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Is routine disinfection efficient in preventing contamination with *Toxocara canis* eggs?

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Research Paper

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Abstract

Toxocara canis (Werner, 1782) is a zoonotic nematode commonly parasitizing dogs worldwide with great public health importance as the aetiological agent of human toxocariasis. In this respect, the aim of this study was to evaluate the effect of six disinfectant products commonly used in kennels, veterinary clinics and as household cleaning products on the embryogenesis and viability of *T. canis* eggs. The composition of active ingredients in these commercial disinfectants was sodium hypochlorite (A); a mix of *N*-(3-aminopropyl)-*N*-dodecylpropan-1.3-diamine and didecyldimethylammonium chloride (B); sodium dichloroisocyanurate dehydrate (C); a mix of glutaraldehyde, quaternary ammonium compounds, benzyl-c12-18-alkyldimethyl and chlorides (D); a mix of 2-propanol, ethanol, benzalkonium chloride and glucoprotamin (E); a mix of pentapotassium bis (peroxymonosulphate) bis (sulphate), sodium C10-13-alkylbenzenesulphonate, malic acid, sulphamidic acid, sodium toluenesulphonate, dipotassium peroxodisulphate and dipentene (F). After dilution, the tested disinfectants had the maximal concentration recommended by the manufacturer in order to achieve a biocidal effect. Each product was tested on approximately 10,000 *T. canis* eggs, having five different contact times (5, 10, 15, 30, 60 min). Three replicates were tested for each diluted disinfectant and for each contact time. After the treatment, eggs were washed and incubated in distilled water at 27 °C for 2 weeks. None of the tested products had a significant inhibitory effect on the embryogenesis and viability of *T. canis* eggs, regardless of the contact time. Moreover, after 2 weeks, in all tested samples, eggs containing motile infective larvae were identified, showing that routinely used disinfectants do not eliminate risk of infection by *T. canis*.

Introduction

Toxocara canis is a zoonotic nematode and one of the most frequent parasites worldwide, affecting predominantly young dogs under 1 year of age (Barutzki & Schaper, 2013). In Europe, the prevalence in dogs varies between 3.5% and 34% (Overgaauw & van Knapen, 2013). Among risk factors for infection of dogs can be age, coprophagy, unsupervised access to outdoor environment and periodical placement in a kennel (Nijsse *et al.*, 2015).

Dogs can be infected through vertical transmission (transplacental and transmammary), but also through ingestion of embryonated eggs from the environment or by consuming paratenic hosts with viable larvae of *T. canis* (Schnieder *et al.*, 2011). Parasites develop adult stages in the intestinal tract of dogs causing mostly digestive symptoms, but infections may be asymptomatic (Overgaauw & van Knapen, 2013). Nevertheless, one adult female of *T. canis* can lay up to 200,000 unembryonated eggs per day, which are then shed in faeces into the environment. Taking in consideration the cases of high infestation in some dogs, this number can rise up to millions of eggs per day (Glickman & Shofer, 1987). Egg embryonation begins in the environment, under proper temperature and humidity, and it takes between 2 and 5 weeks for the larvae to reach infective stage (Despommier, 2003; Gamboa, 2005). The structure of the eggs provides high resistance to different chemicals and even against high temperature variations for up to 12 months (Bouchet *et al.*, 1986; Azam *et al.*, 2012).

Humans and other paratenic hosts become infected after ingestion of embryonated eggs with second- (Morrondo *et al.*, 2006) or third-stage larvae (Kolbeková *et al.*, 2011). In this case, larvae stop development but they migrate via the bloodstream to different organs and tissues where they remain viable for longer time periods (Schnieder *et al.*, 2011). Nijsse *et al.* (2015) consider that one of the most important infection sources is contaminated soil or artificial surfaces (paved roads, sidewalks, floors, etc.). Consumption of raw meat containing

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somatic larvae, contaminated vegetables and water is also considered a major risk (Overgaauw & Nederland, 1997; Taira *et al.*, 2004; Lee *et al.*, 2010).

