TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 734-739

doi:10.3906/vet-1905-92

Effects of some disinfectants on *Toxocara* spp.eggs viability of dogs and cats

Iasmina LUCA*, Ion OPRESCU, Sorin MORARIU,
Narcisa MEDERLE, Marius Stelian ILIE, Gheorghe DĂRĂBUŞ
Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine,
Banat's University of Agricultural Sciences and Veterinary Medicine, Timişoara, Romania

Received: 28.05.2019 • **Accepted/Published Online:** 03.03.2020 • **Final Version:** 02.06.2020

Abstract: The main cause of *larva migrans syndrome* (LMS) in humans is represented by *Toxocara* spp. found in dogs and cats. In the present study, the effects of three disinfectants based on quaternary ammonium compounds (Kohrsolin® FF, Trioton® rapid AF and Terralin® protect) and one based on propanol and ethanol (Bacillol®), against *Toxocaracanis* and *Toxocaracati* eggs, were tested. These disinfectants have a strong antimicrobial and virucidal action. Two different exposure times and different concentrations for solutions were used. All four disinfectants presented high percentages of destructive effects against *Toxocaracanis* eggs, after 21 days of exposure. The vast majority of eggs with pronounced degenerative changes in the eggshell and embryonic development were observed using Bacillol® for both *Toxocara* species. Terralin® protect was ineffective against *Toxocaracati* eggs. The ovicidal effect of these compounds has been noticed thus allowing their use as disinfectants for surfaces contaminated with *Toxocara* spp. eggs.

Keywords: *Toxocara* spp., disinfectants, exposure, carnivores, efficiency

1. Introduction

Human toxocariasis is a zoonosis caused by the larval stage of *Toxocaracanis* and *Toxocaracati* species, which are gastrointestinal nematodes infectingdogs and cats, found especially in young carnivores under one year of age [1]. People get infected by ingesting the larvated eggs presenting second stage larvae or third stage larvae, found in contaminated soil [2,3], vegetables [4–7], water or animal hair [8,9]. Poorly cooked meat from paratenic hosts (cows, sheep, chickens) is another major source of infection [10]. Children with pica are most commonly diagnosed. They infest themselves with the larvated eggs from sandpits or various playgrounds, where dog or cat faeces are found. The specific human syndromes are *larva migransvisceralis* and *larva migransocularis*.

The morphological aspects of *T. canis* and *T. cati* eggs are very similar. A female ascarid can lay up to 200,000 eggs per day, which are highly resistant in the external environment due to the five-layer eggshell (uterine, vitelline, chitinous, granular, and fibrous layers) [11]. To reach the infective stage, the eggs require approximately 2 to 5 weeks until the larvae forms inside at temperatures between 15 °C and 35 °C and over 85% humidity [12]. The complete development of the eggs can also occur at temperatures of –2 °C or 1 °C and they can last about 6 weeks [13].

In temperate areas, eggs can survive from 6 to 12 months and the most commonly used disinfectants are not effective against them (2% glutaraldehyde, 10% benzalkonium chloride, 1% potassium permanganate, 70% ethyl alcohol, 10% potassium hydroxide and 3% phenol) [11]. Instead, products based on iodine and ethanol can be successfully used against *T. canis* eggs [11,12]. Contradictory results have been observed regarding the action of sodium hypochlorite. Some authors argue its efficacy against *T. canis* eggs [12], while the others sustain the opposite [11].

In hospitals and veterinary clinics, and even in animal shelters, disinfection is a crucial step. The aim of this study was to test the effects of four disinfectants (Kohrsolin® FF, Trioton® rapid AF, Terralin® protect, Bacillol®) on the viability of *T.canis* and *T. cati* eggs. The bactericidal, fungicidal, and virucidal actions of these substances are well-known, but the ovicidal effect is not specified. These products are used intensively in human hospitals for disinfecting surfaces and medical instruments, therefore we considered studying their ovicidal action in hopes of discovering a viable disinfectant for usage in veterinary clinics and hospitals and in other related institutions that come in contact with a possible source of contamination.

