. Parasit. 21: 161-170, 1991.
4. Van Knapen, F, Buijs, J, Kortbeck, LM and Ljungström, I, Larva migrans syndrome: toxocara, ascaris or both. Lancet. 340: 550-551, 1992.
5. Magnaval JV, Galindo V, Glickman LT, Clanet M Human, Toxocara infection of the central nervous system and neurological disorders: a case-control study. Parasitology. 115:537-543, 1997.
6. Barriga OO. A critical look at the importance, prevalence and control of toxocariasis and possibilities of immunological control. Vet Parasitol 29: 195-234, 1988.
7. Bouchet F, Boullard Y, Baccam D, Leger N. Ultrastructural studies of alterations induced by microwaves in *Toxocara canis* eggs: prophylactic interest. Z Parazitenkd 72: 755-765, 1986.
8. Oshima T. Standardisation of techniques for infecting mice with *Toxocara canis* and observations on the normal migration routes of the larvae. J Parasitol 47: 652-656, 1961.
9. Robertson BD, Bianco AT, Mckerrow H, Maizels RM. *Toxocara canis*: Proteolytic enzymes secreted by the infective larvae in vitro. Exp Parasitol 69: 30-36, 1989.
10. Burg WPJ, Borgsteede FH, Van den Burg WPPJ. Effects of various disinfectants on the development and survival possibilities of the pre-parasitic stages of Ostertagia ostertagi, Cooperia oncophora and Ascaris suum. Tijdschr Diergeneeskd 11: 769-778,1987.
11. Juris P, Breza M. Trials with the disinvasive efficiency of some disinfectants in the laboratory conditions. Helminthologia 25: 309-318, 1988.
12. Avramova S, Dinev D, Boeva V, Dakova A, Grigorava M, Khadzhilarska D. Study of the effect of some disinfectants on helminth ova. Epidemiol Mikrobiol Infekt Bolesti 22: 53-56, 1985.
13. Ooi HK, Lin CL, Wang JS. Effect of ozone treatment on *Toxocara canis* eggs. J Vet Med Sci. 60: 169-173, 1998.
14. Barutzki D. Efficacy of commercial disinfectants against coccidial oocycts and sporocysts (Eimeria, Cystoisosporia, Toxoplasma and Sarcocystis) and against eggs of Ascaris and Toxocara in suspension experiments. Inaugural Dissertation, München: Ludwig-Maximillians Universtat pp. 44, 1980.
15. Akao N, Tsukidate S, Kondo K, Fujita K. In vitro effects of benzalkonium-ion intercalated aluminium triphosphate on the second stage larvae and fertilised eggs of *Toxocara canis*. Jpn J Parasitol 44: 138-142, 1995.