Human toxocariasis can have four major forms: 'visceral larva migrans', 'ocular larva migrans', neurotoxocariasis (neural larva migrans) and covert toxocariasis (Fan *et al.*, 2015). During the last decades, the number of companion animals has significantly increased, and therefore so has the possibility of transmission of various zoonotic agents (Paul *et al.*, 2010). According to the Centers for Control Disease and Prevention, from a public health perspective, human toxocariasis remains a major and often neglected zoonotic disease, especially among children, but also for certain occupational categories such as veterinarians and staff working in clinics, hospitals, shelters and owners. Owing to the high risk of *T. canis* infection in dogs, humans and other paratenic hosts, there is a permanent interest in evaluating and finding chemicals or other preventive measures that can inactivate the infective form of the parasite (Morrondo *et al.*, 2006; Araujo *et al.*, 2013; Magaña-López *et al.*, 2016; Von Dohlen *et al.*, 2017). In this context, routine evaluation of the efficient disinfectants in high-risk environments becomes an important task, from both a veterinary and public health perspective. The objective of this study was to evaluate the effect of six disinfectant products commonly used in kennels, veterinary clinics and as household cleaning products on the embryogenesis and viability of *T. canis* eggs.

Material and methods

Preparation of T. canis *eggs*

Live adult parasites of *T. canis* were obtained from seven naturally infected freshly dead puppies at necropsy. The animals died due to other causes, and the necropsy was done in 30–45 min. Female *T. canis* ($n = 18$) were identified, separated and rigorously washed with saline water. The uteri were isolated and both anterior non-bifurcated portions were dissected under magnification (4×) (Oshima, 1961; Alcântara-Neves *et al.*, 2008). Eggs were collected from the uteri and suspended in distilled water in Petri dishes. The exposure of eggs to disinfectants occurred in the same day.

Egg exposure to disinfectants and cultivation

A total of six disinfectant products recommended for their bactericidal and viricidal properties and commonly used in high-risk areas (i.e. veterinary clinics, hospitals, kennels and shelters) were tested. Even if manufacturers do not specify antiparasitic effects on the label, some veterinarians assume (off-label) that the biocidal effect also includes parasites. The compositions and concentrations of the tested products were (A) 4.5% sodium hypochlorite; (B) mix of 9.9% *N*-(3-aminopropyl)-*N*-dodecylpropan-1.3-diamine and 6% didecyldimethylammonium chloride; (C) 77.14% sodium dichloroisocyanurate dehydrate; (D) mix of 10–30% glutaraldehyde and 5–10% quaternary ammonium compounds, benzyl-c12-18-alkyldimethyl, chlorides; (E) mix of 20% 2-propanol, 10% ethanol, 0.2% benzalkonium chloride, 0.01% glucoprotamin; (F) mix of 40–55% pentapotassium bis (peroxymonosulphate) bis (sulphate), 10–12% sodium C10-13-alkylbenzenesulphonate, 7–10% malic acid, 4–6% sulphamidic acid, 1–5% sodium toluenesulphonate, <3% dipotassium peroxodisulphate and <0.25% dipentene; (G) control group, represented by eggs solely exposed to distilled water.

For testing, the commercial products were diluted to maximal concentration recommended by the manufacturer for a biocidal effect: A – 2.4%, B – 1%, C – 0.05%, D – 0.06%, E – 95%, F – 1%. From each diluted product samples of 1.9 ml were mixed in sterile tubes with 0.1-ml aliquots containing 10,000 *T. canis* eggs in a total volume of 2 ml. In order to mimic the real-life situations (daily disinfections) that occur in veterinary clinics, five different contact times were applied for each mixture: 5, 10, 15, 30 and 60 min, at a constant temperature of 25 °C.