* Correspondence: iasminaluca0@gmail.com

2. Materials and methods

2.1. Gathering the eggs

The female ascarids were longitudinally sectioned at tegumentary level in order to highlight the uterine horns. These were separately collected and longitudinally sectioned, after which they were milled with distilled water and filtered through a 0.5 mm mesh sieve, obtaining the eggs of *T. canis* and *T. Cati* [14].

2.2.Exposure of unembryonated eggs to disinfectants

The concentrations used for each disinfectant and their chemical composition are shown schematically in Table 1. These were established according to the manufacturers of each product, choosing those concentrations most commonly used in human hospitals for short exposure times (15 and 30 min, respectively).

Approximately 10^4 *T. canis* and *T. cati* eggs were obtained and subsequently placed in 48 glass tubes with 5 mL from each disinfectant.

For each disinfectant, 3 samples were prepared. Thereby, from the total of 48 samples, 24 were with *T. canis* eggs and 24 with *T. cati* eggs.

The eggs were then exposed for 15 min and 30 min, at room temperature (22–24 °C), as follows: from a total of 24 samples of *T. canis* eggs, 12 were exposed to the action of disinfectants for a period of 15 min and the other 12, for a period of 30 min. The same procedure was also performed for *T. cati* eggs.

After the exposure, the samples were centrifuged for 7 min, at 2500 rpm, a greater centrifugal force than the one used by Gautam et al. [15], for a more efficient and correct sedimentation. The centrifugation process and the removal of the supernatant followed by a replacement with distilled water was repeated 4 times. The eggs immersed in distilled water were subsequently kept at room temperature (22–24 °C) for 21 days, being sputtered daily in order to ensure the necessary oxygen. Periodical examinations were performed [15].The light/darkness cycle provided throughout the experiment was 10/14 h, daily.

A total of 6 control samples (3 with *T. canis* eggs and 3 with *T. cati* eggs) were maintained in 5 mL of physiological serum for the same period of time.

2.3. Egg classification / disinfectant efficiency

After 21 days the eggs were microscopically examined in order to check their viability. From each sample, a total number of 100 eggs were classified as viable and nonviable and directly expressed in percentage. The viable eggs include the ones completely or partially embryonated. The nonviable eggs include the unembryonated and the ones with degenerative changes at structural level.

From each sample 50 µL were collected and the microscopic examination was performed using 10x and 40x objectives. All possible structural changes of the eggshell and of the embryonic development were tracked. All the degenerative processes observed are illustrated in Figure (the pictures attached being from our own study).

2.4. Statistical analysis

Statistical analysis of the results was made using GraphPad Prism program, QuickCalcs, with two-tailed Fischer's exact test to obtain the P-value. The results obtained in the samples exposed to disinfectants (the number of nonviable eggs) were compared with those from the control group. Moreover, the results regarding the general action of disinfectants against the ascarid eggs and the individual action of the solutions against each ascarid species (*T. canis*, *T. cati*) were compared.

For each disinfectant, the standard deviation and the average of the results expressed in percentage obtained from 3 samples were calculated.

3. Results

The efficacy of each product is expressed in Table 2. All four disinfectants had negative effects on the viability of *T. canis* eggs, causing eggshell decortication and internal cellular content degranulation and extravasation (Figure). The percentages of *T. canis* nonviable eggs were higher in samples exposed to disinfectants for 15 and 30 min

Table 1. Chemical composition and concentration of disinfectants.

Disinfectant	Chemical composition	Solution
Kohrsolin FF (BODE Chemie GmbH, Hamburg, Germany)	Synergistic combination of glutaral and quaternary ammonium salts	1%
Terralin protect (Schülke & Mayr GmbH, Norderstedt, Germany)	A combination of quaternary ammonium compounds, aromatic alcohols, glycerin amphiphilic derivatives and nonionic surfactants	2%
Trioton rapid AF (LC Pliwa Ljubomir Cugurovic e.Kfm, Malsfeld, Germany)	Quaternary ammonium compounds and amines	0.25%
Bacillol (BODE Chemie GmbH, Hamburg, Germany)	Propan-1-ol, propan-2-ol, ethanol	1%

