The *in vitro* effect of 1% pentapotassium solution on *Toxocara canis* eggs

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Abstract. The ascarid eggs are very resistant to disinfectants. Virkon® S is an oxygen-based chemical disinfectant containing simple organic salts and organic acids. The active ingredient decomposes in various ways in the environment, soil and water, forming non-harmful substances, such as potassium salts and oxygen. In this study, the eggs of *Toxocara canis* were taken from the uterine horns of female ascarids and exposed to 1% pentapotassium solution, for seven days. The media used for the eggs was PCA agar, in which the disinfectant solution was incorporated. To assess the ovi-cidal effect, the reduction of larval development was calculated. The results showed that the Virkon® S disinfectant presented ovi-cidal action against *Toxocara canis* eggs, with a reduction of larval development of 79.75%.

Keywords: *Toxocara canis*; Virkon® S; ovi-cidal action; pentapotassium solution.

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Introduction

*Toxocara canis* is a gastrointestinal parasite, worldwide spread, which has as a definitive host domestic and wild canines, representing a real danger to their health, with a special impact on puppies (Leutenegger-Aste, 1987).

Also, this nematode poses a health risk to humans because it can cause larva migrans syndrome, such as larva migrans visceralis and larva migrans ocularis (Leutenegger-Aste, 1987).

The prevalence of *Toxocara canis* infestation in dogs (pets, dogs from shelter, stray dogs, dogs from rural areas) in Europe ranges between 3.5% and 34% (Parsons, 1987; Overgaauw, 1997; Fok et al., 2001; Habluetzel et al., 2003; Le Nobel et al., 2004; Martínez-Carrasco et al., 2007; Dubná et al., 2017).

In optimal conditions, *Toxocara canis* eggs can develop over a relatively short time period, about 21 days (Okoshi and Usui, 1968). At 30°C, the *Toxocara canis* eggs can develop completely in about 4 days (Onorato, 1932). The ascarid eggs have a five-layer wall (Ayçicek et al., 2001), which makes them very resistant to the external environment, under varied conditions of temperature and humidity and to most disinfectants used in veterinary clinics and kennels (Bouchet et al., 1986; Oshima, 1961).
Considering the particular resistance of the ascarid eggs and starting from the results obtained by El-Dakhly et al., in 2017, in which they observed the negative action of Virkon® S solution on the embryogenesis of *Toxascaris leonina* eggs, the aim of this study was to investigate the ovicidal effect of 1% pentapotassium solution (Virkon® S) on *Toxocara canis* eggs.

**Materials and methods**

**Gathering the eggs**

The adult ascarids were gathered after deworming four puppies, approximately a month old, from the public shelter in Prahova County, with a product based on praziquantel, pyrantel pamoate and febantel (Drontal® Plus). The adult parasites removed from the faeces were examined at the magnifying glass to identify the females based on their morphology.

**Exposure of unembryonated eggs to 1% pentapotassium solution (Virkon® S, Dupont company)**

The female adult ascarids were longitudinally sectioned at the skin level to reveal the uterine horns, which were taken out, grated and the resulting contents were filtered to obtain the eggs, which were subsequently plated on solid medium (PCA – plate count agar).

To obtain the solid medium, 3.5 g of agar was used in 100 ml of distilled water and approximately 8000 eggs were spread on the agar at a depth of less than 1 mm, according to Okoshi and Usui (1967). For the tested samples, in the agar composition, 1% pentapotassium solution (Virkon® S) was incorporated.

The pentapotassium solution was prepared using a 1% concentration, meaning one gram of pentapotassium powder combined with 100 ml of distilled water.

Eight samples were obtained, four control samples and four samples that were exposed to the disinfectant. After seven days at room temperature (24 ± 3°C), the samples were examined microscopically to calculate the percentage of larvated and unembryonated eggs.

**Evaluation of effects on larval development**

The amount of oxygen required for the embryonation of *Toxocara canis* eggs was provided daily by shaking the samples. After 7 days, the percentage of larvated and unembryonated eggs was calculated according to the formula used by El-Dakhly et al. (2017):

\[
\frac{\text{larvated eggs of the control group} - \text{larvated eggs of the treated group}}{\text{larvated eggs of the control group}} \times 100
\]

**Results**

**The effects of 1% Virkon® S solution on unembryonated eggs**

The Virkon® S solution determined a 76.24% reduction of larval development (table 1), with 79.75% of unembryonated eggs and 20.25% of larvated eggs (figure 2). The *Toxocara canis* unembryonated eggs were observed with internal content and wall degeneration after 1-2 days of exposure. In the control samples, the percentage of unembryonated and larvated eggs were 14.75% and 85.25%, respectively (figure 1).

**The effects on embryonated eggs**

The Virkon® S solution on *Toxocara canis* embryonated eggs determined wall alteration and the release of larvae from the eggs.

**Discussions**

Starting from the results obtained by El-Dakhly et al. (2017), in which the Virkon® S solution showed high ovicide effect on *Toxascaris leonina* eggs, in this study the negative effect on *Toxocara canis* eggs was investigated.
Table 1. The percentage of the identified eggs (unembryonated and larvated), in control and treated samples, correlated with standard deviation and the reduction of larval development

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage of unembryonated eggs (mean±SD)</th>
<th>Percentage of larvated eggs (mean±SD)</th>
<th>Reduction of larval development (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control untreated</td>
<td>14.75±17.59</td>
<td>85.25±23.20</td>
<td>0</td>
</tr>
<tr>
<td>Virkon S</td>
<td>79.75±40.26</td>
<td>20.25±2.75</td>
<td>76.24%</td>
</tr>
</tbody>
</table>

The results were encouraging, the reduction of larval development being 79.75%. However, El-Dakhly et al. have obtained a 100% reduction of larval development, using Virkon® S and Dettol® against *Toxascaris leonina* eggs.

In a study conducted by El-Sayed in 2017, the negative effect of *Zingiber officinale* extract on *Toxocara canis* eggs was observed. The author tested multiple concentrations and the most pronounced effect was observed using the 100 mg/ml concentration, after 24 hours of egg exposure, with an efficacy of 98.2%. At concentrations like 25, 50 mg/ml of *Z. officinale* extract, after 24 hours, ovicidal activity was 59.22% and 82.5%.
Also in 2017, Von Dohlen et al. tested the effect of 5.25% sodium hypochlorite solution on *Toxocara canis* eggs. They were exposed for 15, 30, 60 and 120 minutes. After 18 days, mobile larvae have been identified, so the results obtained showed a resistance of *Toxocara canis* eggs to this disinfectant, as such it cannot be used in kennels for cleaning cages and surfaces.

In 2001, Aycicok et al. noticed that iodine-based disinfectants are effective against *Toxocara canis* embryonated eggs, compared to other commonly used disinfectants such as: glutaraldehyde, benzalkonium chloride, sodium hypochloride, potassium permanganate, ethyl alcohol, potassium hydroxide, phenol solutions.

References


Oshima T. 1961. Standardization of techniques for infecting mice with *Toxocara canis* and observations on the normal migration routes of the larvae. J. Parasitol. 47:652-656.