Permanent stirring was provided to avoid egg sedimentation. After exposure, mixtures were centrifuged for 5 min at 1015 RCF (relative centrifugal force). The supernatant was removed and the egg sediment was washed twice with distilled water and twice centrifuged for 5 min at 1015 RCF. Three replicates were tested for each diluted disinfectant and for each contact time. Each procedure was also repeated for the control group. All samples were transferred to 12 multiwell culture plates, each containing 3 ml of distilled water, and incubated for 2 weeks at 27 °C. Daily oxygenation and 10/14 h light/darkness cycles were provided.

Larval development assay/disinfectant efficacy

Egg morphology and larval motility were assessed by daily examination of 20 µl of each sample under light microscopy (10×, 20×, 30×, 40×). As eggs may become infective in 2 weeks under controlled temperature, all statistical analyses were set at this time point (Gamboa, 2005). The percentage of larval development was calculated applying the formula: % larval development = number of fully embryonated eggs/total number of eggs × 100 (Oh *et al.*, 2016). Degenerated eggs were not counted.

For every disinfectant tested, a mean of three samples of 50 µl of each replicate was used in obtaining the larval development percentage. For the control group, a mean of all samples was calculated in the same manner. Effect on embryogenesis was expressed by subtracting the larval development percentage from 100.

Statistical analyses

The statistical software package GraphPad© InStat was used to perform the statistical evaluations using one-way analysis of variance. The comparison was made by using the Tukey–Kramer multiple comparisons test. The significance level for all statistical tests was predetermined at $P < 0.05$.

Results

The efficacy of each disinfectant product, for all exposure times, is shown in table 1 as the arithmetic mean of the three replicates. In all samples, embryonation of *T. canis* started on the second day of incubation. Fully embryonated eggs were observed starting at day 7 post exposure. All samples contained L2 motile *T. canis* larvae on day 14. Regardless of the contact time, none of the tested disinfectants succeeded in completely stopping embryonation nor in destroying all eggs. Statistical analysis of egg embryonation inhibition did not reveal any significance, regardless of the disinfectant used or contact time. Comparing the results between disinfectants, the probability factor was without statistical significance ($P > 0.05$). Results proved that product C, containing sodium dichloroisocyanurate dehydrate, with a 30-min contact time, was the most efficient (31.44%). Morphological changes,

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Table 1. Efficacy of disinfectants for each exposure time after 2 weeks of incubation (replicates arithmetic mean).

	Exposure time									
	5 min		10 min		15 min		30 min		60 min	
Disinfectant	%LD	E%	%LD	E%	%LD	E%	%LD	E%	%LD	E%
A	86.37	13.63	84.14	15.86	85.95	14.02	85.55	14.45	91.40	8.60
B	91.30	8.70	76.52	23.48	79.32	20.68	70.78	29.22	72.67	27.33
C	77.31	22.69	74.54	25.46	68.95	31.05	68.56	31.44	77.7	22.30
D	88.56	11.44	75.11	24.89	85.10	14.90	81.55	18.45	89.25	10.75
E	83.95	16.05	84.75	15.25	69.58	30.42	82.92	17.05	82.67	17.33
F	81.39	18.61	79.21	20.79	80.67	19.33	79.48	20.52	80.36	19.64
G	79.28	20.72	79.28	20.72	79.28	20.72	79.28	20.72	79.28	20.72
SD	±5.05		±4.09		±6.77		±6.30		±6.54	

LD, larval development; E, efficacy; SD, standard deviation.