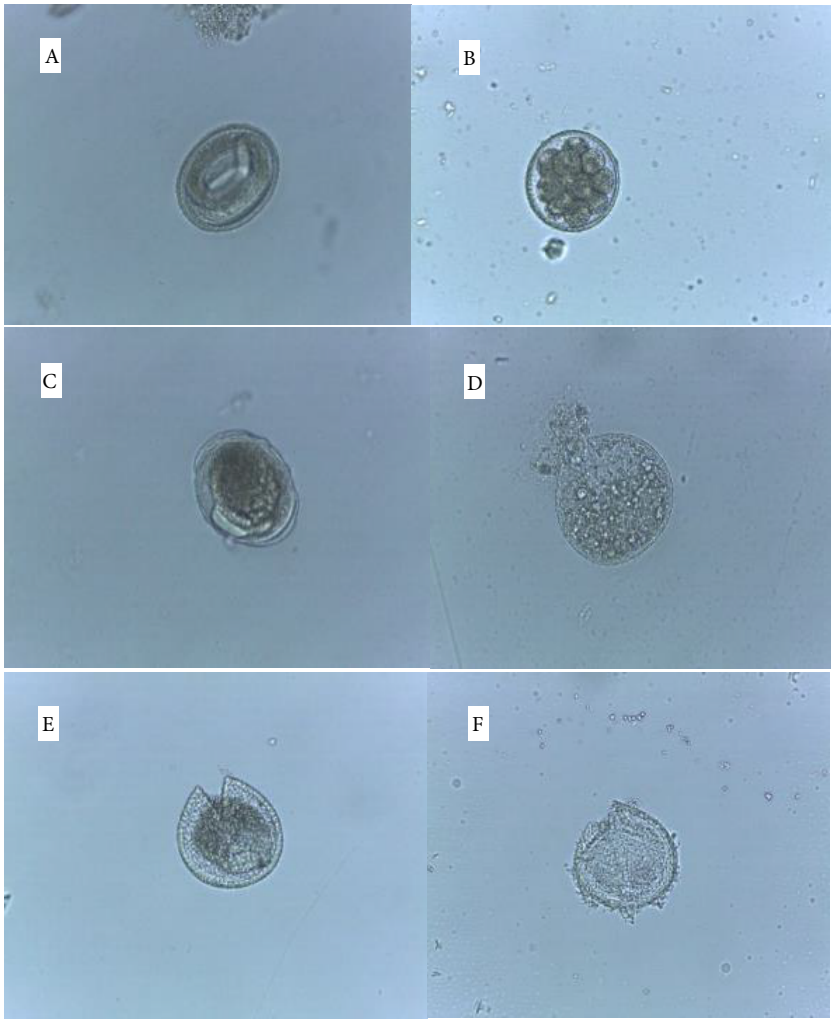

Figure. A –*Toxocaracanis*egg with larvae (control); B – *Toxocaracati* partially embryonated egg (control); C - *Toxocaracanis* eggs with destroyed walls and degranulated content (Kohrsolin® FF); D –*Toxocaracanis* eggs with destroyed walls and degranulated content (Bacillol®); E – *Toxocaracati* eggs with destroyed walls and degranulated content (Trioton® rapid AF); F –*Toxocaracati* eggs with destroyed walls and degranulated content (Bacillol®).

compared to the control group with extremely significant differences ($P < 0.0001$) after 21 days of exposure. Only Terralin® protect was ineffective against *T. cati* eggs, the percentages of nonviable eggs being similar to those found in control samples ($P > 0.005$), after 21 days.

The most effective disinfectant against *T. canis* eggs was Bacillol® 1%, regardless of the exposure time, followed by Terralin® protect 2%, Kohrsolin® FF 1% and Trioton® rapid AF 0.25% solutions. No significant differences were identified between the individual actions of disinfectants based on quaternary ammonium compounds ($P > 0.05$). Instead, extremely significant differences were noticed regarding the individual action of Bacillol® compared to the other products ($P < 0.001$).

After 21 days, the percentages of *T. cati* nonviable eggs were also higher in samples exposed to Kohrsolin® FF, Trioton® rapid AF and Bacillol® compared to the control group regardless of the exposure time, with extremely significant differences ($P < 0.001$). No significant differences were identified regarding the action of Terralin® protect solution compared to control values ($P > 0.05$).

Regarding the action of disinfectants against *T. cati* eggs, after a 15-min exposure, the percentages of nonviable eggs observed in samples exposed to Bacillol® compared to those identified in samples exposed to Kohrsolin® were similar ($P > 0.05$). Higher percentages of nonviable eggs have been noted in samples exposed to Bacillol® compared to those observed in samples exposed to Trioton® rapid AF,

Table 2. Effect of disinfectants on *Toxocara spp.* after 21 days.

Disinfectant	*Toxocaracanis*		*Toxocaracati*	
	Exposure time		Exposure time	
	15 min	30 min	15 min	30 min
	Mean[1] ± SD[2]	Mean ± SD	Mean ± SD	Mean ± SD
Kohrsolin FF	65.33 ± 0.57	66.66 ± 0.57	95.33 ± 0.57	97.33 ± 2.51
Terralin protect	65.66 ± 1.15	69.66 ± 1.52	21.33 ± 1.52	28.33 ± 0.57
Trioton rapid AF	57.66 ± 1.52	68.66 ± 1.52	84.66 ± 1.52	97 ± 2.64
Bacillol	87.66 ± 1.52	93.66 ± 1.15	96.66 ± 3.05	98.33 ± 1.52
Control	27.33 ± 2.51		23.33 ± 1.15	

[1] = the mean value was obtained by summing up the nonviable eggs in the three samples and dividing them by three, [2] = standard deviation.

the differences being very significant($P < 0.01$). Significant differences ($P < 0.05$) were observed by comparing the individual action of Kohrsolin® to Trioton®'s rapid AF. After a 30-min exposure, Kohrsolin® FF, Trioton® rapid AF and Bacillol® determined similar percentages of nonviable eggs, the differences between their action being insignificant ($P > 0.05$).

Considering the action of the same disinfectant both on *T. canis* eggs and on *T. cati* eggs, extremely significant differences ($P < 0.0001$) regarding the effect of Kohrsolin® FF, Terralin® protect and Trioton® rapid AF, exposed for 15 or 30 min, were observed. Regarding Bacillol®'s action, significant differences ($P < 0.05$), after 15-min exposure and insignificant differences ($P > 0.05$), after 30-min exposure were identified.

4. Discussion

In a similar study, Laciak et al.[16] tested a disinfectant based on quaternary ammonium compounds against *T. canis* eggs. Saniten® product was used in a 10% concentration, a higher concentration compared to 0.25% (Trioton® rapid AF), 1% (Kohrsolin® FF) and 2% (Terralin® protect) used in the current study. *T. canis* eggs had been exposed for a longer time to chemicals (60, 90, and 180 min, respectively). The percentages of nonviable eggs observed at 21 days in the samples exposed to disinfectant were 56.32% (60 min), 63.07% (90 min), and 56.01% (180 min). In the current study, similar results were obtained for *T. canis* nonviable eggs examined after the same period of time. Higher percentages of *T. cati* nonviable eggs in samples exposed to Kohrsolin®FF and Trioton® rapid AF were obtained. This suggests a greater sensitivity of *T. cati* eggs to these disinfectants, except Terralin® protect. The most effective disinfectant, identified in the study conducted by Laciak et al. [16], was H1 100% that contains hydrogen peroxide, tenside and sodium hydroxide, with 94.18% (60 min), 97.96% (90 min), and 98.04% (180 min) nonviable eggs.

Short exposure times were used in this current study in order to mimic the real-time conditions from veterinary clinics and hospitals, where a surface disinfection is performed after each patient and the period between two consultations is 30 min maximum. Perhaps a longer exposure of *T. canis* and *T. cati* eggs to disinfectants would have led to an increase in percentage of nonviable eggs.