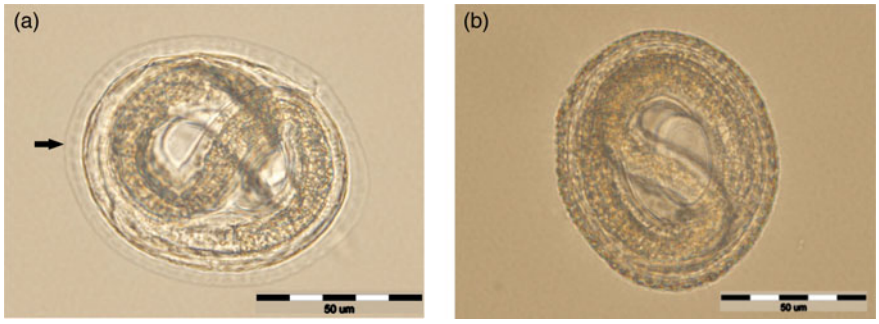


Fig. 1. Egg exposed to sodium hypochlorite with changes in the eggshell (arrow), but with a normal larval morphology (a) compared with untreated egg with an intact outer layer (b).

consisting of decorticated eggs with a more transparent outer layer, were detected for product A, containing sodium hypochlorite (fig. 1). Furthermore, for this product, egg development was faster than the control group.

Discussion

Hygiene and preventive measures for biological risks in veterinary clinics or hospitals, veterinary diagnostic laboratories, shelters and kennels include disinfection against bacterial, viral and fungal agents. Nevertheless, surfaces, soil and dog playgrounds are also potential sources of parasitic infections for both humans and animals. In the present study, six routinely used disinfectants were tested at different exposure times in order to assess their effect on embryogenesis of *T. canis* eggs. The results of the study revealed that preventive measures based on common disinfectants against other various pathogens are inefficient against *T. canis* eggs, with the zoonotic risk still present. None of the chemicals used, regardless of the contact time, were able to destroy all eggs, nor to completely stop their development to infective stages. Shorter or prolonged contact time had no influence on egg development inhibition.

Studies concerning the efficacy of various disinfectants against *T. canis* eggs concluded in different, often contrasting, results. Sodium hypochlorite is frequently used due to its availability and efficacy against different microorganisms. Morrondo *et al.* (2006) reported that, when kept for 15 days in 2% sodium hypochlorite, only 2% of *T. canis* eggs reach the L2 stage, and if contact time is prolonged to 24 days, L2 stage egg development reaches 8%. Mice infection with these L2 stage eggs did not reveal migratory capacities in the nervous system. Verocai *et al.* (2010) a noticed low embryonation percentage when using 2–2.5% sodium hypochlorite. The same authors also noticed total egg degeneration when 70% ethanol was used. Variability of scientific data concerning disinfectant efficacy can be explained by the use of different testing methods: incubation within the diluted disinfectant for a certain time period or using different contact times followed by removal of chemicals before incubation. Von Dohlen *et al.* (2017) found results similar to the current study, noting that the use of sodium hypochlorite was not sufficient to inhibit *T. canis* eggs embryogenesis at different contact times (15–120 min). Moreover, the same authors reported that sodium hypochlorite solution induced morphological changes of the egg shell and faster embryonation, emphasizing the possibility of a more intense egg oxygenation. Disinfectants containing benzalkonium chloride and glutaraldehyde proved to be ineffective in inhibiting *T. canis* larval development (Verocai *et al.*, 2010).

The disinfectant containing a mix of pentapotassium, sulphamidic acid, sodium dodecylbenzenesulphonate and dipotassium peroxodisulphate (product F) has never been tested on *T. canis* eggs before. The product was only tested on *T. leonina* and proved effective in stopping larval development (El-Dakhly *et al.*, 2018). Sodium hypochlorite, in concentrations of 0.2% and 0.02%, showed no efficacy in inhibiting *Ascaris suum* egg development (Oh *et al.*, 2016). Oh *et al.* (2016) obtained the same results for ethanol, methanol and chlorhexidine.

Different concentrations of iodine (2.5%, 5%, 7.5% and 10%) did not induce morphological changes in eggs of *T. canis*, but

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succeeded in inhibiting larvae motility *in vitro* and migratory capacities in the brains of infected mice, whereas eggs treated with 10% glutaraldehyde, 3% phenol, 7% sodium hypochlorite, 10% benzalkonium chloride, 1% potassium permanganate or 70% ethyl alcohol solutions still showed larval motility (Ayçiçek *et al.*, 2001). Povidone iodine solutions only managed to inactivate the majority of *A. suum* eggs after 5 min of exposure at a concentration of 10%, but it did not completely destroy all eggs (Oh *et al.*, 2016).