Since Terralin® protect contains the same basic ingredients (quaternary ammonium salts) as the other two disinfectants and the conditions of egg exposure and storage were identical, further studies are required to identify the mechanism of action of these solutions, especially on *T. cati* eggs.

Other researchers revealed that the hydrogen peroxide 3% solution has an inhibitory activity on 99.73% of*T. canis*unembryonated eggs after a 24-h exposure [17].

Von Dohlen et al. [18] looked at the effect of sodium hypochlorite 5.25% solution on *T. canis* eggs. The results obtained have shown that this disinfectant is ineffective against *T. canis* eggs and that they can later develop into the infestation stage. Higher concentrations, such as 7% sodium hypochlorite solution were ineffective against *T. canis* eggs [11]. Instead, Verocai et al. [19] showed that 2%–2.5% sodium hypochlorite and 70% ethanol solutions negatively influenced the embryogenesis of *T. canis* eggs, which also caused changes in their integrity.Similar results were obtained by Morrondo et al., indicating the possibility of using sodium hypochlorite solution against *T. canis* eggs [12].

El-Sayed observed that *Zingiber officinale* ethanol extract, with 100 mg/mL concentration has ovicidal activity on *T. canis* eggs with an efficiency of 98.2% after

24 hours. The efficacy was 59.22% and 82.5%, respectively, after 24 h, in case of 25, 50 mg/mL concentrations usage [20].

In a recent study, El-Dakhly et al. [21] tested the effects of 6 commercial disinfectants on *Toxascarisleonina* embryogenesis and larval development. Only 4 of them caused a significant reduction of larval development ($P \leq 0.05$): 58.8% (TH4 - glutaraldehyde), 85.8% (70% ethanol) and 100% (Dettol® - chloroxylenol and Virkon® - potassium peroxymonosulfate, sodium chloride). Sodium hypochlorite and phenol have led to an insignificant reduction of larval development (2.8% and 21.0%, respectively).

In conclusion, these solutions based on quaternary ammoniumcompounds, propanol and ethanol have ovicidal action and can be used for disinfection of surfaces contaminated with *Toxocara*spp. eggs, except Terralin® protect which was ineffective against *Toxocaracati* eggs. Further studies are needed to identify the chemical and pathophysiological mechanism of action of these disinfectants on ascarid egg (especially *T.cati* eggs) and the morphological changes that occur.

Conflict of interest

The authors have no conflicts of interest to disclose.

References

1. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clinical Microbiology Reviews 2003; 16 (2): 265-272. doi: 10.1128/cmr.16.2.265-272.2003
2. Horiuchi S, Paller VG, Uga S. Soil contamination by parasite eggs in rural village in the Philippines. Tropical Biomedicine 2013; 30 (3): 495-503.
3. Thomas D, Jeyathilakan N. Detection of *Toxocara* eggs in contaminated soil from various public places of Chennai city and detailed correlation with literature. Journal of Parasitic Diseases 2014; 38 (2): 174-180. doi: 10.1007/s12639-012-0217-x
4. Amaral HL,Rassier GL,Pepe MS,Gallina T,Villela MM et al. Presence of *Toxocaracanis* eggs on the hair of dogs: a risk factor for Visceral Larva Migrans. Veterinary Parasitology 2010; 174 (1-2): 115-118. doi: 10.1016/j.vetpar.2010.07.016
5. Rojas TO, Romero C, Heredia R, Bautista LG, Sheinberg G. Identification of *Toxocaraspp*. eggs in dog hair and associated risk factors. Veterinary World 2017; 10 (7): 798-802. doi: 10.14202/vetworld.2017.798-802
6. Abougrain AK,Nahaisi MH,Madi NS, Saied MM, Ghenghesh KS. Parasitological contamination in salad vegetables in Tripoli-Libya. Food Control 2010; 21 (5): 760-762. doi: https://doi.org/10.1016/j.foodcont.2009.11.005
7. Adanir R, Tasci F. Prevalence of helminth eggs in raw vegetables consumed in Burdur, Turkey. Food Control 2013; 31: 482-484. doi: https://doi.org/10.1016/j.foodcont.2012.10.032
8. Hotez PJ, Wilkins PP. Toxocariasis: America's Most Common Neglected Infection of Poverty and a Helminthiasis of Global Importance?. PLoS Neglected Tropical Diseases 2009; 3 (3): e400. doi: https://doi.org/10.1371/journal.pntd.0000400
9. Holland C, O'Connor P, Taylor MR, Hughes G, Girdwood RW et al. Families, parks, gardens and toxocariasis. Scandinavian Journal of Infectious Diseases 1991; 23 (2): 225-231. doi: https://doi.org/10.3109/00365549109023405
10. Chen J, Liu Q, Liu GH, Zheng WB, Hong SJ et al. Toxocariasis: a silent threat with a progressive public health impact. Infectious Diseases of Poverty 2018; 7 (1): 59. doi: 10.1186/s40249-018-0437-0
11. Aycicek H, Yarsan E, Sarimehmetoglu HO, Tanyuksel M, Girginkardesler N et al. 2001. Efficacy of some disinfectants on embryonated eggs of *Toxocaracanis*. Turkish Journal of Medical Sciences; 31: 35-39.
12. Morrondo P, Díez-Morrondo C, Pedreira J, Díez-Baños N, Sánchez-Andrade R et al. *Toxocaracanis* larvae viability after disinfectant-exposition. Parasitology Research 2006; 99 (5): 558-561.
13. Azam D, Ukpai OM, Said A, Abd-Allah GA, Morgan ER. Temperature and the development and survival of infective *Toxocaracanis* larvae. Parasitology Research 2012; 110: 649. doi: https://doi.org/10.1007/s00436-011-2536-8
14. Alcântara-Neves NM, Barbosa dos Santos A, Mendonca LR, Figueiredo CV, Pontes-de-Carvalho L. An improved method to obtain antigen-excreting *Toxocaracanis* larvae. Experimental Parasitology 2008; 119: 349-351. doi: 10.1016/j.exppara.2008.03.006
15. Gautam S, Petkeviciute E, Storm NT, Thapa S, Mejer H. Effect of disinfectants on viability of *Ascaris suum* and *Ascaridiagalli* eggs. In: Spring Symposium of the Danish Society for Parasitlogy; Copenhagen, Denmark; 2014.
16. Laciak V, Laciaková A, Máté D, Severa J, Pagáč M. Action of selected disinfectants on *Toxocaracanis* eggs. Medycyna Weterynaryjna 2009; 65 (2): 102-106.
17. Shalaby HA, Abdel-Shafy S,Ashry HM, El-Moghazy FM. Efficacy of hydrogen peroxide and dihydroxy benzol mixture (disinfectant) on *Toxocaracanis* eggs. Research Journal of Parasitology 2011; 6: 144-150. doi: 10.3923/jp.2011.144.150
18. Von Dohlen AR, Houk-Miles AE, Zajac AM, Lindsay DS. Flotation of *Toxocaracanis* eggs in commercial bleach and effects of bleach treatment times on larval development in these eggs. Journal of Parasitology 2017; 103 (2): 183-186. doi: https://doi.org/10.1645/16-123

19. Verocai GG, Tavares PV, De Ribeiro FA, Correia TR, Scott FB. Effects of disinfectants on *Toxocaracanis* embryogenesis and larval establishment in mice tissues. Zoonoses and Public Health 2010; 57 (7-8): 213-216. doi: https://doi.org/10.1111/j.1863-2378.2010.01330.x

20. El-Sayed NM. Efficacy of *Zingiber officinale* ethanol extract on the viability, embryogenesis and infectivity of *Toxocaracanis* eggs. Journal of Parasitic Diseases 2017; 41: 1020. doi: https://doi.org/10.1007/s12639-017-0928-0

21. El-Dakhly KM, Aboshinaf ASM, Arafa WM, Mahrous LN, El-Nahass E et al. In vitro study of disinfectants on the embryonation and survival of *Toxascarisleonina* eggs. Journal of Helminthology 2018; 92 (5): 530-534. doi: https://doi.org/10.1017/S0022149X17000839