The tested exposure times were chosen to mimic the real-life situations that occur in veterinary clinics during daily disinfections. The difference between this study and other studies on disinfectant efficacy is that the majority of cited authors tested the disinfectants by keeping them for several days in contact with the eggs.

Further and more complex studies are required in order to assess efficient methods to completely destroy or inhibit *T. canis* embryogenesis and prevent animal and human contamination, especially in areas with high biological risk. The disinfectants chosen in this study were used off label for decontaminant purposes, but they are frequently used for disinfecting potentially contaminated areas in veterinary clinics, hospitals, laboratories, kennels and shelters or as household products.

In conclusion, current measures of disinfection used in places with high risk of contamination are not sufficient for infective stages of *T. canis*; therefore, the persistence of zoonotic and animal-to-animal infection hazards still remains. Further studies on other active compounds, new combinations and concentrations, optimal exposure times or even new disinfectants are required in order to completely inactivate *T. canis* eggs or inhibit embryogenesis.

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Ethical standards. This article does not contain any studies with human participants or animals performed by any of the authors.

References

Alcântara-Neves NM, Barbosa dos Santos A, Mendonca LR, Figueiredo CV and Pontes-de-Carvalho L (2008) An improved method to obtain antigen-excreting Toxocara canis larvae. *Experimental Parasitology* **119**, 349–351. doi: 10.1016/j.exppara.2008.03.006.

Araujo JM, Araújo JVD, Braga FR, Ferreira SR and Tavela ADO (2013) Predatory activity of chlamydospores of the fungus *Pochonia chlamydosporia* on *Toxocara canis* eggs under laboratory conditions. *Revista Brasileira de Parasitologia Veterinária* **22**(1), 171–174.

Ayçiçek H, Yarsan E, Sarimehmetoğlu HO, Tanyüksel M, Girginkardeşler N and Özyurt M (2001) Efficacy of some disinfectants on embryonated eggs of *Toxocara canis*. *Turkish Journal of Medical Sciences* **31**(1), 35–39.

Azam D, Ukpai OM, Said A, Abd-Allah GA and Morgan ER (2012) Temperature and the development and survival of infective *Toxocara canis* larvae. *Parasitology Research* **110**(2), 649–656. doi: 10.1007/s00436-011-2536-8.

Barutzki D and Schaper R (2013) Age-dependant prevalence of endoparasites in young dogs and cats up to one year of age. *Parasitology Research* **112**, 119–131. doi: 10.1007/s00436-013-3286-6.

Bouchet F, Boulard Y, Baccain D and Leger N (1986) Ultrastructural studies of alterations induced by microwaves in *Toxocara canis* eggs: prophylactic interest. *Zeitschrift für Parasitenkunde* **72**(6), 755–764.

Despommier D (2003) Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clinical Microbiology Reviews* **16**(2), 265–272. doi: 10.1128/CMR.16.2.265-272.2003.

El-Dakhly KM, Aboshinaf ASM, Arafa WM, Mahrous LN, El-Nahass E, Gharib AF and Craig TM (2018) In vitro study of disinfectants on the embryonation and survival of *Toxascaris leonina* eggs. *Journal of Helminthology* **92**(5), 530–534. doi: 10.1017/S0022149X17000839.

Fan CK, Holland CV, Loxton K and Barghouth U (2015) Cerebral toxocariasis: silent progression to neurodegenerative disorders? *Clinical Microbiology Reviews* **28**(3), 663–686. doi: 10.1128/CMR.00106-14.

Gamboa MI (2005) Effects of temperature and humidity on the development of eggs of *Toxocara canis* under laboratory conditions. *Journal of Helminthology* **79**(4), 327–331.

Glickman LT and Shofer FS (1987) Zoonotic visceral and ocular larva migrans. *Veterinary Clinics of North America: Small Animal Practice* **17** (1), 39–53.

Kolbeková P, Větvička D, Svoboda J, Skírnisson K, Leissová M, Syrůček M and Kolářová L (2011) *Toxocara canis* larvae reinfecting BALB/c mice exhibit accelerated speed of migration to the host CNS. *Parasitology Research* **109**(5), 1267–1278. doi: 10.1007/s00436-011-2371-y.

Lee IH, Kim ST, Oh DK, Kim HJ, Kim KH, Jeon P and Byun HS (2010) MRI findings of spinal visceral larva migrans of *Toxocara canis*. *European Journal of Radiology* **75**(2), 236–240. doi: 10.1016/j.ejrad.2009.04.024.

Magaña-López R, Luna-Pabello VM, Barrera-Godínez JA, de Velásquez MO and Fernández-Villagómez G (2016) Effect of mineral aggregates on the morphology and viability of *Toxocara canis* eggs. *Ecological Engineering* **90**, 125–134. doi: 10.1016/j.ecoleng.2016.01.071.

Morrondo P, Díez-Morrondo C, Pedreira J, Díez-Baños N, Sánchez-Andrade R, Paz-Silva A and Díez-Baños P (2006) *Toxocara canis* larvae viability after disinfectant-exposition. *Parasitology Research* **99**(5), 558–561.

Nijsse R, Ploeger HW, Wagenaar JA and Mughini-Gras L (2015) *Toxocara canis* in household dogs: prevalence, risk factors and owners' attitude towards deworming. *Parasitology Research* **114**(2), 561–569. doi: 10.1007/s00436-014-4218-9.

Oh KS, Kim GT, Ahn KS and Shin SS (2016) Effects of disinfectants on larval development of *Ascaris suum* eggs. *The Korean Journal of Parasitology* **54** (1), 103–107. doi: 10.3347/kjp.2016.54.1.103.

Oshima T (1961) Standardization of techniques for infecting mice with *Toxocara canis* and observations on the normal migration routes of the larvae. *The Journal of Parasitology* **47**(4), 652–656.

Overgaauw PA and Nederland V (1997) Aspects of *Toxocara epidemiology*: human toxocarosis. *Critical Reviews in Microbiology* **23**(3), 215–231.

Overgaauw PA and van Knapen F (2013) Veterinary and public health aspects of *Toxocara spp*. *Veterinary Parasitology* **193**(4), 398–403. doi: 10.1016/j.vetpar.2012.12.035.

Paul M, King L and Carlin EP (2010) Zoonoses of people and their pets: a US perspective on significant pet-associated parasitic diseases. *Trends in Parasitology* **26**(4), 153–154. doi: 10.1016/j.pt.2010.01.008.

Schnieder T, Laabs EM and Welz C (2011) Larval development of *Toxocara canis* in dogs. *Veterinary Parasitology* **175**(3-4), 193–206. doi: 10.1016/j.vetpar.2010.10.027.

Taira K, Saeed I, Permin A and Kapel CMO (2004) Zoonotic risk of *Toxocara canis* infection through consumption of pig or poultry viscera. *Veterinary Parasitology* **121**(1-2), 115–124.

Verocai GG, Tavares PV, De Ribeiro FA, Correia TR and Scott FB (2010) Effects of disinfectants on *Toxocara canis* embryogenesis and larval establishment in mice tissues. *Zoonoses and Public Health* **57**(7–8), e213–e216. doi: 10.1111/j.1863-2378.2010.01330.x.

von Dohlen AR, Houk-Miles AE, Zajac AM and Lindsay DS (2017) Flotation of *Toxocara canis* eggs in commercial bleach and effects of bleach treatment times on larval development in these eggs. *Journal of Parasitology* **103**(2), 183–186. doi: 10.1645/16-123.

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